Pulmonary disorders, including vocal cord dysfunction

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The lung is a very complex immunologic organ and responds in a variety of ways to inhaled antigens, organic or inorganic materials, infectious or saprophytic agents, fumes, and irritants. There might be airways obstruction, restriction, neither, or both accompanied by inflammatory destruction of the pulmonary interstitium, alveoli, or bronchioles. This review focuses on diseases organized by their predominant immunologic responses, either innate or acquired. Pulmonary innate immune conditions include transfusion-related acute lung injury, World Trade Center cough, and acute respiratory distress syndrome. Adaptive immunity responses involve the systemic and mucosal immune systems, activated lymphocytes, cytokines, and antibodies that produce $CD4^+$ T_H1 phenotypes, such as for tuberculosis or acute forms of hypersensitivity pneumonitis, and $CD4^+$ T_H2 phenotypes, such as for asthma, Churg-Strauss syndrome, and allergic bronchopulmonary aspergillosis.
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Abbreviations used

ABPA:	Allergic bronchopulmonary aspergillosis
ANCA:	Antineutrophil cytoplasmic antibody
ARDS:	Acute respiratory distress syndrome
BAL:	Bronchoalveolar lavage
COPD:	Chronic obstructive pulmonary disease
CSS:	Churg-Strauss syndrome
CT:	Computed tomography
FVC:	Forced vital capacity
HDAC:	Histone deacetylase
LT:	Leukotriene
PMN:	Polymorphonuclear leukocyte
RADS:	Reactive airways dysfunction syndrome
TLR:	Toll-like receptor
TRALI:	Transfusion-related acute lung injury
VCD:	Vocal cord dysfunction

Pulmonary disorders can be organized according to whether the primary immune responses are characterized by innate or adaptive immune responses. The innate responses use complement activation or activation of polymorphonuclear leukocytes (PMNs) and occur without a period for sensitization. The adaptive responses include T_H1 or T_H2 lymphocytes, eosinophils, antibody mediated, and granuloma formation.[1] This chapter will review the various pulmonary disorders with a predominant immunologic pattern and also discuss vocal cord dysfunction (VCD), which can coexist with asthma or occur independently and results in cough, shortness of breath, and dyspnea.

INNATE IMMUNE RESPONSES

Transfusion-related acute lung injury

Transfusion-related acute lung injury (TRALI) is a nonhemolytic transfusion reaction that occurs within 10 minutes to as long as 6 hours after infusion of a blood product and causes very severe noncardiogenic pulmonary edema, cyanosis, arterial hypoxemia, and respiratory failure.[2,3] The donor plasma typically contains antibodies to human neutrophil antigens or HLA class I or II antigens.[2,3] Neutrophil alloantibodies are found in 10% to 20% of female donors and 1% to 4% of male donors, yet the incidence of TRALI is about 1:5000 transfusions.[3] Alloantibodies are generated during pregnancy, but of course that would not explain the presence of such antibodies in men. Some recipients have antineutrophil antibodies. The immediate reaction, which might resemble anaphylaxis, involves sequestration of PMNs in the pulmonary vasculature, complement activation, and generation of TGF-β, IL-8, and IL-13.[2] Immune complexes activate PMNs and cause disruption of the endothelium barrier to plasma. TRALI is extremely rare after intravenous immunoglobulin infusions but occurs with infusions of platelets (suspended in plasma), whole blood, cryoprecipitates, and fresh frozen plasma.

The immediate management includes stopping the infusion, oxygen, mechanical ventilation if indicated, and treatment of hypotension with vasopressors. Donors should be deferred from future donations. Indeed, some transfusion experts have recommended that the donor pool should not include women who have been pregnant and that donor plasma be tested for alloantibodies.[2,3] Neither of these suggestions are standard practice.

Acute respiratory distress syndrome and acute lung injury

Acute respiratory distress syndrome (ARDS) and acute lung injury represent diffuse pulmonary disease that can be fatal.[4] ARDS is a more severe form of acute lung injury. Causes include sepsis, pneumonia, trauma, or aspiration pneumonia.[4] Patients experience severe dyspnea, tachypnea, and hypoxemia. The chest roentgenogram and computed tomographic (CT) examination demonstrate bilateral infiltrates, alveolar consolidation, and "white out" of the lung. The alveoli collapse as they become filled with protein and fibrin-rich exudates (hyaline membranes), which inactivate surfactant.[4,5] Neutrophils release oxidant proteases, which damage the capillary endothelium. Bronchoalveolar lavage (BAL) reveals the presence of PMNs, procoagulant activity, IL-8 (chemotactic for PMNs), IL-2, IL-6, and TGF-β. There is reduced apoptosis of

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PMNs, which is attributable to increased concentrations of BAL fluid IL-2, IL-8, granulocyte colony-stimulating factor, GM-CSF, and growth-related oncogene α.[6] Alternatively, there is enhanced apoptosis of epithelial cells, resulting in the lack of a sufficient barrier between the alveoli and capillaries. TNF-related apoptosis-induced ligand levels are increased in BAL fluid in patients with ARDS and are recognized as proapoptotic for epithelial cells.[6]

Patients requiring mechanical ventilation benefit from smaller volumes, such as a tidal volume of 6 mL/kg, with positive end-expiratory pressures of 5 to 10 cm H_2O. Fluid replacement should be conservative. Corticosteroids and other interventions, such as nitric oxide and surfactants, are not effective.[7]

Community-acquired pneumonia

Community-acquired pneumonia presents with a productive cough, fever, pleuritic chest pain, and abnormal chest roentgenographic results.[8] On auscultation, there can be crackles and bronchial breath sounds. Most pathogens include viruses, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, and *Legionella pneumophila*.[8] There might be no recovered organisms in some patients. Comorbidities influence survival.[8]

Levels of proinflammatory cytokines, such as TNF-α and IL-6, and the anti-inflammatory cytokine IL-10 are increased in those patients who succumb compared with survivors.[9] Impaired recognition of molecular patterns of bacteria is associated with decreased activation of innate immunity and worse clinical outcomes.[10] Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns, and genetic polymorphisms have been identified in patients who had invasive *S pneumoniae* infections.[10] For example, polymorphisms of TLR4 impair its function in recognition of *S pneumoniae* pneumolysin, whereas polymorphisms of CD14, a coreceptor on monocytes for both TLR2 and TLR4, are associated with invasive *S pneumoniae* infections.[10] Polymorphisms in FCγRIIA increase the susceptibility to invasive disease. Current therapy includes early administration of antibiotics and supportive care. Future diagnosis might identify at-risk subjects proactively, and therapies will be able to strengthen the innate immune system.

NONINFECTIOUS PULMONARY CONDITIONS

Byssinosis occurs from the inhalation of dusts from flax, cotton, sisal, and hemp. The dusts produce bronchoconstriction, typically on the first day of the workweek, but then tachyphylaxis develops with continued exposure. Byssinosis is not asthma or hypersensitivity pneumonitis.[1] At-risk workers include those who are exposed to endotoxin during the processing of raw cotton. In contrast, workers who spin cotton are not exposed to the high concentrations of endotoxin and are considered at low risk. Long-term exposure can result in symptoms of chronic bronchitis and cough. Modest reductions in FEV_1 and forced vital capacity (FVC) have been found, but concurrent smoking appears to be the major contributor as opposed to workplace exposures. Prevention includes methods to reduce the generation of endotoxins from gram-negative bacteria by reducing exposure to waste from cotton.

In contrast to byssinosis, the organic toxic dust syndrome is a toxic alveolitis that produces influenza-type symptoms of sudden-onset headache, chills, nonproductive cough, myalgias, arthralgias, and dyspnea. Crackles can be present on lung auscultation. The onset of symptoms is within 12 hours of inhalation of organic dusts. Although the clinical presentation might mimic that of acute hypersensitivity pneumonitis, there is no requirement for prior exposure or immunologic sensitization (see the later section on hypersensitivity pneumonitis). Various circumstances of exposure have been described, such as from organic mulch, endotoxin-rich vegetables and grass seeds, and contaminated seaweed. Massive inhalation of microbial products can cause an ARDS-like presentation, and this is designated as organic dust toxic syndrome or pulmonary mycotoxicosis.[11]

In patients with silo-unloader's disease, there is inhalation of nonorganic gases, such as NO, NO_2, or N_2O_4. These nitrogen oxides then generate nitric and nitrous acids that cause noncardiac pulmonary edema and, in some patients, methemoglobinemia. Deaths can occur, whereas survivors might have bronchiolitis obliterans.

Grain-handler's disease occurs in agricultural workers with a chronic cough, symptoms of chronic bronchitis, or wheeze after exposure to grain dusts. Concurrent cigarette smoking appears to be more injurious to the lung and associated with reductions in spirometric values. Measures to reduce exposure to dust are beneficial. Because of less implementation of safety standards, there is a major concern that workers will experience grain-handler's disease and other respiratory disorders in the world's emerging economies.

Reactive airways dysfunction syndrome

The reactive airways dysfunction syndrome (RADS) describes a single unexpected inhalation of high concentrations of irritant fumes, vapors, fog, or smoke that results in acute cough, dyspnea, and wheezing within 24 hours.[12] An asthma-like syndrome begins that can last for months or years. Bronchial hyperreactivity can be demonstrated by means of methacholine challenge testing, and spirometry reveals normal or decreased FEV_1, FVC, and FEV_1/FVC ratio. There might be little to no bronchodilator response to albuterol. Bronchial biopsy specimens demonstrate loss of epithelium, subepithelial fibrosis, and infiltrates with lymphocytes but not eosinophils (as would be characteristic of asthma).

RADS might be confused with occupational asthma, where there is a sensitization period of months or years before symptoms begin, and with aggravation of underlying asthma. But RADS refers to the acute irritant-induced asthma.

World Trade Center cough

The first responders to the 2001 collapse of the World Trade Center in New York City experienced a very troublesome cough within 24 hours of beginning rescue operations.[13,14] The exposures included acrid smoke, fires that burned for 3 months, asbestos, glass fibers, lead, and aromatic compounds. Many responders did not use protective masks. Subsequent evaluations identified methacholine hyperreactivity in 24% and a reduced FEV_1/FVC ratio of less than 0.75 in 16% of affected subjects.[13] The high exposures would be consistent with a diagnosis of RADS in some subjects.[14]

VCD

VCD is a form of "functional" or nonanatomic upper airway obstruction.[15] The inspiratory tracing on a flow-volume loop is truncated (Fig 1) or incompletely performed. Other causes of nonanatomic inspiratory obstruction include vocal cord paralysis,

neuromuscular disorders, and sleep disorders.[15] In contrast, some anatomic abnormalities that cause a truncated inspiratory loop include a large goiter, tracheal stenosis, and an obstructing tumor. Symptoms of VCD include dyspnea, wheeze, tightness in the neck, shortness of breath, inability to breathe deeply or satisfactorily, and coughing. Some patients with VCD have concurrent asthma and chronic rhinosinusitis with postnasal drainage or gastroesophageal reflux or atypical (laryngopharyngeal) reflux. VCD can be intermittent and might not be present when the patient is distracted, sedated, or asleep. VCD can masquerade as or coexist with severe asthma.[16]

Recognition of VCD might begin with the truncated inspiratory loop of the flow-volume tracing, especially when the patient is symptomatic. Alternatively, it can be suspected when the patient's difficulty breathing surpasses the physical findings, such as clear chest on auscultation, wheezes over the neck but not lower airways, whispering instead of talking loudly, and refusal to inspire to total lung capacity or produce an appropriate forced expiratory maneuver. Bronchoscopy might be of value in excluding other causes. Fiberoptic laryngoscopy can help demonstrate the adduction of vocal cords during inspiration. When methacholine challenge tests are performed in patients with VCD, there might or might not be apparent flattening of the inspiratory flow-volume loop or, in fact, quite severe airways obstruction, even stridor or respiratory arrest. The latter can occur in patients with major psychiatric diagnoses and even should be anticipated in considering a methacholine challenge test in such patients with VCD.

Some patients benefit from speech therapy, which can emphasize breathing through the abdomen as opposed to thoracic breathing. Nevertheless, other patients with psychologic or psychiatric conditions might not overcome their VCD. When this is the case, it is important to avoid continued treatment with systemic corticosteroids unless it is demonstrated that there is both persistent asthma and VCD.

GRANULOMATOUS T_H1 INFLAMMATORY CONDITIONS

The granulomatous T_H1 conditions comprise sarcoidosis, tuberculosis, berylliosis, and hypersensitivity pneumonitis. CD4 T_H1 lymphocytes participate in granuloma formation. Some cytokines include IL-2, IL-12, and IFN-γ. IFN-γ, which is generated by CD4 T_H1 and $CD8^+$ lymphocytes, can be measured in patients with tuberculosis, and the US Food and Drug Administration has approved an assay that helps in the diagnosis of tuberculosis.[17] Class I MHC–restricted $CD8^+$ lymphocytes can function as memory cells to *Mycobacterium tuberculosis*.[18] In patients with advanced pulmonary tuberculosis, the BAL fluid reveals increased numbers of $CD4^+$ lymphocytes and increased $CD4^+$/$CD8^+$ ratios. There is evidence for pulmonary sequestration or compartmentalization of the $CD4^+$ lymphocytes because the peripheral blood $CD4^+$ lymphocytes can be decreased relatively and the $CD4^+$/$CD8^+$ ratio is reduced because of increases in the numbers of $CD8^+$ lymphocytes.[19] In patients with HIV/AIDS, the low numbers of $CD4^+$ lymphocytes are associated with greater susceptibility and more severe tuberculosis,[20] including decreased delayed hypersensitivity responses (type IVa1).

Granulomas help limit the replication of mycobacteria; however, lung architecture is destroyed in the process. $CD4^+CD25^+$ regulatory T cell numbers are increased in patients with tuberculosis and are thought to help control or attempt to control the intensity of the $CD4^+$ T_H1 granulomatous responses.[21] The expression of the transcription factor forkhead box protein 3 is increased and is indirect evidence of regulatory T-cell suppression of the granulomas.

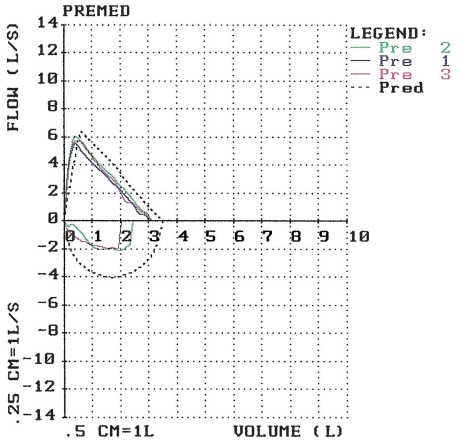

FIG 1. Flow-volume loop of a 26-year-old woman with shortness of breath, wheezing, and cough. Note blunting of the inspiratory phase versus predicted value. FVC was 3.19 L (91%), FEV_1 was 2.75 L (91%), and FEV_1/FVC ratio was 0.86. Notably, forced expiratory flow at 50%/forced inspiratory flow at 50% of FVC was increased at 1.62 (normal value is <1).

Sarcoidosis remains a disease of unknown cause that produces noncaseating, epithelioid granulomas that can affect most organ systems.[22] BAL fluid recoveries demonstrate very high numbers of activated $CD4^+$ lymphocytes, which are sustained by IL-2.[22] $CD4^+$ T_H1 lymphocytes participate in formation of the granuloma, in association with IFN-γ, and activated macrophages. IL-18, derived from monocyte/macrophages and airway epithelial cells, upregulates expression of IL-2 and supports IFN-γ production.[23] IL-18 levels are increased in BAL fluid and plasma and have been associated with progression of sarcoidosis.

Although not all patients are treated because up to two thirds have a spontaneous remission, initial pharmacotherapy is with oral corticosteroids. In an attempt to reduce the granulomatous response, TNF-α inhibitors have been administered to patients with sarcoidosis[22]; their role is not established, however. Endobronchial sarcoidosis is a rare cause of cough and wheezing.

GRANULOMATOUS T_H2 INFLAMMATORY CONDITIONS

Churg-Strauss syndrome (CSS) is a systemic, necrotizing, eosinophil-laden granulomatous vasculitis. The presentation can be that of (1) asthma with pulmonary infiltrates, (2) peripheral blood eosinophilia, (3) peripheral neuropathy (mononeuritis multiplex), or (4) palpable purpura. When a patient with asthma experiences palpable purpura on the shins or upper extremities or if foot or wrist drop occurs, CSS should be suspected. A decrease in oral corticosteroids or in high-dose inhaled corticosteroids might be associated with onset of fever and eosinophilic pneumonia, purpura, or wrist drop, any of which should raise the possibility of CSS. Histologic evidence for CSS can be obtained by means of skin biopsy or biopsy of nerves (eg, sural) or pulmonary tissue.

Laboratory findings demonstrate peripheral blood eosinophilia (20% to 60%), $CD4^+$ T_H2 lymphocytes, increased total IgE concentrations, and antineutrophil cytoplasmic antibodies (ANCAs). Approximately 60% of patients have the perinuclear pattern of

ANCAs, which on ELISA is positive for antibodies to myeloperoxidase, whereas 10% of patients have positive results for cytoplasmic staining, with antibodies directed against proteinase-3.[1] Although the presence of a perinuclear pattern of ANCAs is helpful in supporting a diagnosis, the ANCA titers do not provide prognostic information for disease management.[24,25] Similarly, eosinophil-derived major basic protein and cationic protein have not been demonstrated to have utility in guiding treatment.[24] Urinary concentrations of leukotriene (LT) E_4, the major metabolite of LTC_4 and LTD_4, and eosinophil-derived neurotoxin and 3-bromotyrosine, a marker for oxidation of eosinophils, are increased in patients with CSS.[26]

The 6-year survival has been reported to be 70%.[24] Long-term survival, up to 26 years, has also been reported.[27] The most effective therapy has been with oral corticosteroids.[24,27] Additional corticosteroid-sparing and immunosuppressive therapies include cyclophosphamide, azathioprine, IFN-α, mepolizumab (anti–IL-5), omalizumab (anti-IgE), and rituximab (anti-CD20 B lymphocytes). There are potential untoward effects from cyclophosphamide (cytopenias, hemorrhagic cystitis, and malignancy potential), azathioprine (cytopenia, nausea, and vomiting), and IFN-α (depression and progressive multifocal leukoencephalopathy). Often patients can be managed long-term with prednisone administered on an alternate-day schedule with or without immunosuppressive therapy, such as with azathioprine. Abrupt discontinuation of prednisone is not advisable because it can result in fever, eosinophilia, and pulmonary infiltrates within a few days, demonstrating that the CSS might be controlled but is not in remission.

T_H1-RELATED INFLAMMATORY CONDITIONS

Hypersensitivity pneumonitis

Hypersensitivity pneumonitis is a $CD4^+$ T_H1 and $CD8^+$ lymphocyte–predominant alveolitis that results in noncaseasting granulomas and pulmonary fibrosis. Clinical stages include acute, subacute (clinically similar to acute), and chronic. In the acute and subacute stages inhalation of organic antigens causes cough, shortness of breath, myalgias, and fever within 4 to 6 hours. The physical examination would reveal pulmonary crackles. A patient might self-treat for "flu" or be given an improper diagnosis of community-acquired pneumonia. When there is continued or repeated exposure to antigens, such as bird excreta, patients might have subacute episodes or evolve into chronic hypersensitivity pneumonitis where typical flu-like illness does not occur. The latter patients experience a nonproductive cough and progressive dyspnea and, in advanced cases, oxygen requirements. Pulmonary function tests in the acute and subacute stages typically are described as restrictive; however, especially with bird fanciers, obstructive findings can occur and mimic asthma. The restrictive findings are associated with a decreased diffusing capacity for carbon monoxide. In contrast, the diffusing capacity for carbon monoxide in patients with asthma is normal or even increased.

High-resolution CT scans demonstrate small nodules (<5 mm) that indicate alveolitis or areas of pulmonary fibrosis. Mosaic findings of fibrosis are present in patients with chronic hypersensitivity pneumonitis. An example of pulmonary fibrosis and traction bronchiectasis from avian hypersensitivity pneumonitis is shown in Fig 2.

There is striking BAL lymphocytosis of 60% to 80% from acutely ill patients.[28,29] The classic finding is a CD4/CD8 ratio of less than 1, whereas in patients with sarcoidosis, the CD4/CD8 ratio is as high as 8 because of the $CD4^+$ alveolitis.[30] In patients with hypersensitivity pneumonitis, levels of T_H1 cytokines are increased, including IL-12, IL-18, and TNF-α. $CD8^+$ lymphocytes serve as effector cells but are not sufficiently functional.[28,31,32] In contrast, in patients with chronic hypersensitivity pneumonitis, there can be an increase in the CD4/CD8 ratio as the CD4 (and T_H2) lymphocytes increase and $CD8^+$ lymphocytes decrease.[32] It has been suggested that the effector $CD8^+$ lymphocytes become "exhausted." These data suggest that chronic hypersensitivity pneumonitis is associated with "skewing" toward T_H2 lymphocytes, IL-4 production, and pulmonary fibrosis.[32] IL-17, which is proinflammatory, increases activation and numbers of neutrophils, and upregulates IL-6, IL-8, and TNF-α, might participate in hypersensitivity pneumonitis.[33,34]

Treatment includes early identification of patients with hypersensitivity pneumonitis, avoidance/remediation of the antigens involved, oral corticosteroids for short-term use, and monitoring of overall respiratory status depending on the stage that is present.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterized by fixed dyspnea, lack of fully reversible airways obstruction, and progressive loss of FEV_1 over time. Cessation of cigarette smoking and use of oxygen have proved of value. Pharmacotherapy includes short- and long-acting bronchodilators and anticholinergic medications. For patients with moderate–to–very severe COPD, when the FEV_1 is less than 50% and the FEV_1/FVC ratio is less than 70%, combination inhaled corticosteroid/long-acting β-agonist therapy is recommended. Treatment with combination fluticasone propionate and salmeterol has resulted in fewer exacerbations but not fewer deaths.[35] In a study of patients with COPD in whom fluticasone/salmeterol or salmeterol was added to tiotropium, there was no additional benefit over tiotropium in the primary outcome of exacerbations of COPD.[36] Secondary outcomes did demonstrate increases in FEV_1, fewer hospitalizations, and improved quality-of-life measures in those patients receiving fluticasone/salmeterol.[36] An unexpected finding has been increased numbers of cases of pneumonia in patients with COPD receiving high-dose fluticasone propionate.[35,37]

The pathogenesis of COPD includes cigarette smoking (most cases), viral or bacterial infections (or a combination), genetic susceptibility, oxidative stress, and little to no response to high-dose corticosteroids. Sputum often harbors PMNs, but eosinophils can be present with either viral or combined viral and bacterial infections.[38] In patients with COPD, not only is there presence of PMNs and macrophages, there are also increases in CD4 T_H1 and CD8 lymphocyte numbers.[39]

The impaired response to corticosteroids helps differentiates COPD from asthma in most cases. After absorption, the corticosteroid binds to its receptor and traverses the cytoplasm and enters the nucleus, where it interacts with glucocorticoid response elements of DNA.[40] Then corticosteroids can reduce levels of the proinflammatory transcription factors nuclear factor κB and activator protein 1. It is thought that these transcription factors would have been upregulated by viral upper respiratory tract infections. Transcription factors can be generated as the DNA-histone complex "unwinds" during a process of acetylation by histone acetyltransferase.[40] Histone acetyltransferase levels are increased in some but not all cases of COPD, whereas in patients

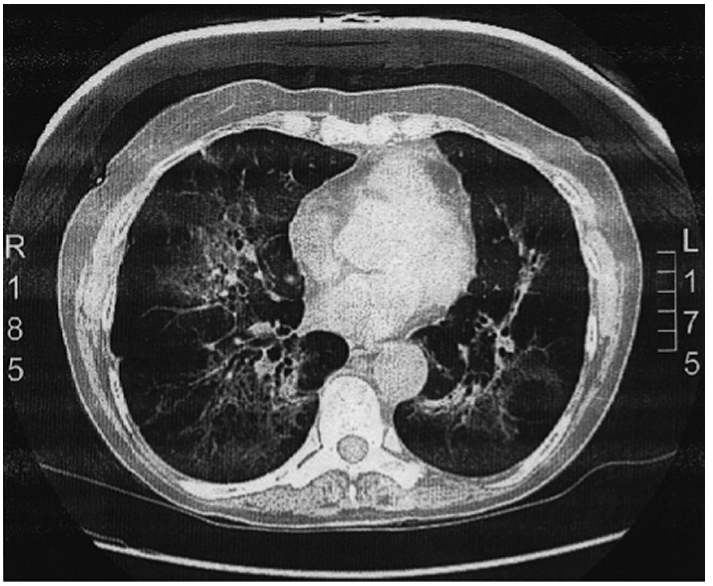

FIG 2. A 62-year-old woman who presented with "uncontrolled asthma" and had pulse oxygenation of 83% on room air reported shortness of breath for 6 years. She had 5 birds at home and worked at an exotic animal house. The CT examination revealed widened (bronchiectactic) bronchi, honeycomb fibrosis, and some opacities near the bronchi. The bronchiectasis occurred because of traction by the lung parenchyma/interstitium on the bronchi. The diffusion capacity of the lung for carbon monoxide was 39%, and the FVC was 74%. FEV_1 was 84% of predicted value, with a 6% improvement with albuterol.

with asthma, they are increased consistently.[40] Gene repression can occur when the DNA is deacetylated by histone deacetylase (HDAC) as the DNA is compacted. HDAC levels are reduced in both patients with COPD and those with asthma, but corticosteroids will increase HDAC levels in patients with asthma but not those with COPD.[40] Lack of deacetylation of the DNA in patients with COPD can favor sustained proinflammatory action and lack of response to corticosteroids, which is in contrast to what occurs in patients with asthma.

T_H2-RELATED INFLAMMATORY CONDITIONS

It has been reported that the half-life of eosinophils in peripheral blood is 8 to 18 hours and 2 to 5 days or longer in tissue.[41] In addition, perhaps there are at least 100 times as many eosinophils in tissue than in peripheral blood.[41] In the bone marrow eosinophils differentiate and proliferate from $CD34^+$ progenitors (see Chapter 6) with the major cytokines IL-3, IL-5, and GM-CSF.[42] Potent chemoattractants for eosinophils include RANTES, CCL11 (eotaxin-1), platelet-activating factor, and LTB_4.[42] The interaction of very late antigen 4 on eosinophils with vascular cell adhesion molecule 1 on endothelium results in firm adhesion to the endothelial cells. During allergic reactions, IL-4, IL-13, and TNF-α will upregulate vascular cell adhesion molecule 1, enhancing this process.

In Table I there is a list of prototype pulmonary eosinophilia syndromes or conditions. One prototype condition is allergic bronchopulmonary aspergillosis (ABPA), which complicates both asthma and cystic fibrosis.[43,44] ABPA might overlap with either hyper-IgE syndrome or chronic granulomatous disease.[45] Patients with asthma who have ABPA typically experience pneumonias or pulmonary infiltrates with eosinophilia (10% to 30%) but not peripheral blood eosinophilia as high as 40% to 60%, which occurs with CSS or parasitism. All patients have immediate skin reactivity to *Aspergillus fumigatus*. Because some commercial mixtures of *Aspergillus* species or mold mixes contain little or no *A fumigatus*, it is advisable to use a reactive extract for screening. Negative skin test results help to exclude ABPA for nearly all patients unless there is an allergic bronchopulmonary mycosis present. High-resolution CT examination demonstrates proximal bronchiectasis (inner two thirds of the lung field) in contrast to the distal bronchiectasis that occurs in some patients with COPD or recurrent infections. In patients with cystic fibrosis, there is proximal and distal bronchiectasis, and such a finding should suggest the possibility of concomitant (usually pancreatic sufficient) cystic fibrosis. In patients with ABPA, the predominant response is that of $CD4^+$ T_H2 lymphocytosis; eosinophilia; increased total serum IgE and anti–*A fumigatus* IgE, IgG, and IgA antibody levels; precipitating antibodies *to A fumigatus* and a genetically restricted susceptibility profile; and increased responsiveness to IL-4 stimulation.[43,46,47]

Treatment includes avoidance/remediation of areas in a home/workplace of obvious mold growth that can occur from unplanned water entry, oral corticosteroids to clear the pulmonary infiltrates and manage asthma, antiasthma medications as indicated, monitoring of the total serum IgE concentration because doubling over baseline values indicates a possible current new pulmonary infiltrate, and assessment of pulmonary function and respiratory status over time.[43] For initial treatment of a patient with newly diagnosed ABPA, the dose of prednisone is 0.5 mg/kg given each morning for 1 to 2 weeks, with conversion to alternate day-therapy for 2 months. The radiographic findings can be expected to clear or be reduced, as demonstrated by means of high-resolution CT examination in 2 months. Then the prednisone can be tapered and discontinued. It is not necessary to continue prednisone indefinitely in the absence of new infiltrates or development of severe (prednisone dependent) asthma. With use of the alternate-day prednisone, serious adverse effects are avoided or minimized.

TABLE I. Pulmonary eosinophilia syndromes or conditions

Asthma (allergic and nonallergic)
Asthma with atelectasis from mucus plugging
ABPA
Allergic bronchopulmonary mycosis
CSS
Collagen vascular disease (rare)
Drug allergy with pulmonary eosinophilia
Eosinophilic pneumonia
Acute (BAL fluid eosinophilia 25% to 60% with little or no peripheral blood eosinophilia)
Chronic (high peripheral blood eosinophilia)
Simple eosinophilia (Loffler syndrome)
Tropical pulmonary eosinophilia
Hypereosinophilic syndromes (interstitial infiltrates and pleural effusions, thromboembolism)
Neoplasms
Parasitism (helminthic)
Sarcoidosis (very rare)

Adapted with permission from Greenberger.[1]

Antifungal therapies have been used for the treatment of ABPA and are considered adjunctive.[48,49] A potentially good candidate for antifungal therapy is a patient with sputum plugs harboring *A fumigatus* despite prednisone therapy. There are reports of the use of omalizumab[50] for patients with ABPA, but it remains to be established whether this treatment will help to prevent new infiltrates or improve asthma symptoms.

Eosinophilic pneumonias are divided into 4 types: acute, chronic, simple, and tropical (Table I). Acute eosinophilic pneumonia can masquerade as severe community-acquired pneumonia and present with respiratory failure. When there is no or little peripheral blood eosinophilia, the diagnosis can be made with bronchoscopy and BAL showing eosinophilia of 25% to 60%. Alternatively, there might be peripheral blood eosinophilia as high as 42%.[51] Drugs, nonprescription products, parasitism, and other causes of widespread pulmonary infiltrates should be considered.

Chronic eosinophilic pneumonia is characterized by respiratory symptoms for at least 2 weeks, peripheral blood eosinophilia of at least 1000/mm^3 or BAL eosinophilia of greater than 25%, and bilateral pulmonary infiltrates.[52] In classic presentations the infiltrates are in the periphery, suggesting the photographic negative of pulmonary edema. Most patients require years of oral corticosteroid treatment. The radiographic infiltrates and surges of peripheral blood eosinophilia can be controlled with modest doses of prednisone.

Simple pneumonia (Loffler syndrome) is a mild condition lasting less than 4 weeks and has transient pulmonary infiltrates.

Tropical pulmonary eosinophilia is characterized by widespread pulmonary infiltrates and high levels of peripheral blood eosinophilia. Mediastinal lymph nodes might be enlarged and can harbor activated eosinophils. Patients typically have lived in endemic areas of parasites before tropical pulmonary eosinophilia occurs.

SUMMARY

The immunologic features of pulmonary disorders can be used to categorize various conditions and provide focus for potential innovative therapies. Although usually there is not a single treatment that antagonizes a critical component of either the innate or acquired immune system and results in clinical improvement, complex conditions might be amenable to immunologically based treatments in the future. A more ambitious goal is primary prevention of many of the pulmonary conditions discussed in this chapter. The ability to diagnose pulmonary conditions and the masquerader of asthma, VCD, continues to improve, which should result in earlier diagnoses and improved outcomes.

REFERENCES

1. Greenberger PA. 7. Immunologic lung disease. J Allergy Clin Immunol 2008; 121(suppl):S393-7.
2. Eder AF, Herron R, Strupp A, Dy B, Notari EP, Chambers LA, et al. Transfusion-related acute lung injury surveillance (2003-2005) and the potential impact of the selective use of plasma of male donors in the American Red Cross. Transfusion 2007;47:599-607.
3. Eder AF, Benjamin RJ. TRALI risk reduction: donor and component management strategies. J Clin Apher 2009;24:122-9.
4. Tsushima K, King LS, Aggarwal NR, De Gorordo A, D'Alessio FR, Kubo K. Acute lung injury review. Intern Med 2009;48:621-30.
5. Bastarache JA, Wang L, Geiser T, Wang Z, Albertine KH, Matthay MA, et al. The alveolar epithelium can initiate the extrinsic coagulation cascade through expression of tissue factor. Thorax 2007;62:608-16.
6. Lee KS, Choi YH, Kim YS, Baik SH, Oh YJ, Sheen SS, et al. Evaluation of bronchoalveolar lavage fluid from ARDS patients with regard to apoptosis. Respir Med 2008;102:464-9.
7. Calfee CS, Matthay MA. Nonventilatory treatments for acute lung injury and ARDS. Chest 2007;131:913-20.
8. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007;44(suppl):S27-72.
9. Reade MC, Yende S, D'Angelo G, Kong L, Kellum JA, Barnato AE, et al. Differences in immune response may explain lower survival among older men with pneumonia. Crit Care Med 2009;37:1655-62.
10. Yuan FF, Marks K, Wong M, Watson S, de Leon E, McIntyre PB, et al. Clinical relevance of TLR2, TLR4, CD14 and FCγRIIA gene polymorphisms in *Streptococcus pneumoniae* infection. Immunol Cell Biol 2008;86:268-70.
11. Perry LP, Iwata M, Tazelaar HD, Colby TV, Yousem SA. Pulmonary toxicosis: a clinicopathologic study of three cases. Mod Pathol 1998;11:432-6.
12. Brooks SM, Weiss MA, Bernstein IL. Reactive airways dysfunction syndrome (RADS). Persistent asthma syndrome after high level irritant exposures. Chest 1985;88:376-84.
13. Prezant DJ, Weiden M, Banauch GI, McGuinness G, Rom WN, Aldrich TK, et al. Cough and bronchial responsiveness in firefighters at the world trade center site. N Engl J Med 2002;347:806-15.
14. Banauch GI, Alleyne D, Sanchez R, Olender K, Cohen HW, Weiden M, et al. Persistent hyperreactivity and reactive airways dysfunction in firefighters at the world trade center. Am J Respir Crit Care Med 2003;168:54-62.
15. Sterner JB, Morris MJ, Sill JM, Hayes JA. Inspiratory flow-volume curve evaluation for detecting upper airway disease. Respir Care 2009;54:461-6.
16. Moore WC, Peters SP. Severe asthma: an overview. J Allergy Clin Immunol 2006; 117:487-94.
17. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med 2007;146:340-54.
18. Guyot-Revol V, Innes JA, Hackforth S, Hinks T, Lalvani A. Regulatory T cells are expanded in blood and disease sites in patients with tuberculosis. Am J Respir Crit Care Med 2006;173:803-10.
19. Tsao TCY, Chen H-C, Jong J-H, Hsieh M-J, Tsao K-C, Lee C- H. Shifts of T4/T8 T lymphocytes from BAL fluid and peripheral blood by clinical grade in patients with pulmonary tuberculosis. Chest 2002;122:1285-91.
20. Glynn JR, Murray J, Bester A, Nelson G, Shearer S, Sonnenberg P. Effects of duration of HIV infection and secondary tuberculosis transmission on tuberculosis incidence in the South African gold mines. AIDS 2008;22:1859-67.
21. Richeldi L. An update on the diagnosis of Tuberculosis infection. Am J Respir Crit Care Med 2006;174:736-42.
22. Callejas-Rubio JL, Lopez-Perez L, Ortego-Centeno N. Tumor necrosis factor-alpha inhibitor treatment for sarcoidosis. Ther Clin Risk Manag 2008;4:1305-13.
23. Kieszko R, Krawczyk P, Jankowska O, Chocholska S, Krol A, Milanowski J. The clinical significance of interleukin 18 assessment in sarcoidosis patients. Respir Med 2007;101:722-8.

24. Klion AD, Bochner BS, Gleich GJ, Nutman TB, Rothenberg ME, Simon H-U, et al. Approaches to the treatment of hypereosinophilic syndrome: a workshop summary report. J Allergy Clin Immunol 2006;117:1292-302.
25. Guillevin L, Cohen P, Gayraud M, Lhote F, Jarrousse B, Cassassus P. Churg-Strauss syndrome. Clinical study and long-term follow-up of 96 patients. Medicine (Baltimore) 1999;78:26-37.
26. Highashi N, Mita H, Taniguchi M, Turikisawa N, Higashi A, Ozawa Y, et al. Urinary eicosanoid and tyrosine derivative concentrations in patients with vasculitides. J Allergy Clin Immunol 2004;114:1353-8.
27. Vemuri P, Greenberger PA, Patterson R. Churg-Strauss syndrome: survival for 26 years. Ann Allergy Asthma Immunol 2002;88:640-3.
28. Fink JN, Ortega HG, Reynolds HY, Cormier YF, Fan LL, Franks TJ, et al. Needs and opportunities for research in hypersensitivity pneumonitis. Am J Respir Crit Care Med 2005;171:792-8.
29. Agostini C, Calabrese F, Poletti V, Marcer G, Facco M, Miorin M. CXCR3/CXCL10 interactions in the development of hypersensitivity pneumonitis. Respir Res 2005;6:20.
30. Hamagami S, Miyagawa T, Ochi T, Tsuyuguchi I, Kishimoto S. A raised level of soluble CD8 in bronchoalveolar lavage in summer-type hypersensitivity pneumonitis. Am J Respir Crit Care Med 1999;159:1830-4.
31. Chen B, Tong Z, Nakamura S, Costabel V, Guzman J. Production of IL-12, IL-18 and TNF-alpha by alveolar macrophages in hypersensitivity pneumonitis. Sarcoidosis Vasc Diffuse Lung Dis 2004;21:199-203.
32. Barrera L, Mendoza F, Zuniga J, Estrada A, Zamora AC, Melendro EI, et al. Functional diversity of T-cell subpopulations in subacute and chronic hypersensitivity pneumonitis. Am J Respir Crit Care Med 2008;177:44-55.
33. Joshi AD, Fong DJ, Oak SR, Trujillo G, Flaherty KR, Martinez FJ, et al. Interleukin-17-mediated immunopathogenesis in experimental hypersensitivity pneumonitis. Am J Respir Crit Care Med 2009;179:705-16.
34. Selman M, Pardo A, Barrera L, Estrada A, Watson SR, Wilson K, et al. Gene expression profiles distinguish idiopathic pulmonary fibrosis from hypersensitivity pneumonitis. Am J Respir Crit Care Med 2006;173:188-98.
35. Calverly PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. N Engl J Med 2007;356:775-89.
36. Aaron SD, Vandemheen KL, Fergusson D, Maltais F, Bourdeau J, Goldstein R, et al. Tiotropium in combination with placebo, salmeterol, or fluticasone-salmeterol for treatment of chronic obstructive pulmonary disease: a randomized trial. Ann Intern Med 2007;146:545-55.
37. Weddzicha JA, Calverly PM, Seemungal TA, Hagan G, Ansari Z, Stockley RA, et al. The prevention of chronic obstructive pulmonary disease exacerbations by salmeterol/fluticasone propionate or tiotropium bromide. Am J Respir Crit Care Med 2008;177:19-26.
38. Papi A, Bellettato CM, Braccioni F, Romagnoli M, Casolari P, Caramori G, et al. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. Am J Respir Crit Care Med 2006;173:1114-21.
39. Fabbri LM, Luppi F, Beghe B, Rabe KF. Update in chronic obstructive pulmonary disease 2005. Am J Respir Crit Care Med 2006;173:1056-65.
40. Ito K, Ito M, Elliott WM, Cosio B, Caramori G, Kon OM, et al. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. N Engl J Med 2005;352:1967-76.
41. Kita H, Adolphson CR, Gleich GJ. Biology of eosinophils. In: Adkinson NF, Yunginger JW Jr, Busse WW, Bochner BS, Holgate ST, Simons FER, editors. 6th ed. Philadelphia: Mosby; 2003. p. 305-32 Middleton's allergy: principles and practice.
42. Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 2006;117(suppl):S450-6.
43. Greenberger PA. Allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 2002;110:685-92.
44. Stevens DA, Moss RB, Kurup VP, Knutsen AP, Greenberger PA, Judson MA, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: Cystic Fibrosis Foundation Consensus Conference. Clin Infect Dis 2003;37(suppl 3):S225-64.
45. Eppinger TM, Greenberger PA, White DA, Brown AE, Cunningham-Rundles C. Sensitization to Aspergillus species in the congenital neutrophil disorders chronic granulomatous disease and hyper-IgE syndrome. J Allergy Clin Immunol 1999;104:1265-72.
46. Knutsen AP, Kariuki B, Consolino JD, Warrier MR. IL-4 alpha chain receptor (IL-4Ralpha) polymorphisms in allergic bronchopulmonary aspergillosis. Clin Mol Allergy 2006;4:3.
47. Kurup VP, Knutsen AP, Moss RB, Bansal NK. Specific antibodies to recombinant allergens of *Aspergillus fumigatus* in cystic fibrosis patients with ABPA. Clin Mol Allergy 2006;4:11.
48. Wark PA, Hensley MJ, Saltos N, Boyle MJ, Toneguzzi RC, Epid GD, et al. Anti-inflammatory effect of itraconazole in stable allergic bronchopulmonary aspergillosis: a randomized controlled trial. J Allergy Clin Immunol 2003;111:952-7.
49. Moss RB. Critique of trials in allergic bronchopulmonary aspergillosis and fungal allergy. Med Mycol 2006;44:269-72.
50. van der Ent CK, Hoekstra H, Rijkers GT. Successful treatment of allergic bronchopulmonary aspergillosis with recombinant anti-IgE antibody. Thorax 2007;62:276-7.
51. Shorr AF, Scoville SL, Cersovsky SB, Shanks GD, Ochenhouse CF, Smoak BL, et al. Acute eosinophilic pneumonia in US Military personnel deployed in or near Iraq. JAMA 2004;292:2997-3005.
52. Tsuirkisawa N, Saito H, Tsuburai T, Oshikata C, Ono E, Mitomi H, et al. Differences in regulatory T cells between Churg-Strauss syndrome and chronic eosinophilic pneumonia with asthma. J Allergy Clin Immunol 2008;122:610-6.

Mucosal immunology, eosinophilic esophagitis, and other intestinal inflammatory diseases

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The gastrointestinal mucosa constitutes the largest host-environment interface of the body. It uses both innate and adaptive immune mechanisms to provide protection from the diverse onslaught of foods, microbes, and other ingested products. The innate immune system is genetically encoded and evolutionarily ancient, possesses no memory, and lacks diversity. In contrast, the adaptive immune system is quite diverse, develops memory, and undergoes expansion after stimulation. The gastrointestinal mucosa is charged with the difficult task of mounting protective responses against invading microorganisms while simultaneously maintaining an overall state of nonresponsiveness or tolerance to innocuous substances, such as commensal bacteria and food antigens. Perturbation or malfunction of these complex protective mechanisms results in diseases, such as inflammatory bowel diseases, celiac disease, or eosinophilic gastrointestinal diseases. (J Allergy Clin Immunol 2010;125:S255-61.)

Key words: *Mucosal immunity, eosinophilic esophagitis eosinophilic gastrointestinal diseases*

Abbreviations used

DC:	Dendritic cell
EGID:	Eosinophilic gastrointestinal disease
EoE:	Eosinophilic esophagitis
FAE:	Follicle-associated epithelium
Foxp3:	Forkhead box protein 3
GALT:	Gut-associated lymphoid tissue
IBD:	Inflammatory bowel disease
IEL:	Intraepithelial lymphocyte
LP:	Lamina propria
M:	Microfold
NOD:	Nucleotide oligomerization domain
PP:	Peyer patch
TCR:	T-cell receptor
Treg:	Regulatory T

OVERVIEW OF GUT-ASSOCIATED LYMPHOID TISSUE

Mucosa-associated lymphoid tissues comprise the largest immune organ of the body and are active at multiple host-environment interfaces, such as the gastrointestinal tract and the genitourinary and bronchopulmonary systems. A discussion of the site-specific aspects of each component of the mucosa-associated lymphoid tissue is beyond the scope of this Primer, but the reader is referred to a number of outstanding reviews on these topics.[1-6] Here we will briefly review the gastrointestinal mucosal immune system and its gut-associated lymphoid tissue (GALT).

The human gastrointestinal tract is presented daily with a seemingly overwhelming load of diverse substances, including commensal bacteria and dietary antigens. Typically, the GALT is able to discriminate pathogens that require an immediate immune response from normal microbial flora or nutritive products. This process of maintaining a state of nonresponsiveness is known as oral tolerance. The mechanisms that govern tolerance are not only interesting and important aspects of this homeostatic process but are being potentially harnessed as therapeutic approaches for the treatment of certain autoimmune and inflammatory diseases.

The mucosal system is characterized as a site where antigen is selectively sampled and tolerance develops to maintain a state of controlled and protective inflammation. To accomplish these goals, the mucosa is composed of luminal protective molecules, the epithelial barrier, and the immunologically rich lamina propria (LP; Table I). The overall anatomy of the GALT is presented in Fig 1. This general overview shows important elements of the system, including the sampling of luminal antigens by microfold (M) cells, dendritic cells (DCs), and epithelia and the antigen-driven priming and maturation of naive T and B lymphocytes.

ANATOMY OF GALT

Although the primary responsibility of the intestinal epithelial cell is nutrient absorption, its role in mucosal immunity has previously been relegated to barrier function and the transport of secretory IgA. However, it is now clear that epithelia possess the ability to actively participate in mucosal immune responses.[7] Intestinal epithelial cells act as nonprofessional antigen-presenting cells, recognize and respond to bacterial and viral motifs by virtue of the expression of nucleotide oligomerization domain (NOD) and Toll-like receptors, and produce cytokines/chemokines that influence immune responses.[7] In addition, intestinal epithelial cells likely influence expansion of intestinal regulatory T (Treg) cells and cytokine expression.[8,9]

The epithelial surface overlying the Peyer patches (PPs) and lymphoid follicles is composed of a single layer of columnar cells termed the follicle-associated epithelium (FAE). Within the FAE reside specialized M cells derived from enterocytes under the influence of lymphotoxin. Human M cells differ from absorptive epithelium in that they do not harbor microvilli or membrane-

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TABLE I. Elements of the mucosal immune system

Innate mechanisms of defense
Mucus
Trefoil factors
IgA
Peristalsis
Tight junctional proteins
Antimicrobial peptides
Adaptive elements of defense
B cells
$CD4^+$ T cells
$CD8^+$ T cells
Treg cells
DCs
Cellular components
Eosinophils
Mast cells
Neurons
Epithelial cells

associated hydrolytic enzymes and contain less glycocalyx but do express cathepsin E and Toll-like receptors. Regional differences in M cells (ie, differences in M cells in the colon compared with those of the small intestine) are thought to exist, suggesting accommodations to changing microflora; however, the functional significance of this is unknown. A distinctive characteristic of the M cell is the presence of an invaginated subdomain at the basolateral membrane forming an intraepithelial "pocket."[10] At this site, predominantly $CD4^+$ CD45RO memory T cells and both naive ($sIgD^+$) and memory ($sIgD^-$) B cells interact with the M cell.

The major function of M cells is the transepithelial vesicular transport of antigens from the lumen directly to the subepithelial lymphoid tissues. M cells have been shown to transport particulate proteins, bacteria, viruses, and noninfectious particles.[11] This sampling of luminal antigens and microorganisms is thought to be important in the development of immune responses and tolerance. Although various pathogenic organisms can exploit the propensity for vectorial transport of M cells as a mechanism to gain entry for infection, M cells also transport commensal flora as a potential mechanism to regulate immune responses to endogenous flora.

Microenvironmental anatomic differences within the different parts of the gastrointestinal tract are well described. Although the esophagus is lined by a stratified squamous epithelium, M cells have not yet been identified, and no resident eosinophils are present in the mucosal surface (see the eosinophilic esophagitis [EoE] section below). In contrast, the small intestine and colon are lined by a columnar epithelium, and the cellular components of the GALT are localized within microenvironments, such as PPs or interstitial lymphoid follicles. Formation of PPs is dependent on several factors, such as the IL-7 receptor and TNF, along with TNF receptor family members. These miniorgans are covered by M cells and FAE that participate in antigen trafficking, as described above. Within the barrier are also unique cell types, including intraepithelial lymphocytes (IELs) and the antimicrobial-filled Paneth cells that reside at the crypt base. The LP is populated by T and B cells, along with unique populations of DCs. Mesenteric lymph nodes are a robust site of antigen processing and form a filter that separates the mucosa from other mucosal organs.

T lymphocytes localize in the small intestine as a result of selective expression of $\alpha4\beta7$ and CCR9. $CD4^+$ and $CD8^+$ T cells occupy the LP, whereas $CD8^+$ T cells preferentially reside in the intraepithelial space. IELs are a heterogeneous population of lymphocytes that are predominantly effector/effector memory cells made up of $\gamma\delta$ T-cell receptor (TCR) $CD8^+$ T cells and 2 distinct subsets of $\alpha\beta$ TCR cells: $\alpha\beta$ TCR $CD4^+$ or $CD8^+$ cells and those that lack coreceptor expression, the so-called double-negative cells.

One subset of T cells receiving recent recognition is the Treg cell.[12] Treg cells generally have suppressive capacities that participate in the maintenance of self-tolerance. Surface marker studies have identified several subtypes, including the forkhead box protein 3 (Foxp3)–positive T cell. Mutations in the gene encoding Foxp3, a Treg-specific transcription factor, have been associated with autoimmunity in murine models and a clinical syndrome termed immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Patients with this disease have severe diarrhea and small and large intestinal inflammation.

B cells secreting IgA^+ originate in the PPs, ultimately taking up residence in the intestinal LP. This journey is regulated by the interaction of site-specific adhesion molecules, an $\alpha4\beta7$ on the lymphocytes, and mucosal addressin cell-adhesion molecule 1 on the high endothelial venules in the LP. A smaller percentage of IgA-producing cells in the gut (about 25%) are derived from peritoneal B1 lymphocytes driven by commensal bacteria in a T cell–dependent manner and are thought to be important in modulating the mucosal immune response to bacterial flora.

Mast cells are abundant throughout the gastrointestinal tract, and although important in the host response to parasitic infection, they might participate also in innate immune responses to bacteria.[13] LP mast cells and lymphocytes interface with the enteric nervous system, providing another pathway that can influence mucosal immune responses.[14]

Eosinophils are absent in the normal esophagus but are resident cells of the stomach and small and large bowel. Chemotactic factors contributing to this population include the constitutive expression of eotaxin-1.[15,16] The exact numbers that define normalcy are open to debate but likely depend on a number of different factors. Like mast cells, eosinophils can perform important effector functions during parasitic infection and allergic responses but can also contribute to normal gut homeostasis.[17]

INNATE MECHANISMS OF DEFENSE

Often ignored are a host of mediators that participate in the innate defense mechanisms.[18] These molecules participate in nonspecific actions that limit antigens and microbes from communicating with the epithelium and LP. Mucus is composed of a number of glycoproteins that form a viscoelastic blanket that covers the epithelial surface. The inner mucus layer ranges between 50 and 100 μm, whereas the outer layer measures up to 500 μm. This mucus blanket is primarily composed of mucin-2 but also harbors a number of different mediators, including trefoil factors, secretory IgA, and antimicrobial peptides.

Trefoil factors are shamrock-shaped proteins held together by 3 disulfide bonds.[19] Several types of trefoil factors have been described that localize to different mucosal surfaces to assist in barrier repair and wound healing. Numerous stimuli induce the production of trefoil factors, including hypoxia and epithelial disruption.

IgA antibodies are divided into 2 subclasses, IgA1 and IgA2, with IgA2 representing the predominant form on intestinal surfaces. Secretory IgA, which is secreted by B cells, binds to

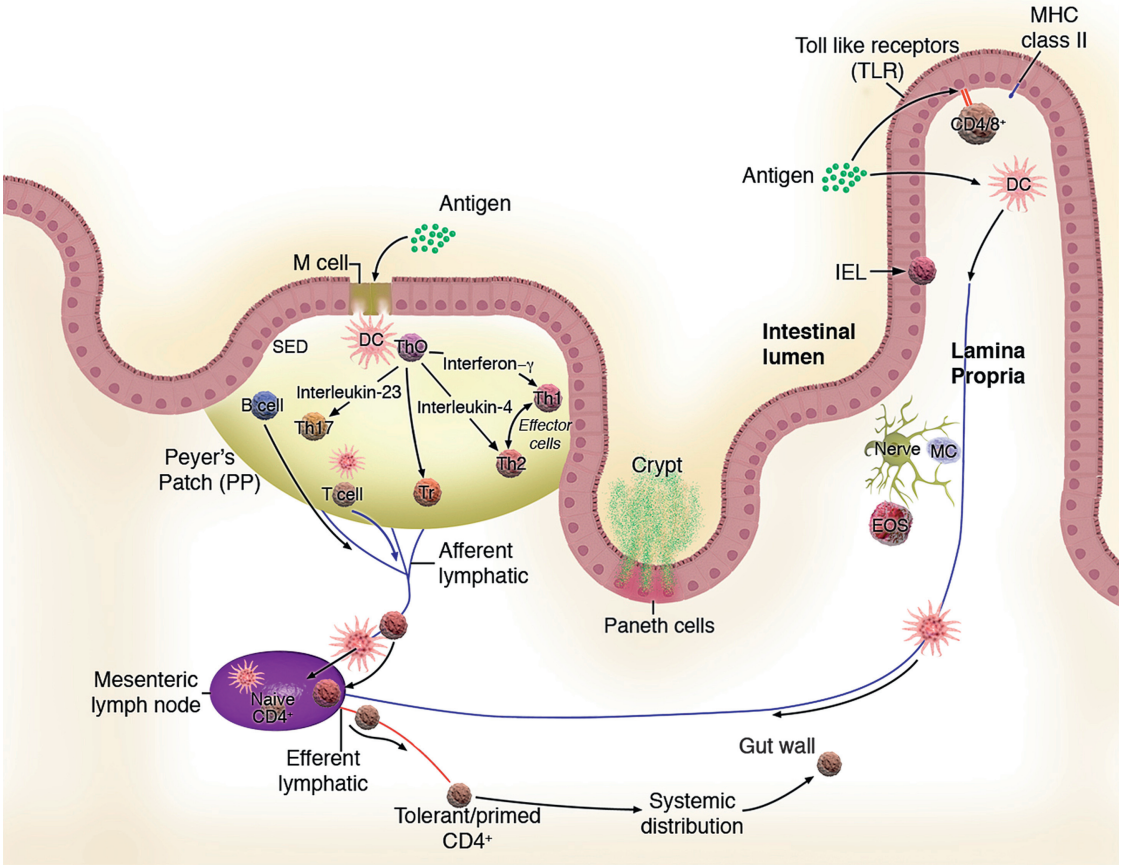

FIG 1. Anatomy of the gastrointestinal mucosa. Antigen can cross the epithelium through the M cell or DC. The subepithelial dome *(SED)* is occupied by a number of lymphocytes, including T_H0 cells that, under the direction of specific cytokines, differentiate into T_H1, T_H2, Treg, or T_H17 cells. Additional lymphocyte populations include the IELs that reside juxtaposed to the intestinal epithelial cells. Other resident cells in the LP that likely participate in the immune response include mast cells *(MC)* and eosinophils *(EOS)*.

and forms a covalent complex with the polymeric immunoglobulin receptor expressed on basolateral aspects of intestinal epithelia. Within the epithelia, IgA forms dimers that are connected by a J segment. This complex is actively transported across the epithelia to the apical surface, where it is released after proteolytic cleavage from the polymeric immunoglobulin receptor. Its exact function is unclear, but sIgA has been shown to bind microbes and toxins, preventing them from contacting the apical surface of the epithelium. Newer observations suggest that IgA might also regulate the composition of the microbial environment of the gut and limit local inflammation induced by pathogen-associated molecular patterns, such as LPS.[20,21]

Antimicrobial proteins are composed of a number of highly charged proteins called defensins.[22,23] These molecules are synthesized by Paneth cells and the epithelia. The antimicrobial properties of these highly charged molecules are attributed to their ability to increase bacterial membrane permeability. Six human α-defensins have been identified that possess selective activity against gram-positive and gram-negative bacteria and possibly viruses.[24] These cells likely participate in innate immunity, as was demonstrated in mice deficient in a Paneth cell–processing enzyme, rendering them unable to produce mature α-defensins. These mice were more susceptible to orally administered *Salmonella typhimurium* than wild-type mice.

INDUCTION OF A MUCOSAL IMMUNE RESPONSE

PPs are well-defined lymphoid aggregates composed of a large B-cell follicle surrounded by an interfollicular T-cell region. Interspersed throughout this organ are numerous macrophages and DCs. The subepithelial dome is an area rich in T and B lymphocytes and DCs. DCs migrate to basolateral surfaces of the M cell to acquire antigen and then travel to the interfollicular zone T-cell area, presumably where they participate in antigen presentation. DCs can migrate to distant sites, including mesenteric lymph nodes and the intestinal LP, where they can orchestrate an effector immune response. Experimental evidence supports an immunomodulatory role for DCs that includes both induction of oral tolerance and protective immune responses,[25] as described in a recent report in which $CD103^+$ DCs participated in the conversion of intestinal naive T cells to $Foxp3^+$ T cells.[26]

DISEASES

Inflammatory bowel diseases

Clinical description. Inflammatory bowel diseases (IBDs) are a heterogeneous group of diseases characterized by signs and symptoms related to immune-mediated inflammation of the gastrointestinal tract. The incidence of IBD ranges from 5 to 10 per 10,000 persons depending on the population examined.[27]

Typical symptoms include abdominal pain and bloody diarrhea in addition to other extraintestinal symptoms, such as fever, fatigue, arthralgias, and uveitis. In children growth failure can be an early sign. Physical examination reveals abdominal tenderness, particularly in the right lower quadrant.

Mucosal inflammation associated with Crohn disease can occur anywhere along the length of the gastrointestinal tract, with preponderance in the terminal ileum. Histologic hallmarks of tissues affected by Crohn disease include transmural inflammation and often noncaseating granulomas. Endoscopic features include skip lesions consisting of aphthous ulcerations, and radiographic studies show terminal ileal narrowing.

Suggestive laboratory abnormalities include anemia, increased sedimentation rate or C-reactive protein level, hypoalbuminemia, and increased liver enzyme levels. Ulcerative colitis has many clinical features in common with Crohn disease, but intestinal involvement is limited to the colon. In addition, the intestinal inflammation is limited to the superficial mucosa without granulomas, involves the rectum, and extends proximally. Other forms of IBD include microscopic colitis, lymphocytic colitis, and diversion colitis. Long-term complications include colorectal dysplasia and cancer.

Neutrophilic crypt abscesses are one of the most characteristic histologic features of both forms of IBD. In addition, eosinophils might be present, although to a seemingly lesser degree.

Pathophysiology. A complete review of the pathophysiology of IBD is beyond the scope of this Primer; the reader is referred to excellent reviews for more detailed descriptions.[5,18,28-32]Although the exact pathophysiology of IBD has not been determined, it is thought to develop when a genetically predisposed host is exposed to a luminal/environmental trigger. Over the course of the last few years, a number of genes have been identified in patients with Crohn disease, in particular genes linked to epithelial responses to luminal bacteria, autophagy, IL-10, and IL-23/IL-17 pathways. For instance, pathogen recognition receptors are present on the epithelial surface. One group of pathogen recognition receptors, the NOD molecules, recognize pathogen-associated molecular patterns that are present on bacterial membranes. A specific mutation of the *NOD2* gene allows for inappropriate sensing of bacteria with subsequent epithelial activation, leading to increased proinflammatory cytokine production within the mucosa. One gene associated with autophagy, *ATG16L1*, has been associated with Crohn disease. IL-10 suppresses deleterious intestinal inflammation, and recent studies have linked *IL10*mutations to IBD.[33] IL-23 and IL-17 mediate innate microbial defense and are linked to IBD.[34] In addition, loss of tolerance might also play a role because the mucosa affected by IBD contains fewer Treg cells than the healthy mucosa. IgE and food allergies are not thought to play a role in the underlying pathogenesis of these diseases.

Treatment. Goals of treatment of IBD include reducing inflammation, maintaining remission, enhancing quality of life, and avoiding the potential toxicity associated with treatments.[35-37] With this in mind, acute exacerbations are typically managed with systemic corticosteroids, whereas remission is addressed with the use of either aminosalicylates or immunomodulators, such as mercaptopurine or azathioprine. Recently, biological treatments, including anti–TNF-α antibodies (ie, infliximab and adalimumab), have significantly affected the clinicopathological features of IBD. A number of other agents, such as antidiarrheal agents, bile binders, and antispasmodics, might enhance quality of life.

Celiac disease

Clinical description. Celiac disease is an immune-mediated enteropathy that occurs as a result of gluten sensitivity in genetically predisposed individuals (DQ2/DQ8-positive HLA).[38] The incidence is 1 in 133 persons in the United States. Manifestations include those related to the gastrointestinal tract, such as chronic/recurrent diarrhea, abdominal pain, constipation, and slow growth, and nongastrointestinal symptoms, including dermatitis herpetiformis, seizures with occipital calcifications, dental hypoplasia, osteopenia, short stature, iron deficiency anemia, hepatitis, infertility, and arthritis.[39,40]

Other conditions associated with celiac disease include type 1 diabetes; Williams, Down, and Turner syndromes; IgA deficiency; autoimmune diseases; and a family history of first-degree relatives with celiac disease. Without treatment, there is an increased risk of intestinal lymphoma. Although serologic test results, such as increased IgA anti-endomysial antibody or tissue transglutaminase levels, provide strong evidence for celiac disease, the diagnosis rests on the finding of villous blunting and increased IEL numbers in mucosal biopsy specimens of the duodenal mucosa.

Pathophysiology. Recent studies have shown that a 33-amino-acid peptide in gliadin that is resistant to digestion contains the epitopes critical to the development of abnormal small intestinal mucosa in patients with celiac disease.[41,42] After uptake by the epithelium, processing of this 33-mer leads to activation of $CD4^+$ LP T cells, upregulation of the IL-2 receptor, increased production of IFN-γ and IL-15, and infiltration of the epithelia with γδ T cells. The resultant inflammatory process leads to villous blunting, crypt elongation, and loss of absorptive surfaces. Celiac disease is a cell-mediated and not IgE-mediated food allergic disease.

Treatment. Complete elimination of gliadin from the diet is the primary treatment of celiac disease.[43] Of paramount importance is attention to education and support of patients with respect to dietary elimination of gluten-containing products, review of alternative diets, adequacy of caloric and nutrient intake, and psychological support. For instance, although a number of foods should obviously be avoided, a number of products, including candies, gravies, food colorings, soy sauce, medications, play dough, and cosmetics, contain gluten in quantities sufficient to cause inflammation resulting in symptoms. In addition, many products that have been deemed wheat free, such as oats, are frequently contaminated with gliadin and should not be ingested by patients with celiac disease. Patients might also have iron, zinc, folic acid, and B complex vitamin deficiencies.

Eosinophilic gastrointestinal diseases

Clinical description. Eosinophilic gastrointestinal diseases (EGIDs) are heterogeneous diseases characterized by a diverse set of symptoms that occur in association with intestinal eosinophilia.[44] These diseases have been termed EoE, eosinophilic gastritis, eosinophilic gastroenteritis, and eosinophilic colitis depending on the anatomic location in which eosinophil numbers are increased. Over the last decade, EoE has been recognized as the most common EGID. The remainder of this section will focus on EoE. For further information, the reader is referred to recent reviews on other EGIDs.[44-46] Recent reports have expanded the association of esophageal eosinophilia with other diseases,

including celiac disease; the exact pathogenetic mechanisms and therapeutic implications of this are uncertain.[47-50]

EoE is a clinicopathological disease characterized by upper intestinal symptoms that occur in association with dense esophageal eosinophilia; other potential causes must have been ruled out as causes of symptoms and eosinophilia.[51] Children with EoE present with a wide range of symptoms, including vomiting, abdominal pain, feeding dysfunction, and dysphagia.[46,52] Feeding dysfunction is often overlooked and requires specific questioning regarding how patients eat foods (eg, dysphagia, food sticking, requiring water to wash food down, and prolonged chewing).[53] Adults present with stereotypical features of food impaction or dysphagia.[54] Patients presenting with food impaction, especially when recurrent, should be evaluated for a diagnosis of EoE. EoE occurs in all age groups and has been reported in all continents except Africa, with a reported prevalence of EoE ranging between 1 and 4 per 10,000 persons. Although the natural history is unknown, the one identified complication is esophageal stricture or narrowing.[55]

The physical examination should be directed toward excluding other causes of esophageal eosinophilia, such as IBDs, celiac disease, and connective tissue diseases. No single marker, including peripheral eosinophilia, provides diagnostic support for or against the diagnosis of EoE, although one study suggests that the combination of peripheral eosinophilia and increased serum eotaxin-3 and eosinophil-derived neurotoxin levels correlates with esophageal eosinophil density.[56] Upper gastrointestinal series can screen for other causes of vomiting and for evidence of esophageal stricture or long-segment narrowing, features associated with EoE.

Pathophysiology. Esophageal eosinophilia is a nonspecific finding that reflects a state of injury. Although a variety of diseases have been associated with this type of inflammation, including gastroesophageal reflux disease, EoE, celiac disease, infections, and IBDs among others, the exact mechanism driving this response is not certain.[44] For instance, recent evidence suggests that specific cytokines, including IL-6 and IL-1, might participate in acid-induced injury.[57] In contrast, as discussed below, IL-5 is critical to this response in murine models, and eotaxin-3 contributes to human disease.[58,59] The acute inflammatory infiltrate in patients with EoE is exclusively composed of eosinophils, with the virtual complete absence of neutrophils.

A series of recent studies have identified potential mechanisms for the pathogenesis of EoE. Mishra et al[59] provided the first murine model of aeroallergen-induced esophageal eosinophilia by sensitizing and challenging with *Aspergillus fumigatus*. By applying this system to IL-5 null mice, the investigators were able to demonstrate that esophageal eosinophilia was dependent on IL-5, as well as T cells.[59] The inflamed mucosa contains increased $CD4^+$ effector T cells and decreased Treg cells.[60,61] In addition, the same investigators have determined the effect of IL-5 on tissue remodeling associated with this eosinophilic inflammation.[62]- Together, these findings provided support for the development of therapeutics targeting IL-5 in the treatment of this disease. Addressing this need are 2 ongoing studies of anti–IL-5 in the treatment of pediatric EoE.

Translational studies have also brought increased understanding of EoE. For instance, a number of studies have begun to define the immunomicroenvironment of the esophageal mucosa. Although diagnostic criteria have solely focused on eosinophil numbers, other studies are examining associated inflammatory features, including eosinophil degranulation, that appear to be increased compared with those seen in gastroesophageal reflux disease.[63] In addition, the mucosa from patients with EoE contains increased T_H1 and T_H2 proinflammatory cytokines (IL-5 and TNF-α), CD8 lymphocytes (CD8 and CD1a), B cells, and mast cell and basophil infiltration.[64] The exact role of Treg cells in EoE is uncertain because one study demonstrated immunohistochemical evidence of $Foxp3^+$ cells in both patients with EoE and those with gastroesophageal reflux disease.[60] One genome-wide microarray analysis revealed that the most upregulated gene in the esophageal epithelia was eotaxin-3, a chemokine critical for eosinophil migration.[65] Another study identified increased eotaxin expression in the affected mucosa, providing further support for eotaxin's role in EoE's pathogenesis.[66]

Other studies have focused on remodeling in EoE, showing an increased level of esophageal fibrosis in children with EoE.[67,68] Although the exact mechanisms of this response are not certain, Aceves et al[68] showed increased TGF-β expression with activation of the SMAD pathway. Therapeutic studies suggest that fibrosis might be reversible.[69]

IgE and non-IgE immune mechanisms might participate in the pathogenesis of EoE, an important point to be kept in mind when evaluating these patients for allergen sensitization. Although skin prick testing and the measurement of food allergen–specific IgE levels are often useful in identifying potential culprit foods, they are not helpful in the detection of causative foods in non–IgE-mediated reactions. However, atopy patch testing to foods has been proposed as a useful method to potentially identify foods causing symptoms through a non–IgE-mediated immune mechanism.[70,71]

In summary, evidence to date supports a role for both IgE-mediated and non–IgE-mediated mechanisms in the pathogenesis of EoE, with eotaxin-3 and IL-5 being central mediators and fibrosis being one of the potential outcomes.

Treatment. Treatment goals have been directed toward symptom elimination and reduction/normalization of esophageal inflammation. The rationale for the later end point has been that complete histologic remission might reduce the incidence of complications. To date, the incidence of esophageal complications is unknown, and potential emotional and developmental effects of chronic treatments and repeated endoscopic analyses are beginning to be recognized.[72] Prospective studies will provide data to illuminate this area of controversy.

Despite this issue, at least 2 effective treatments, corticosteroids and dietary elimination of suspected culprit foods, have been identified. The reader is referred to a number of recent reviews on this topic for further details regarding the specifics of each treatment.[46,73] Regardless, evidence to date suggests that EoE is a chronic disease, and without continuous treatment, symptoms and inflammation will persist or return. To date, no medical maintenance treatment has been identified.[51]

SUMMARY

The mucosal immune system has a unique anatomy and physiology aimed at providing a mechanism that will allow tolerance to food antigens and commensal bacteria along with the capacity to respond to pathogenic microbes, other injurious agents, or both. The monolayered epithelium forms the initial interface between the environment and host that forms not only a barrier but also a sensor providing bidirectional communication

with other resident mucosal lymphoid cells. The lymphocytes, DCs, mast cells, and eosinophils in the LP interact to form a pluripotent network that orchestrates an innate and adaptive immune response to potential pathogens. Further delineation of the mechanisms governing the normal responses of the mucosal immune system will provide insight into disease states, such as food allergies, IBDs, and EGIDs.

REFERENCES

1. Cario E. Innate immune signalling at intestinal mucosal surfaces: a fine line between host protection and destruction. Curr Opin Gastroenterol 2008;24:725-32.
2. Boirivant M, Amendola A, Butera A. Intestinal microflora and immunoregulation. Mucosal Immunol 2008;1(suppl 1):S47-9.
3. Artis D, Grencis RK. The intestinal epithelium: sensors to effectors in nematode infection. Mucosal Immunol 2008;1:252-64.
4. Macpherson AJ, McCoy KD, Johansen FE, Brandtzaeg P. The immune geography of IgA induction and function. Mucosal Immunol 2008;1:11-22.
5. Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology 2009; 136:65-80.
6. Mason KL, Huffnagle GB, Noverr MC, Kao JY. Overview of gut immunology. Adv Exp Med Biol 2008;635:1-14.
7. Dahan S, Roth-Walter F, Arnaboldi P, Agarwal S, Mayer L. Epithelia: lymphocyte interactions in the gut. Immunol Rev 2007;215:243-53.
8. Allez M, Brimnes J, Dotan I, Mayer L. Expansion of CD8+ T cells with regulatory function after interaction with intestinal epithelial cells. Gastroenterology 2002; 123:1516-26.
9. Dotan I, Allez M, Nakazawa A, Brimnes J, Schulder-Katz M, Mayer L. Intestinal epithelial cells from inflammatory bowel disease patients preferentially stimulate CD4+ T cells to proliferate and secrete interferon-gamma. Am J Physiol Gastrointest Liver Physiol 2007;292:G1630-40.
10. Neutra MR, Mantis NJ, Kraehenbuhl JP. Collaboration of epithelial cells with organized mucosal lymphoid tissues. Nat Immunol 2001;2:1004-9.
11. Jang MH, Kweon MN, Iwatani K, Yamamoto M, Terahara K, Sasakawa C, et al. Intestinal villous M cells: an antigen entry site in the mucosal epithelium. Proc Natl Acad Sci U S A 2004;101:6110-5.
12. Himmel ME, Hardenberg G, Piccirillo CA, Steiner TS, Levings MK. The role of T-regulatory cells and Toll-like receptors in the pathogenesis of human inflammatory bowel disease. Immunology 2008;125:145-53.
13. Galli SJ, Tsai M. Mast cells: versatile regulators of inflammation, tissue remodeling, host defense and homeostasis. J Dermatol Sci 2008;49:7-19.
14. Koon HW, Pothoulakis C. Immunomodulatory properties of substance P: the gastrointestinal system as a model. Ann N Y Acad Sci 2006;1088:23-40.
15. Blanchard C, Rothenberg ME. Biology of the eosinophil. Adv Immunol 2009;101: 81-121.
16. Blanchard C, Rothenberg ME. Chemotactic factors associated with eosinophilic gastrointestinal diseases. Immunol Allergy Clin North Am 2009;29:141-8, xi.
17. Hogan SP, Rothenberg ME. Eosinophil function in eosinophil-associated gastrointestinal disorders. Curr Allergy Asthma Rep 2006;6:65-71.
18. McGuckin MA, Eri R, Simms LA, Florin TH, Radford-Smith G. Intestinal barrier dysfunction in inflammatory bowel diseases. Inflamm Bowel Dis 2009;15: 100-13.
19. Kjellev S. The trefoil factor family—small peptides with multiple functionalities. Cell Mol Life Sci 2009;66:1350-69.
20. Suzuki K, Fagarasan S. How host-bacterial interactions lead to IgA synthesis in the gut. Trends Immunol 2008;29:523-31.
21. Evolution Fagarasan S. development, mechanism and function of IgA in the gut. Curr Opin Immunol 2008;20:170-7.
22. Braff MH, Hawkins MA, Di Nardo A, Lopez-Garcia B, Howell MD, Wong C, et al. Structure-function relationships among human cathelicidin peptides: dissociation of antimicrobial properties from host immunostimulatory activities. J Immunol 2005;174:4271-8.
23. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. N Engl J Med 2002;347:1151-60.
24. Ericksen B, Wu Z, Lu W, Lehrer RI. Antibacterial activity and specificity of the six human α-defensins. Antimicrob Agents Chemother 2005;49:269-75.
25. Bilsborough J, Viney JL. Gastrointestinal dendritic cells play a role in immunity, tolerance, and disease. Gastroenterology 2004;127:300-9.
26. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med 2007;204:1757-64.
27. Shanahan F, Bernstein CN. The evolving epidemiology of inflammatory bowel disease. Curr Opin Gastroenterol 2009;25:301-5.
28. van Lierop PP, Samsom JN, Escher JC, Nieuwenhuis EE. Role of the innate immune system in the pathogenesis of inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2009;48:142-51.
29. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 2009;9:313-23.
30. Weinstock JV, Elliott DE. Helminths and the IBD hygiene hypothesis. Inflamm Bowel Dis 2009;15:128-33.
31. Yoshida H, Granger DN. Inflammatory bowel disease: a paradigm for the link between coagulation and inflammation. Inflamm Bowel Dis 2009;15:1245-55.
32. Abraham C, Cho J. Inflammatory bowel disease. N Engl J Med 2009;361:2066-78.
33. Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schaffer AA, Noyan F, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med 2009;361:2033-45.
34. Abraham C, Cho JH. IL-23 and autoimmunity: new insights into the pathogenesis of inflammatory bowel disease. Annu Rev Med 2009;60:97-110.
35. Yamamoto T, Nakahigashi M, Saniabadi AR. Review article: diet and inflammatory bowel disease—epidemiology and treatment. Aliment Pharmacol Ther 2009;30:99-112.
36. Regueiro M. Management and prevention of postoperative Crohn's disease. Inflamm Bowel Dis 2009;15:1583-90.
37. Rutgeerts P, Vermeire S, Van Assche G. Biological therapies for inflammatory bowel diseases. Gastroenterology 2009;136:1182-97.
38. Hunt KA, van Heel DA. Recent advances in coeliac disease genetics. Gut 2009;58: 473-6.
39. Barker JM, Liu E. Celiac disease: pathophysiology, clinical manifestations, and associated autoimmune conditions. Adv Pediatr 2008;55:349-65.
40. Freeman HJ. Pearls and pitfalls in the diagnosis of adult celiac disease. Can J Gastroenterol 2008;22:273-80.
41. Molberg O, Uhlen AK, Jensen T, Flaete NS, Fleckenstein B, Arentz-Hansen H, et al. Mapping of gluten T-cell epitopes in the bread wheat ancestors: implications for celiac disease. Gastroenterology 2005;128:393-401.
42. Schumann M, Richter JF, Wedell I, Moos V, Zimmermann-Kordmann M, Schneider T, et al. Mechanisms of epithelial translocation of the alpha(2)-gliadin-33mer in coeliac sprue. Gut 2008;57:747-54.
43. Haines ML, Anderson RP, Gibson PR. Systematic review: the evidence base for long-term management of coeliac disease. Aliment Pharmacol Ther 2008;28:1042-66.
44. Fleischer DM, Atkins D. Evaluation of the patient with suspected eosinophilic gastrointestinal disease. Immunol Allergy Clin North Am 2009;29:53-63, ix.
45. Straumann A. Idiopathic eosinophilic gastrointestinal diseases in adults. Best Pract Res Clin Gastroenterol 2008;22:481-96.
46. Atkins D, Kramer R, Capocelli K, Lovell M, Furuta GT. Eosinophilic esophagitis: the newest esophageal inflammatory disease. Nat Rev Gastroenterol Hepatol 2009; 6:267-78.
47. Leslie C, Mews C, Charles A, Ravikumara M. Celiac disease and eosinophilic esophagitis: a true association. J Pediatr Gastroenterol Nutr 2009 [Epub ahead of print].
48. Verzegnassi F, Bua J, De Angelis P, Dall'oglio L, Di Leo G, Ventura A. Eosinophilic oesophagitis and coeliac disease: is it just a casual association? Gut 2007; 56:1029-30.
49. Ooi CY, Day AS, Jackson R, Bohane TD, Tobias V, Lemberg DA. Eosinophilic esophagitis in children with celiac disease. J Gastroenterol Hepatol 2008;23:1144-8.
50. Kagalwalla AF, Shah A, Ritz S, Melin-Aldana H, Li BU. Cow's milk protein-induced eosinophilic esophagitis in a child with gluten-sensitive enteropathy. J Pediatr Gastroenterol Nutr 2007;44:386-8.
51. Furuta GT, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, et al. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology 2007;133:1342-63.
52. Spergel JM, Brown-Whitehorn TF, Beausoleil JL, Franciosi J, Shuker M, Verma R, et al. 14 years of eosinophilic esophagitis: clinical features and prognosis. J Pediatr Gastroenterol Nutr 2009;48:30-6.
53. Haas A, Creskoff-Naune N. Feeding dysfunction in children with eosinophilic esophagitis. Immunol Allergy Clin North Am 2009. In press.
54. Desai TK, Stecevic V, Chang CH, Goldstein NS, Badizadegan K, Furuta GT. Association of eosinophilic inflammation with esophageal food impaction in adults. Gastrointest Endosc 2005;61:795-801.
55. Straumann A. The natural history and complications of eosinophilic esophagitis. Gastrointest Endosc Clin North Am 2008;18:99-118, ix.
56. Konikoff MR, Blanchard C, Kirby C, Buckmeier BK, Cohen MB, Heubi JE, et al. Potential of blood eosinophils, eosinophil-derived neurotoxin, and eotaxin-3 as biomarkers of eosinophilic esophagitis. Clin Gastroenterol Hepatol 2006;4:1328-36.
57. Souza RF, Huo X, Mittal V, Schuler CM, Carmack SW, Zhang HY, et al. Gastroesophageal reflux may cause esophagitis through a cytokine-mediated mechanism, not by caustic (acid) injury. Gastroenterology 2009;137:1776-84.

58. Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, et al. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest 2006;116:536-47.
59. Mishra A, Hogan SP, Brandt EB, Rothenberg ME. IL-5 promotes eosinophil trafficking to the esophagus. J Immunol 2002;168:2464-9.
60. Tantibhaedhyangkul U, Tatevian N, Gilger MA, Major AM, Davis CM. Increased esophageal regulatory T cells and eosinophil characteristics in children with eosinophilic esophagitis and gastroesophageal reflux disease. Ann Clin Lab Sci 2009;39:99-107.
61. Zhu X, Wang M, Crump CH, Mishra A. An imbalance of esophageal effector and regulatory T cell subsets in experimental eosinophilic esophagitis in mice. Am J Physiol Gastrointest Liver Physiol 2009;297:G550-8.
62. Mishra A, Wang M, Pemmaraju VR, Collins MH, Fulkerson PC, Abonia JP, et al. Esophageal remodeling develops as a consequence of tissue specific IL-5-induced eosinophilia. Gastroenterology 2008;134:204-14.
63. Protheroe C, Woodruff SA, de Petris G, Mukkada V, Ochkur SI, Janarthanan S, et al. A novel histologic scoring system to evaluate mucosal biopsies from patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol 2009;7:749-55, e11.
64. Vicario M, Blanchard C, Stringer KF, Collins MH, Mingler MK, Ahrens A, et al. Local B cells and IgE production in the esophageal mucosa in eosinophilic esophagitis. Gut 2009 [Epub ahead of print].
65. Blanchard C, Durual S, Estienne M, Emami S, Vasseur S, Cuber JC. Eotaxin-3/CCL26 gene expression in intestinal epithelial cells is up-regulated by interleukin-4 and interleukin-13 via the signal transducer and activator of transcription 6. Int J Biochem Cell Biol 2005;37:2559-73.
66. Bhattacharya B, Carlsten J, Sabo E, Kethu S, Meitner P, Tavares R, et al. Increased expression of eotaxin-3 distinguishes between eosinophilic esophagitis and gastroesophageal reflux disease. Hum Pathol 2007;38:1744-53.
67. Chehade M, Sampson HA, Morotti RA, Magid MS. Esophageal subepithelial fibrosis in children with eosinophilic esophagitis. J Pediatr Gastroenterol Nutr 2007;45:319-28.
68. Aceves SS, Newbury RO, Dohil R, Bastian JF, Broide DH. Esophageal remodeling in pediatric eosinophilic esophagitis. J Allergy Clin Immunol 2007;119:206-12.
69. Aceves SS, Newbury RO, Chen D, Mueller J, Dohil R, Hoffman H, et al. Resolution of remodeling in eosinophilic esophagitis correlates with epithelial response to topical corticosteroids. Allergy 2009 [Epub ahead of print].
70. Spergel JM, Andrews T, Brown-Whitehorn TF, Beausoleil JL, Liacouras CA. Treatment of eosinophilic esophagitis with specific food elimination diet directed by a combination of skin prick and patch tests. Ann Allergy Asthma Immunol 2005;95:336-43.
71. Spergel JM, Beausoleil JL, Mascarenhas M, Liacouras CA. The use of skin prick tests and patch tests to identify causative foods in eosinophilic esophagitis. J Allergy Clin Immunol 2002;109:363-8.
72. Klinnert MD. Psychological impact of eosinophilic esophagitis on children and families. Immunol Allergy Clin North Am 2009;29:99-107, x.
73. Rothenberg ME. Biology and treatment of eosinophilic esophagitis. Gastroenterology 2009;137:1238-49.

Complement disorders and hereditary angioedema

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The term *complement* was introduced more than 100 years ago to refer to a group of plasma factors important in host defense and in the destruction of microorganisms. We now know that there are 3 separate activation pathways that appeared at different times in evolution: the classical, alternative, and lectin pathways. Two of these appear before the evolution of the adaptive immune system and do not require antibody for initiation. All pathways come together to activate C3, the principle opsonic protein of the complement cascade, and all continue together to the generation of biologically active factors, such as C5a, and to lysis of cells and microbes. In general, complete deficiencies of complement proteins are rare, although partial or complete deficiencies of one of the proteins that initiates the lectin pathway, mannose-binding lectin, are far more common. Although genetically controlled complement defects are rare, defects in the proteins in the circulation and on cell membranes that downregulate complement so as to limit uncontrolled inflammation are more common. A number of these are discussed, and because new methods of treatment are currently being introduced, one of these defects, CI inhibitor deficiency associated with hereditary angioedema, is discussed in some detail. (J Allergy Clin Immunol 2010;125:S262-71.)

Key words: *Complement, complement deficiencies, hereditary angioedema, atypical hemolytic uremic syndrome*

Abbreviations used
C1-INH: C1 inhibitor
C': complement
FDA: US Food and Drug Administration
HAE: Hereditary angioedema
iC3b: Inactivated C3b
MASP: Mannose-binding lectin–associated serine protease
MBL: Mannose-binding lectin
MCP: Membrane cofactor protein
PNH: Paroxysmal nocturnal hemoglobinuria

Complement is a term originally introduced a hundred years ago to define a group of factors present in fresh plasma that, when activated by a specific antibody, were able to kill microorganisms.[1] Later work showed the bacteria studied were lysed and that the killing principle was heat labile. We now define complement as a collective term for a group of about 30 known proteins and protein regulators, some of which circulate in the blood and some of which are cell membrane bound. The complement proteins play a major role in host defense and innate immunity. Although all of the early studies focused on the role of complement in host defense, in recent years, we have learned that complement is also important in the generation of a normal immune response. Phylogenetically, the complement proteins are ancient, serving a host defense function even in primitive animals in the absence of any adaptive immune system. The adaptive immune system appears in evolution at the level of the fish, and by this point in evolution, all the various complement proteins are arrayed to produce their regulatory and host defense functions.[2]

We have come to recognize 3 pathways of complement activation (Fig 1).[3,4] The first pathway was defined almost a century ago and, for this reason, is termed the *classical pathway*. This pathway is usually activated by antibody and was the first pathway identified because of its ability to kill antibody-sensitized bacteria. A second pathway, now termed the *alternative pathway*, was first observed in the 1950s but was studied in detail in the 1970s and 1980s.[5] The alternative pathway has been shown to be phylogenetically older than the classical pathway. It does not require antibody to function and is found in organisms as primitive as sea squirts.[2] Although antibody is not required for its function, the presence of antibody usually allows this pathway to function more efficiently. A third pathway described in the past 2 decades, the *lectin pathway*, is still being defined in detail. This pathway appears in development sometime after the alternative pathway and also does not require antibody to function.[6] All 3 pathways proceed through a series of proteins that are discussed below to the activation and binding of the plasma protein C3, which is central to all 3 pathways. The pathways then proceed together through the binding of an additional series of proteins to the lytic and inflammation-promoting steps in complement action.

Most reviews focus on the 3 major effector functions of complement in host defense. First is its ability to lyse cells, second is its ability to opsonize particles (ie, to render them easy for phagocytes to engulf), and third is the ability of the proteins on activation to generate cleavage fragments that have potent inflammatory activity. For example, the small fragment of C5, C5a, can cause mast cells to degranulate and release histamine, as if they were coated with IgE and antigen. It can cause migration of phagocytic cells toward the place where the peptide is generated (ie, to induce chemotaxis) and can cause cytokine and biologically active peptide release from cells.[7,8] The biological basis of these 3 complement effector activities is defined below.

It is rare to find patients with deficiencies of classical or alternative pathway proteins, although deficiencies of some of the proteins of the lectin pathway are surprisingly more common. In most cases, when complement contributes to disease it is acting appropriately (ie, the system is being activated and causing tissue damage and cell death in a normal fashion), but it is being activated inappropriately.[9,10] Thus, for example, a patient might produce an abnormal antibody to the basement membrane of the glomerulus. The antibody can bind to the glomerulus, activate complement, and cause inflammatory damage. In this case complement is acting normally; it is the antibody that is inappropriate. Table I lists some

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diseases in which complement deficiency is associated with clinical illness. Because of the many untoward effects of inappropriate complement activation, there are many control proteins that act to downregulate activated complement proteins at each step in the various reaction cascades. The importance of these proteins is that they prevent unwanted damage of one's own tissues and cells. Although the absence of complement proteins is unusual, the absence of control proteins is more common, and many of the patients who have absent control proteins have poorly controlled inflammatory disease. Moreover, as mentioned in our discussion of the lectin pathway below, there is a sizable group of patients with allotypic variations of mannose-binding lectin (MBL) that lead to very low levels of this circulating protein.

Although effector functions of complement in host defense have received the most attention over the years, more recently, it has been found that complement also functions in the induction of adaptive immunity, but here there has been far less study, and less information is available.[11] One reason that this information is coming to light slowly is that there are so few complement-deficient subjects to study. With the advent of knockout gene technology, it has been possible to develop murine strains missing 1 or more complement proteins or complement receptors, and it has been found that these animals have defects in the development of many aspects of the normal murine immune response.[12] This is discussed further toward the end of this chapter.

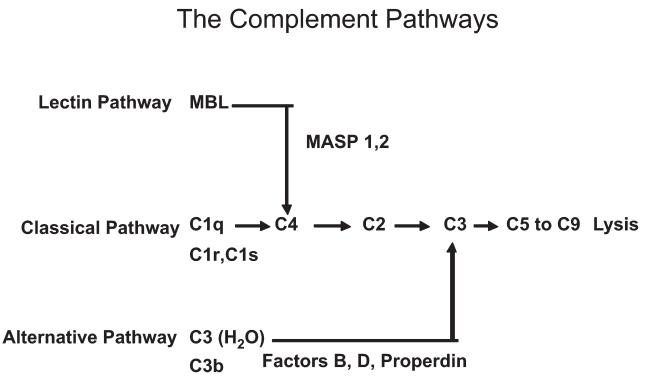

FIG 1. The 3 complement activation pathways. The classical pathway is usually activated by antibody. The lectin pathway is activated by the recognition molecule MBL binding to structures with the appropriate repetitive sugars. The ficolins are MBL-like molecules that can also activate this pathway. The alternative pathway does not have a recognition molecule as such. It is initiated by the binding of factor B to C3, which can then be cleaved by factor D. Because C3 always undergoes slow hydrolysis, the pathway is always undergoing some degree of activation. Properdin stabilizes the complex and can also initiate alternative pathway activation. C3b itself is an efficient activator of the alternative pathway, and classical pathway activation leading to C3 deposition on a target rapidly activates the alternative pathway.

THE CLASSICAL PATHWAY

The classical pathway is usually activated by antibody. IgM and the IgG subclasses IgG1, IgG2, and IgG3 bind the first component of complement, C1, to initiate activation of the classical pathway.[3] C1 exists in serum as a 3-part molecule (C1q, C1r, and C1s) held together in the presence of ionic calcium. C1q has a central protein core and 6 radiating arms, each ending in a pod-like protein domain that can bind to the Fc fragment of IgG or IgM. Each of the 6 arms is made up of 3 intertwined chains, C1q A, B, and C, and has a triple-helix structure like collagen, providing flexibility. In the case of IgG, the binding of multiple IgG molecules to an antigenic surface allows binding of multiple arms of C1q, each to an Fc fragment, with sufficient affinity of the C1q to allow C1 activation. In the case of IgM, a single molecule bound to an antigenic surface by multiple binding sites with the availability of multiple Fc fragments within this one polymeric molecule is sufficient to bind C1q and activate this pathway. On binding of C1q to antibody, a distortion of the C1q molecule takes place that in turn causes autoactivation of C1r, which then activates C1s. C1s, like C1r, acquires enzymatic activity and continues the complement cascade sequence. C1 requires calcium for self-association and therefore the classical pathway requires calcium for initiation. The function of activated C1 is to bind and cleave C4, the next protein in the classical pathway activation sequence. C4 is cleaved into a large fragment (C4b) and a small fragment (C4a). The large fragment continues the complement cascade and the small fragment, like the small fragments of C3 (C3a) and the small fragment of the next protein (C5) in the sequence (C5a), has anaphylatoxic activity. All of these fragments are able to cause mast cell degranulation with resulting histamine release.[7]

On activation of C4, a thioester-containing binding site is exposed on C4b that allows covalent attachment of C4b to the target. The nature of the binding site on C4 and C3 is similar and is discussed in further detail in the section on the alternative pathway. The site now containing C1 and C4 bound to a target allows the next protein, C2, to bind to C4b. On C2 binding to the C14b site, C1s cleaves C2 also into a large and small fragment. Again, the large fragment remains bound to the assembling protein complex. C2 binding to C4 requires the presence of ionic magnesium. The new site consisting of C4b and C2 (C4b2a) no longer requires C1 for activity. Enzymatic activity resides in the C2 fragment. This site is termed the C3 convertase of the classical pathway because it can bind the next complement protein in the sequence (C3) and, as in the earlier steps, cleave it into a large fragment (C3b) and a small fragment (C3a), which again has inflammatory activity. Just as in the case of C4b, C3b can bind covalently to the target of attack. In many cases it binds directly to C4b on the target. As mentioned earlier, C3 is the central component of all 3 complement pathways and is present at high concentration in serum, about 1.2 mg/mL.

An important function of complement is the ability to opsonize particles, which means to coat them with complement-derived protein fragments that allow them to be phagocytosed easily. Phagocytic cells have on their surface specific receptors for complement-derived peptides, often cleavage fragments of C3. When these fragments are deposited on microbes, they can link the microbe to the phagocyte receptors; the adherence facilitates the phagocytic process.

On the addition of C3b to the C4bC2 site, a new binding site is created that can bind C5, the next protein in the sequence. Again, C5 is cleaved into a large fragment and a small fragment. The large fragment, C5b, continues the complement cascade, although it does not form a covalent bond with the target and remains associated with C3b. The small fragment released, C5a, is one of the most potent inflammatory peptides released by complement activation and has strong neutrophil-aggregating activity, strong neutrophil chemotactic activity and is an excellent anaphylatoxin.[7] Injection of sufficient purified C5a into an animal

TABLE I. Functional and clinical consequences of complement deficiency

Defect	Classical pathway	Lectin pathway	Alternative pathway	C3 and factors that control C3 levels	Late-acting proteins: C5-C9
Functional Consequence	Delayed C′ activation, decreased immune response, poor antibody activation of C′	Decreased activation in the absence of antibody	Decreased C′ activation in the absence of antibody	Decreased opsonization: if control factors are abnormal, increased C′ mediated pathology	Inability to form lytic Lesions; C5 important in PMN chemotaxis
Clinical consequences	Increased incidence of autoimmune disease: infection with high-grade pathogens (eg, pneumococcus)	Infection in the newborn: question of increased rheumatic disease Question of risk of increased infection with unusual pathogens like cryptosporidium and aspergillis	Increase in infection with high-grade pathogens: some increase in *Neisseria* species infections	Marked increase in infection with high-grade pathogens: failure to downregulate C3 associated with hemolytic uremic syndrome and adult-onset macular degeneration	Marked increase in neisserial infection

may cause anaphylaxis and death from neutrophil aggregation in the circulation and massive histamine release.[7,13]

The complement cascade continues after C5b binding with the binding of C6, C7, C8, and C9. One molecule of C6 and C7 each bind to C5b on the target surface. If this binding takes place at the surface of a cell or microbe, the introduction of C7 to the binding site leads to an increase in hydrophobicity of the C5-7 complex and insertion of the complex into the lipid cytoplasmic membrane of the cell. Under these circumstances, the cell is targeted for lysis. With the binding of one molecule of C8 to the C5-7 complex, a slow leak in cells such as erythrocytes appears, and with the binding of up to 16 molecules of C9, a cylinder or donut-like structure is formed, containing all the proteins C5b through C9, that penetrates the cell membrane. The pore-like interior of the donut allows free fluid transfer and destroys the ability to maintain its osmotic equilibrium, and it lyses.

Cells protect themselves from complement attack in a variety of ways. The many complement control proteins will be discussed in greater detail below, but also the lytic C5b–9 complex can be shed from the surface of some cells or internalized and destroyed as the cell acts to protect itself from damage. Cells such as erythrocytes with little intracellular protein synthetic machinery to help repair their membranes rely on the control proteins for protection. Cells such as macrophages and endothelial cells have these extra mechanisms for clearing their membranes of deposited complement proteins.

THE LECTIN PATHWAY

The lectin pathway, unlike the classical pathway, does not require antibody to function and is developmentally more primitive than the classical pathway. It is quite similar in function to the classical pathway.[6] In the more evolved classical pathway, the recognition molecule that that sees foreign antigen with great specificity and induces complement activation is antibody. The lectin pathway does not use antibody but has its own more primitive recognition molecule. The pathway is initiated by the plasma protein MBL or by the related proteins, the ficolins. MBL has a structure remarkably similar to C1q, with a central core and a series of radiating arms composed of a flexible triple helix, each ending in a binding structure. Unlike C1q, in MBL the helix contains 3 copies of a single chain. In the case of C1q, the binding structure at the end of the arms recognizes the Fc fragment of immunoglobulin, and antibody is the recognition protein that triggers the activation sequence. In the case of MBL, there are 3 lectin-binding sites at the termination of each of the arms of the MBL. Each lectin-binding site has low affinity for sugars like mannose, but with the binding of multiple arms of the MBL, each with 3 binding sites to, for example, the repeating polysaccharides on the surface of a bacterium, the association is stabilized and the complement pathway is activated. Therefore the protein that recognizes the foreign structure is not a specific antibody but MBL itself. MBL circulates as a series of multimers and can have 2, 4, or 6 arms. In general, it is thought that the 4-arm structure predominates.

Associated with MBL in the circulation are proteins termed mannose-binding lectin–associated serine protease (MASPs). The functional structure again resembles that of C1 because C1q, the subunit with collagen-like arms that binds to antibody, also associates with serine proteases, C1r, and C1s. In the case of MBL, the associated serine proteases are MASP1, MASP2, and MASP3, as well as some other related molecules. Recent work further demonstrates the similarity of the classical and lectin pathways. C1r and C1s are reported to have some affinity for MBL, and the MASPs have an affinity for C1q. It is believed that MASP2 is the principle serine protease involved in continuation of the complement cascade, with MASP1 also active. MASP3's function is still being explored, but it might have a role in activating the alternative pathway. Currently, it is thought that the main path of activation after MBL binding is through activation of C4 by MASP2. Thus lectin pathway activation is very much like classical pathway activation. In the classical pathway antibody is the recognition molecule. It binds C1, which is then activated and cleaves C4. In the lectin pathway the recognition molecule is MBL, and MASP2 is the C1q like molecule that cleaves C4 into C4a and C4b. C4b then binds C2, the C2 is cleaved by MASP2, and the pathway continues to C9, just as in the classical pathway.

THE ALTERNATIVE PATHWAY

The alternative pathway is probably the oldest of the complement pathways in phylogenetic terms and is more difficult to understand because it operates by means of a mechanism that is fundamentally different and more primitive than that of the

classical and lectin pathways. In these latter 2 cases the pathway is specifically activated by a recognition molecule that binds to the target of attack and activates a serine protease that activates the rest of the complement sequence. In the alternative pathway C3 is itself the recognition molecule, and activation of the pathway is inefficient. C3 is a 2-chain molecule, α and β, with an internal thioester joining a cysteine at position 988 with a glutamine at position 991 in the α chain backbone. The tertiary configuration of the molecule protects the internal thioester from cleavage caused by nucleophilic attack by water; even so, it undergoes slow hydrolysis in the circulation. When water penetrates to the thioester bond, the bond is hydrolyzed, leaving a free sulfhydryl at position 988 and a hydrated carboxyl ion at position 991. This is associated with a marked change in tertiary structure, and the molecule comes to resemble C3b. Hydrolyzed C3, like C3b itself, is capable of binding factor B, a protein of the alternative pathway very much like C2 of the classical pathway. On binding to hydrated C3 or C3b, factor B can be cleaved by a serine protease, very much like C1s of the classical pathway, termed factor D. Thus a protein complex is formed consisting of hydrated C3 or C3b and the large fragment of cleaved factor B, termed Bb, with the release of the small fragment Ba. This complex is the C3 convertase of the alternative pathway. It can bind a new molecule of C3 and cleave it into C3a and C3b. The major difference between the C3 convertase (C3 cleaving enzyme) of the alternative pathway and the C3 convertase of the classical and lectin pathways is that there is no C4b in the convertase of the alternative pathway. C3b itself takes the place of C4b, with factor B acting like C2 and factor D acting like C1. A second difference is that factor D, the molecule that resembles C1s of the classical pathway and MASP2 of the lectin pathway, is not physically bound to the active site but acts as a fluid-phase enzyme.

In summary, the initial alternative pathway C3 convertase ($C3[H_2O]$,Bb), which can form slowly and spontaneously in the circulation, can bind and cleave another molecule of C3. When C3a is cleaved from the C3 to form C3b, the thioester becomes immediately available. If this cleavage occurs close to the surface of a cell or microbe, the carboxyl on the C3b generated can form an ester or amide bond with the surface of a cell or microbe. This target-bound C3b can accept another factor B molecule and, in the presence of factor D, can cleave more C3 into C3a and C3b, with more C3b becoming target bound. In the case of the classical and lectin pathways, the C42 complex is unstable and slowly decays. In the case of the alternative pathway, the C3bBb complex is also unstable. It rapidly decays and is stabilized in the circulation by yet another protein termed properdin. Properdin binds C3b, and it has recently been suggested that properdin bound to a substrate can also bind C3b and initiate alternative pathway attack. Like the classical pathway convertase, the alternative pathway convertase requires magnesium ion to function. Presumably the first pathway to develop in the complement system, in terms of phylogenetic development, was the alternative pathway. Because pathway initiation is not directed and requires the chance hydrolysis of a C3 close to the target of destruction, its binding, and then binding of additional C3 to the target, it is very inefficient. It is believed that the lectin pathway evolved to recognize the target more directly by binding to sugar groups on its surface. With the appearance of antibody, the target could be even more specifically identified. C3b deposited on a target by the lectin or classical pathway can also engage proteins of the alternative pathway to further amplify C3 deposition.

C3b undergoes a complex sequence of degradation steps, with each degradation product having different biological activity. Because these steps are regulated by control molecules, they are considered in the sections below.

COMPLEMENT RECEPTORS AND COMPLEMENT CONTROL MOLECULES

By definition, complement receptors recognize and bind various complement proteins and fragments. As with other receptors, this can cause cellular activation. However, unlike most cellular receptors, some of the complement receptors also act as control molecules and interact with the molecule they bind to allow for further degradation of the bound fragment. In performing this function, the receptors act like the complement control molecules that regulate the degradation of complement proteins to control their biological function. These many receptors and control molecules are discussed below. At virtually each step of the complement cascade, control points are established to downregulate the possibility of untoward complement activation. A few of the control molecules linked to disease are listed in Table II.

Control of activity of C1 and MASPs. In the classical pathway the activation of C1 with cleavage of C4 is downregulated by C1 inhibitor (C1-INH).[14] This single-chain molecule is a serpin (serine protease inhibitor). Enzyme inhibitors of this class present a bait sequence to the enzyme to be inhibited that looks like the enzyme's substrate. When an enzyme cleaves the inhibitor at the site of the bait sequence (amino acid 444 of the C1-INH), the inhibitor springs apart, uncovering a highly reactive site that forms a covalent bond with the active site on the enzyme. C1-INH inhibits C1r and C1s of the classical pathway and MASPs 1 and 2 of the lectin pathway. C1-INH has been termed a suicide inhibitor because it is used up during the inhibition process. During the process of C1 inhibition, the C1 molecule is taken apart, C1r and C1s are removed, and C1q is left bound to the antibody site. As discussed in a later section, C1-INH inhibits enzymes in a number of other mediator pathways in plasma, including the kinin-generating pathway, and patients with abnormalities in even one of the genes for normal C1-INH have hereditary angioedema (HAE), a swelling disorder.

Control of the activity of C4 and C2. The next steps in the classical and lectin complement pathway, the interaction of C4 and C2, are also under the control of a circulating protein, C4-binding protein.[15] This protein binds to C4b, preventing its interaction with C2 and accelerating the decay of the C4b, C2 site once formed. It also is capable of binding to C3b when these reactants are present at high concentration. As discussed, the C4b, C2 site is further controlled because it is subject to spontaneous degradation over time, losing its activity. Loss of activity is accompanied by the release of C2 from the C4b site. The C4b site can accept another C2 and, in the presence of C1, can regenerate the C4b/2 site.

Control molecules and cellular receptors that interact with C3. As an essential component in the lytic pathway, C3 functions in the classical, lectin, and alternative pathways. C3b bound to a target can not only continue the complement cascade but also acts as a potent opsonin by binding its receptor CD35, on phagocytes, aiding the phagocytic process. Because C3b, if deposited on tissue cells, can become a focus of tissue damage, its formation and degradation are under tight regulation. It is

TABLE II. Some regulators of complement activation and their role in disease

C1-INH downregulates the complement, kinin-generating, clotting, and fibrinolytic pathways. Heterozygous deficient individuals have HAE.
MCP (CD46) is a cofactor for the cleavage of C3. Homozygous or heterozygous defects can lead to aHUS.
Factor H is a cofactor for the cleavage of C3. Complete deficiency is associated with glomerulonephritis. Partial and complete deficiencies are associated with aHUS. Polymorphism is associated with age-related macular degeneration and HELLP syndrome.
Factor I is a cofactor for the cleavage of C4 and C3. Complete deficiency is associated with low levels of C3 and infection. Deficiencies are associated with aHUS.
CD59 downregulates formation of the membrane attack complex. Acquired deficiency by hematopoietic progenitors leads to paroxysmal nocturnal hemoglobinuria.

aHUS, Atypical hemolytic uremic syndrome; *HELLP*, hemolytic anemia, elevated liver enzymes, and low platelets, occurring during pregnancy.

simplest to describe the steps in degradation in plasma and then the effect of the receptors (Fig 2).

There are a number circulating proteins and cell-surface receptors that can interact with C3b, and the results of the interaction might differ depending on the set of control proteins with which it interacts.[16,17] Virtually all normal cells have these control molecules. Two plasma proteins, factors H and I, are critical regulators of C3b in plasma and to some extent on certain cells, such as erythrocytes. When C3b is generated, it will bind factor H, and the complex of C3b and H can be attacked by the circulating complement enzyme factor I, which can then cleave the C3b α chain, leading to the formation of inactivated C3b (iC3b). iC3b no longer functions as a C3 or C5 convertase, but it remains cell bound and remains a potent opsonin. The rare patients missing factor I have low C3 levels in the circulation because the alternative pathway stays active and cleaves C3, and these patients also have an increased incidence of infection.

It is interesting that the complement system attempts to discriminate self from nonself in an attempt to minimize unwanted tissue damage. C3b deposited on one's own tissues or cells is often close to a sialic acid which is present in relatively large amounts in normal tissues and cellular membrane carbohydrates. Factor H binding and activity is facilitated by sialic acid. Any C3b deposited on one's own cells therefore tends to be cleaved by factor I, preventing further complement activation. Most microorganism surfaces are not rich in sialic acid. Factor H function is not facilitated. C3b remains on the organism surface, and the C3 convertase of the alternative pathway continues to deposit additional C3b on the microbe to promote phagocytosis. Many pathogens have evolved mechanisms to incorporate sialic acid into surface structures to protect themselves in part from complement attack. For example, *Escherichia coli* K1 has developed sialic acid–containing capsules to mimic the surface of the normal cell and thus protect the bacterium from destruction.[18]

Five different cellular receptors are important in the binding and phagocytosis of C3-coated particles. CD35 (also termed CR1) recognizes C3b, as does a recently described receptor, which is present on Kupffer cells and some monocytes, termed CRIg.[19] The β_2-integrins (CD11b/CD18, which is also termed CR3, and CD11c/CD18, which is also termed CR4) recognize target-bound iC3b, the product formed by the action of factors H and I acting on C3b, and mediate phagocytosis. Receptors for iC3b are present on all phagocytes and dendritic cells, although they are not present on lymphocytes. The β_2-integrins are 2-chain molecules (α and β chain).[20] The α chain (CD11b or CD11c) provides the ligand recognition, and the β chain (CD18) is required for transport of the 2-chain complex to the cell surface. Patients with leukocyte adhesion deficiency have a defect leading to their inability to express these molecules on the cell surface and are highly susceptible to infection. CD11c/CD18 is the signature receptor used in identification of monocytic dendritic cells. Presumably this receptor, acting through complement bound to antigen, is of critical importance in processing of antigen for presentation to the immune system.

The C3b receptor CD35 (CR1) is present on erythrocytes, phagocytes, dendritic cells, and all B cells.[21] As mentioned, binding of a particle to a phagocyte surface by CD35 aids in the phagocytic process. However, if an immune complex forms in the circulation and binds C3b, most often it will bind not to the surface of a phagocyte but to the surface of an erythrocyte through erythrocyte CD35 because of the large number of erythrocytes in the circulation. The immune complex, bound to the surface of the red cell, is effectively out of the circulation and cannot easily leave the intravascular space to be deposited in tissues, such as the kidneys. As the erythrocyte circulates through the liver and spleen, the immune complex comes in contact with the fixed phagocytes in the sinusoids of these organs and is removed from the red cell surface and phagocytosed. The red cell exits the liver or spleen free of the complex and continues to have normal survival. During this process, some of the CD35 is removed from the red cell as the immune complex is removed. The infusion of normal erythrocytes into patients with active systemic lupus erythematosus with circulating immune complexes is followed by those erythrocytes gradually losing their CD35 as the CD35 on the infused erythrocytes binds the circulating immune complexes and transports them to the liver and spleen.

CD35 itself acts as a cofactor protein for degradation of C3, but its function is different from that of the proteins listed above. Like C3b that has bound factor H, C3b bound to CD35 can be cleaved by factor I, but the cleavage leads to a different fragmentation pattern. Cleavage of the α chain leads first to the formation of iC3b, but the process does not stop at this step. Further cleavage of the α chain leads to release from the target-bound C3b of the largest part of the iC3b, C3c, with retention of a 40-kd fragment of the α chain of iC3b, C3dg, which is bound to the target. This fragment can be further trimmed by proteases to C3d. C3dg and C3d do not bind to CD35 or to the β_2-integrins, but do bind to CD21 (CR2), which is present on all B cells, a T-cell subset, and follicular dendritic cells. Because β_2-integrins are not on B cells and CD21 is not present on most phagocytes, the fragmentation pattern of C3 mediated by the various cofactor proteins can direct targets of attack or antigens to phagocytes, antigen-presenting cells, or B cells.

A group of other complement control molecules on the membrane of normal cells also act to dampen the activity of C3 if it is accidently deposited.[21] Thus membrane cofactor protein (MCP; CD46) acts as a cofactor for the cleavage of C3b by factor I, just as factor H does. Another molecule present on most cells, which is bound to the cells by a phosphatidylinositol linkage, decay-accelerating factor (CD55), interacts with both the classical and alternative pathway C3 convertase to increase the rate of degradation of the convertase, destroying its activity. It is interesting that these 2 molecules, which are widely distributed on cells of the body, together have much of the activity of CD35 on immune cells

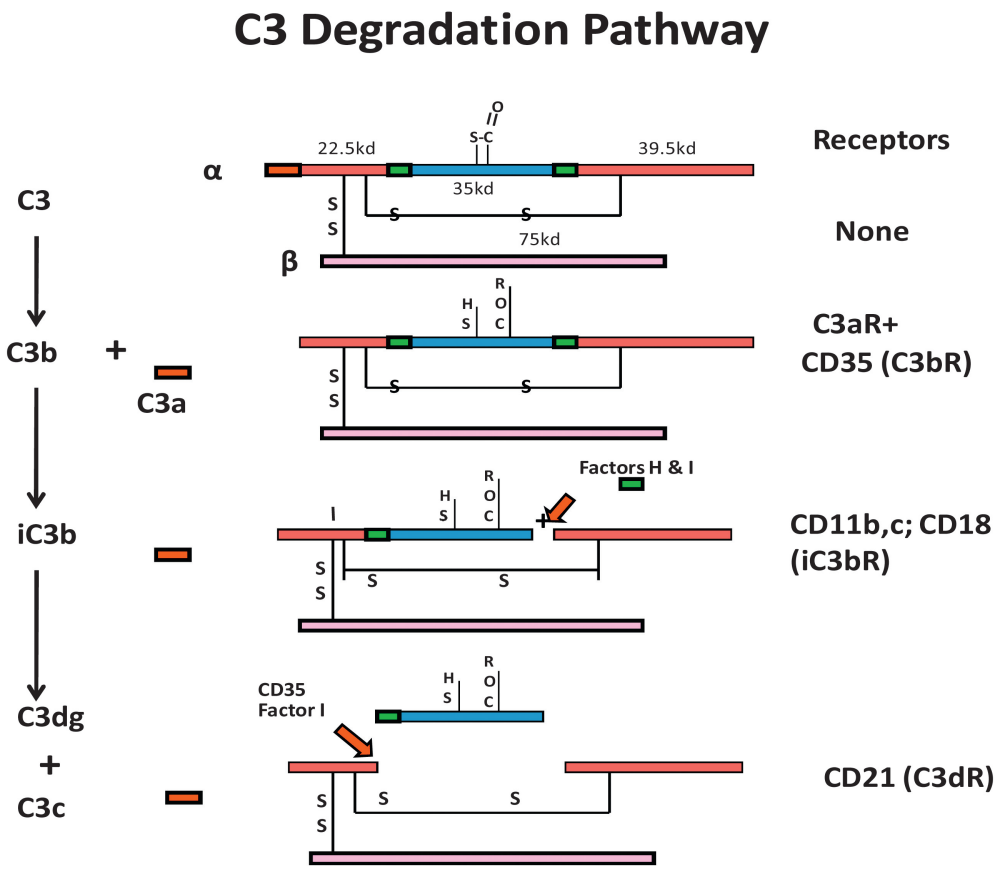

FIG 2. The 2-chain molecule C3 is shown first. There are no receptors that recognize this molecule. The C3 convertase of the classical or alternative pathway cleaves off C3a, an anaphylatoxin. The remainder of the molecule C3b undergoes a marked molecular rearrangement and now is recognized by CD35 (CR1), as well as by the recently recognized receptor on Kupffer cells, CRIg. C3b binds factor H and now can be cleaved by factor I to iC3b. iC3b is recognized by CD11b/CD18 and CD11c/CD18. These 2-chain receptors are on all phagocytes and dendritic cells. They aid in the processing of antigen. In serum the cleavage of C3 stops at this point, but when an immune complex is bound to cellular CD35 or when C3 is deposited on a cell with CD35 or other membrane-bound complement control molecules, such as CD46, it is cleaved further by factor I to C3c and C3dg. C3dg can be trimmed to C3d and C3g. C3d and C3dg are recognized by CD21 found on B cells and dendritic cells. Antigen with multiple bound C3d molecules can interact with both CD21 and the B-cell receptor, which can augment the immune response.

and phagocytes. CD35 has both decay-accelerating and cofactor activity in the same receptor molecule, and these activities are separated and slightly changed in CD46 and CD55.

As discussed, the complement system has been present over much of mammalian evolution, and microorganisms have evolved mechanisms for using these proteins as docking sites for entry into cells. Thus MCP has been shown to be a docking site for measles virus, for certain adenoviruses, and for some *Neisseria* species organisms; CD21 is a docking site for EBV. Each year, the list grows of control molecules that are found to be docking sites for various viruses or bacteria.

Several of the complement receptors are thought to aid directly in cellular activation or inhibition. CD35 has been discussed above as a facilitator of phagocytosis. The β_2-integrins CD11b and c/CD18 are the principle iC3b receptors and, like CD35, provide a signal for phagocytosis. These receptors are present on all phagocytes and natural killer cells. As mentioned, CD11c/CD18 is used as an identifying marker of dendritic cells. Follicular dendritic cells, B cells, and some T cells have CD21 (CR2) on their surface. This receptor recognizes C3d, C3dg, and polymerized iC3b. It is believed that antigens with C3d on their surface can cross-link CD21 with the B-cell receptor, augmenting the ability of antigen to activate B cells by as much as a thousand fold.[22]

As mentioned earlier in the chapter, inherited defects in the control molecules are more common than inherited defects in the complement proteins themselves. Factor H abnormalities have been reported in 2 important medical situations. Lack of normal factor H activity plays a critical role in the development of familial, atypical, hemolytic uremic syndrome, that is hemolytic uremic syndrome that occurs spontaneously and is not associated with bacterial infection and diarrhea.[23] In fact, investigation has shown that 3 different molecules, each of which plays a role in C3 degradation, can be abnormal in various subgroups of these patients. The 3 proteins are factor H, factor I, and MCP. The defects in the proteins can be present in either the heterozygous or homozygous state, probably reflecting the fact that half the normal number of C3 control molecules is not sufficient to protect against untoward immunologic activation. One way of thinking about the pathogenesis of this syndrome is that a toxin enters the circulation and is deposited on endothelial cells, particularly in the kidney, and on

erythrocytes. As the subject makes an immune response to the toxin, in the absence of sufficient control molecules, antibody binds to the toxin, and cells with toxin and antibody are destroyed by poorly regulated complement activation. In truth, no one has shown that this is the mechanism of disease, but it places the disease in a framework that allows the pathophysiology to make sense.

It has also recently been reported that the largest risk factor in the development of macular degeneration in the elderly is an alteration of the amino acid at position 402 in factor H from a tyrosine to a histadine.[24] It is believed from statistical studies of DNA sequences from pedigrees of families with inherited macular degeneration that approximately 50% of cases are associated with this alteration in one amino acid, although the factor H allele with histadine in position 402 is fairly common in the population, and other factors must be involved.

Receptors for the anaphylatoxins C3a, C4a, and C5a

Of the anaphylatoxins, C5a has been studied in the greatest detail.[7] It is a potent chemotactic factor causing the directed migration of phagocytes. It contracts smooth muscle cells and causes mast cells to degranulate in the absence of IgE antibody. It causes neutrophils to adhere to one another and to endothelium in vessels. It clearly plays a part in the damage observed during the course of immunologic lung disease. Mice with a defect in the C5a receptor do not experience all of the manifestations of immunologic or allergic lung disease. It is likely that far more information will become available about this important receptor in the development of asthma. There is less information available on C4a and C3a binding. The membrane receptor for C3a is clearly different from that of C5a and can be triggered to cause mucus secretion in the airways, but its role in immunologic airways disease is still speculative.

Control of the late steps in the complement cascade

The later steps in the complement cascade are also under tight control. The site composed of the C3 convertase with bound C5 will decay if it does not bind C6 rapidly, and there are a series of molecules that downregulate the late-acting proteins both in serum and on cells. S-protein, a plasma protein, interacts with C7 as the C5, C6, and C7 complex forms and becomes hydrophobic.[21] On binding S-protein, this complex is neutralized and can no longer bind to cell surfaces. Similarly, clusterin, another plasma protein, binds to the forming C5-9 complex and prevents its activation and completion. Most cells in the body have membrane-bound CD59, which interacts with the C5b-8 site, decreasing the binding of C9 and preventing polymerization of C9. It protects the cell by preventing effective pore formation. All of these control molecules are important in maintaining homoeostasis, and loss of the control molecules often leads to disease. CD59, like CD55, is linked to cell membranes by a phosphatidylinositol linkage. By not having a transmembrane domain, the protein is free to move rapidly in the fatty hydrophobic plane of the cell membrane to intercept forming C5b-9 and prevent cell lysis. Almost all patients with the disease paroxysmal nocturnal hemoglobinuria (PNH) have an acquired bone marrow defect in which they have a mutation in bone marrow stem cells of the gene *PIGA* (phosphatidylinositol glycan class A), the first enzyme in the development of phosphatidylinositol linkages.[25] A single patient with a genetic deficiency of *CD59* has been reported, and this patient also had PNH. This gene is present on the X-chromosome, and a single gene defect in a bone marrow stem cell leads to an inability to synthesize the first intermediate in this linkage pathway and therefore the failure to have phosphatidylinositol-linked proteins on the cell membrane. A failure to generate hematopoietic cells with CD59 causes all hematopoietic cells of bone marrow origin derived from the abnormal clone to be easily lysed by complement. As mentioned, alternative pathway proteins in the circulation undergo slow activation; CD59 is critical for neutralizing membrane attack proteins when they bind to our own cells. In patients with PNH, this mechanism is defective, and patients have a hemolytic anemia, often thrombocytopenia, and often a low neutrophil count. Recently eculizumab, a humanized monoclonal anti-C5 protein was approved for the treatment of PNH.[26] This antibody binds C5 and prevents complement-mediated lysis while allowing opsonization that occurs at the earlier C3 step to proceed. This is the first medication that improves cell survival in this patient group with a disease that has a generally grim prognosis.

The role of complement in the generation of immunologic lung disease is of particular interest. For many years, it was taken as gospel that complement plays no role in IgE-mediated lung disease or asthma. Recent work has suggested that this might not be the case. Complement can play a number of interesting functions in the generation of lung pathology.

First, it has been suggested that complement functions importantly in directing immune responses toward T_H1- or T_H2-type immunity. T_H1 immunity is generally considered most important in prevention of infection, and T_H2 immunity is associated with asthma and other allergic diseases. It is believed that the activation of C5 and the generation of C5a are important in directing the immune response toward a T_H1 phenotype, and lack of C5 therefore skews the system toward the generation of T_H2 immunity.[27,28] On the other hand, once immunity or allergy is established, it is believed that C5a might be generated during immunologic responses in the lung and, acting as an anaphylatoxin, might cause mast cell degranulation, smooth muscle contraction, and so on, thereby contributing to the asthmatic response.

COMPLEMENT IN THE AFFERENT LIMB OF THE ADAPTIVE IMMUNE RESPONSE

In recent years, attention has turned to the role of complement in the development of immunity.[29,30] This discussion has focused so far on the efferent limb of the response and how tissue damage is caused or controlled by complement. As mentioned early in the chapter, the complement system is phylogenetically older than the adaptive immune system, and many of the complement proteins existed as the adaptive immune system evolved.[31] Therefore it is not surprising that elements of the complement system were incorporated into the adaptive immune system, and these elements are only now being slowly identified. As mentioned in an earlier section, the binding of complement to an antigen allowing cross-linking of CD21 and the B-cell receptor increases antigenicity by up to 1000-fold. In this case complement augments the immune response. It is also known that subjects deficient in complement, although rare, often have major defects in adaptive immunity. Animals deficient in C1q, C4, C3, and CR1/2 make a poor immune response, particularly to T-dependent antigens; have poor germinal center formation; and have poor immunologic memory. Complement aids in the localization and retention of antigens within the germinal center, and it is believed that this localization of antigen to the germinal center facilitates an ongoing immune

response. Perhaps surprisingly, patients deficient in C1, C4, and, to a lesser extent, C2, have a high propensity toward systemic lupus erythematosus.[32,33] In fact, of the relatively few C1q-deficient subjects who have been described, 96% have had systemic lupus. Of the relatively few C4-deficient subjects who have been described, 75% have had lupus. Even heterozygosity of the genes for C4 predispose subjects to the development of lupus. This propensity to cause systemic lupus erythematosus seems to be independent of the genetic localization of C4, C3, and factor B in the major histocompatibility locus as class III genes and therefore their linkage to the MHC. In addition to the above, animals, particularly those deficient in C1q and C4, do not develop normal tolerance as well, although animals deficient in C3 and CR1/2 do not appear to have this defect. Although these are intriguing findings and have been repeated in many laboratories, it is still not completely clear how complement functions in the afferent limb of the adaptive immune response. It is quite likely that this question will be clarified over the next few years.

COMPLEMENT DEFICIENCIES AND CLINICAL ILLNESS

In the preceding paragraphs we have mentioned many diseases associated with defects in complement activation or control. Although recent research has demonstrated that HAE is not a disease whose clinical manifestations are due to defects in complement activation, it has typically been considered in this group. Because enormous progress has been made in defining its pathogenesis and treatment, it is given more detailed consideration.[34,35]

HAE is an inherited disease caused by low functional levels of the complement control plasma protein C1-INH. Patients have spontaneous episodic attacks of angioedema or deep localized swelling, most commonly of a hand or foot, that begin during childhood and become much more severe during adolescence. The edema is nonpitting and nonpruritic and is not associated with urticaria. Patients usually have a prodrome, a tightness or tingling in the area that will swell, lasting most frequently for several hours, followed by the development of angioedema. The swelling typically becomes more severe over about 1½ days and then resolves over about the same time period. In some patients attacks are preceded by the development of an erythematous rash that is not raised and not pruritic: erythema marginatum. The second major symptom complex noted by these patients is attacks of severe abdominal pain caused by edema of the mucosa of any portion of the gastrointestinal tract. The intensity of the pain can approximate that of an acute abdomen, often resulting in unnecessary surgical intervention. The gastrointestinal edema generally follows the same time course to resolution as the cutaneous attacks.

Laryngeal edema is the most feared complication of HAE and can cause complete respiratory obstruction. Although life-threatening attacks are infrequent, more than half the patients with HAE have laryngeal involvement at some time during their lives. Dental work with the injection of a topical anesthetic into the gums is a common precipitant, but laryngeal edema is often spontaneous. The clinical condition can deteriorate rapidly, progressing through mild discomfort to complete airway obstruction over a period of hours. Soft tissue edema can be difficult to see when it involves the larynx. If the swelling progresses to difficulty swallowing secretions or a change in the tone of the voice, this should be considered an emergency and might require emergency intubation or even tracheostomy to ensure an adequate airway. Other presentations are less common, but genital swelling is sometimes noted in male and female patients.

In most cases the cause of the attack is unknown, but some patients note that trauma or emotional stress precipitates attacks. In some female patients menstruation also regularly induces attacks and estrogens increase attack frequency. The frequency of attacks varies greatly among affected subjects and at different times in the same subject, with some experiencing weekly episodes, whereas others might go years between attacks, and attacks can start at any age.

As noted above, C1-INH is a serpin that inactivates its target by forming a stable one-to-one complex with the enzyme to be inhibited. Although hepatocytes are the primary source of C1-INH, the protein is also synthesized by monocytes. The regulation of the protein production is not completely understood, but because patients respond clinically to attenuated androgens with increased serum levels of C1-INH, it is believed that these androgens may stimulate C1-INH synthesis. HAE is an autosomal dominant disease, with as many as 25% of patients providing no family history. Presumably, most of these cases are caused by new gene mutations. Because all C1-INH–deficient patients are heterozygous for this gene defect, it is believed that half the normal level of C1-INH is not sufficient to prevent attacks.

Although named for its action on the first component of complement (C1 esterase), C1-INH also inhibits proteins of the fibrinolytic, clotting, and kinin pathways (Fig 3). Specifically, C1-INH inactivates plasmin-activated Hageman factor (factor XII) and its fragments, activated factor XI, tissue plasminogen activator, and kallikrein. Within the complement system, C1-INH blocks the activation and activity of C1 of the classical pathway and MASPs 1 and 2 of the lectin pathway. Without C1-INH, unchecked activation of complement causes cleavage of the C4 and C2 proteins in the complement sequence, and patients often have low levels of these proteins. Levels of the next protein in the complement cascade, C3, are normal. The major factor responsible for the edema formation is now known to be bradykinin, an important nonapeptide mediator that can induce leakage of post capillary venules. Bradykinin is derived from cleavage of the circulating protein high-molecular-weight kininogen by the plasma enzyme kallikrein, the activity of which is controlled by C1-INH.

There are 2 genetic types of C1-INH deficiency that result in essentially the same phenotypic expression. The C1-INH gene serping 1 is located on chromosome 11 in the p11-q13 region. The inheritance is autosomal dominant with incomplete penetrance. Type 1 is the most common form and accounts for approximately 85% of cases. Synthesis of or secretion C1-INH is blocked at the site of a faulty allele but occurs at the normal allele. The result is transcription of the normal protein, yielding quantitative serum concentrations of C1-INH that are approximately 10% to 40% of normal values. Type 2 HAE accounts for approximately 15% of cases. Mutations near the active site of the inhibitor lead to synthesis and secretion of nonfunctional C1-INH protein. These patients also have a normal functioning allele. Patients with type II HAE have either normal or increased concentrations of the protein.

A clinical syndrome resembling HAE and termed type 3 HAE has been described that affects mostly woman. In this condition no abnormalities of complement or of C1-INH have been described, but one third of patients have been found to have a gain-of-function abnormality of clotting factor XII, and it appears that many of the other patients with type III disease have defects in the proteins that cause normal bradykinin degradation.

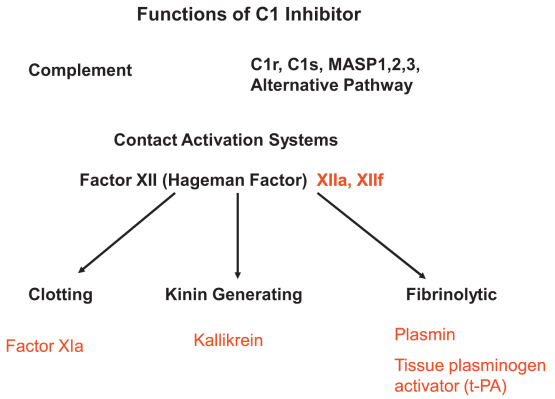

FIG 3. Functions of C1-INH.

In America 3 treatment regimens are available for prophylaxis, and within the last months US Food and Drug Administration (FDA) has approved treatments for acute angioedema attacks. Impeded androgens, such as the gonadotropin inhibitor danazol, have been found to reliably prevent attacks in the vast majority of patients. Impeded or weak androgens have many side effects that, although usually mild, preclude their use in some patients and they are not effective in everyone. In children they can cause premature closure of boney epiphyses, and they are not used in pregnant women. The fibrinolysis inhibitor ϵ aminocaproic acid is also effective in preventing attacks and is often used in children, but its use is attended by the development of severe fatigue and muscle weakness over time.

Recently, purified C1-INH, prepared from human plasma (trade name Cinryze, Viropharma US), given IV has been approved for prophylaxis of HAE, but the half-life of this protein is short, on the order of 40 hours. In clinical trials it was administered intravenously (1000 U) 2 to 3 times a week. A second plasma C1-INH preparation (trade name Berinert, CSL Behring, Australia) at 20 U/kg was recently approved for acute treatment of attacks by the FDA. Recombinant C1-INH (Rhucin) is also in development. Kalbitor, Dyax US (Ecallantide) a 60 amino acid kallikrein antagonist given SQ was recently approved for treatment of acute attacks, and a bradykinin type 2 receptor antagonist (Firazyr, Shize, US) are also reported to be effective in the treatment of acute attacks in preliminary double-blind studies and are in various stages of applying for FDA approval. Thus it is likely that treatment will be greatly modified with the availability of these new agents in the next few years.

Both patients and animals deficient in the classical pathway factors and C3 have an increased propensity to infection, particularly with high-grade pathogenic bacteria like pneumococci, as opposed to viruses (Table I).[9,36] Patients with late component defects, such as of C5-9, have a propensity toward systemic *Neisseria* species infections with *Neisseria gonorrhoeae* or *Neisseria meningitides*. Why opsonization, which only requires complement through C3, is not sufficient to protect against these 2 groups of organisms is not clear, but repeated infection with either of these 2 organisms is often an excellent tip to the clinician that a late complement protein deficiency is present. Alternative pathway defects are rarer, and in fact, no factor B deficiency has ever been described.[37] The few patients with factor D deficiency also have a propensity toward infection, but autoimmunity has not been seen in either animals or patients with defects in this pathway. Defects in the lectin pathway are being defined currently.[6] As discussed earlier in the chapter, MBL has a central core and a series of radiating arms ending in the lectin-binding sites. The radiating arms have the structure of collagen and, like collagen, are composed of 3 intertwined chains; however, unlike collagen, the chains are identical. It has been noted that single-gene defects affecting these chains can lead to improper winding of the chains about one another during the formation of the protein, leading to low levels of MBL. This protein is present normally at very low levels, 2 μg/mL, and patients, commonly with one of 3 genetic defects in the *MBL* gene, even when present in the heterozygous state, have inefficient chain matching and as little as one tenth of the normal level of MBL. Moreover, defects in the promoter region of the gene have been shown to lead to low MBL levels in some patients. It is reported from Europe that children with these defects have a high frequency of infection, although few studies have been done in America to confirm this finding. It is reported that the incidence of other rare infectious disease is increased in this patient group. It is also reported that subjects with MBL abnormalities often die early during the course of cystic fibrosis. Because patients with cystic fibrosis typically have high-titer antibody to their organisms, it is not known why the MBL deficiency should lead to early death. It is also suggested that MBL deficiencies facilitate the pathogenesis of rheumatic disease. All of these observations are intriguing, and all require considerably more study before we understand both the observations and their meaning.

It should be clear from this brief review that complement proteins are capable of having important biological effects and can influence the expression of a wide variety of autoimmune and allergic diseases. We believe that as we develop a clearer understanding of the complex interactions involved in pathogenesis, we will develop a far more insightful approach to the treatment of these important illnesses.

REFERENCES

1. Frank MM. Complement, introduction and historical notes. In: Volanakis JA, Frank MM, editors. The human complement system in health and disease. New York: Marcel Decker; 1998. p. 1-8.
2. Fujita T, Matsushita M, Endo Y. The lectin-complement pathway—its role in innate immunity and evolution. Immunol Rev 2004;198:185-202.
3. Walport MJ. Complement—first of two parts. N Engl J Med 2001;344:1058-66.
4. Walport MJ. Complement—second of two parts. N Engl J Med 2001;344:1140-4.
5. Thurman JM, Holers VM. The central role of the alternative complement pathway in human disease. J Immunol 2006;176:1305-10.
6. Jensenius JC. The mannan-binding lectin (MBL) pathway of complement activation: biochemistry, biology and clinical implications. Adv Exp Med Biol 2005;564:21-2.
7. Hugli TE. Biochemistry and biology of anaphylatoxins. Complement 1986;3:111-27.
8. Nordahl EA, Rydengard V, Nyberg P, Nitsche DP, Morgelin M, Malmsten M, et al. Activation of the complement system generates antibacterial peptides. Proc Natl Acad Sci U S A 2004;101:16879-84.
9. Sjoholm AG, Jonsson G, Braconier JH, Sturfelt G, Truedsson L. Complement deficiency and disease: an update. Mol Immunol 2006;43:78-85.
10. Frank MM. Complement deficiencies. Pediatr Clin North Am 2000;47:1339-54.
11. Carroll M. The complement system in regulation of adaptive immunity. Nat Immunol 2004;5:981-6.
12. Holers VM. Phenotypes of complement knockouts. Immunopharmacology 2000; 49:125-31.
13. Shushakova N, Skokowa J, Schulman J, Baumann U, Zwirner J, Schmidt RE, et al. C5a anaphylatoxin is a major regulator of activating versus inhibitory FcγRs in immune complex-induced lung disease. J Clin Invest 2002;110:1823-30.
14. Davis AE. C1 Inhibitor gene and hereditary angioedema. In: Volanakis JA, and Frank MM, editors. The human complement system in health and disease. Marcel Dekker: New York; 1998. p. 455-80.
15. Blom AM, Villoutreix BO, Dahlback B. Complement inhibitor C4b-binding protein-friend or foe in the innate immune system? Mol Immunol 2004;40:1333-46.

16. Kim DD, Song WC. Membrane complement regulatory proteins. Clin Immunol 2006;118:127-36.
17. Barilla-LaBarca ML, Liszewski MK, Lambris JD, Hourcade D, Atkinson JP. Role of membrane cofactor protein (CD46) in regulation of C4b and C3b deposited on cells. J Immunol 2002;168:6298-304.
18. Joiner KA, Brown EJ, Frank MM. Complement and bacteria: chemistry and biology in host defense. Ann Rev Immunol 1984;2:461-84.
19. He JQ, Wiesmann C, van Lookeren Campagne M. A role of macrophage complement receptor CRIg in immune clearance and inflammation. Mol Immunol 2008; 45:4041-7.
20. Staunton DE, Lupher ML, Liddington R, Gallatin WM. Targeting integrin structure and function in disease. Adv Immunol 2006;91:111-57.
21. Liszewski MK, Atkinson JP. Regulatory proteins of complement. In: Volanakis JA, Frank MM, editors. The human complement system in health and disease. New York: Marcel Dekker; 1998. p. 149-66.
22. Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science 1996;271:348-50.
23. Fang CJ, Richards A, Liszewski MK, Kavanagh D, Atkinson JP. Advances in understanding of pathogenesis of aHUS and HELLP. Br J Haematol 2008;143: 336-48.
24. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, et al. Complement factor H variant increases the risk of age-related macular degeneration. Science 2005;308:419-21.
25. Luzzatto L. Paroxysmal nocturnal hemoglobinuria: an acquired X-linked genetic disease with somatic-cell mosaicism. Curr Opin Genet Dev 2006;16:317-22.
26. Hillmen P, Young NS, Schubert J, Brodsky RA, Socie G, Muus P, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. N Engl J Med 2006;355:1233-43.
27. Kohl J, Baelder R, Lewkowich IP, Pandey MK, Hawlisch H, Wang L, et al. A regulatory role for the C5a anaphylatoxin in type 2 immunity in asthma. J Clin Invest 2006;116:783-96.
28. Peng T, Hao L, Madri JA, Su X, Elias JA, Stahl GL, et al. Role of C5 in the development of airway inflammation, airway hyperresponsiveness, and ongoing airway response. J Clin Invest 2005;115:1590-600.
29. Carroll M. The role of complement and complement receptors in induction and regulation of immunity. Ann Rev Immunol 1998;16:545-68.
30. Carroll MC, Holers VM. Innate autoimmunity. Adv Immunol 2005;86:137-57.
31. Nonaka M. Phylogeny of the complement system. In: Volanakis JA, Frank MM, editors. The human complement system in health and disease. New York: Marcel Dekker; 1998. p. 203-16.
32. Manderson AP, Botto M, Walport MJ. The role of complement in the development of systemic lupus erythematosus. Ann Rev Immunol 2004;22:431-56.
33. Walport M, Davies KA, Botto M. C1q and systemic lupus erythematosus. Immunobiology 1998;199:256-85.
34. Frank MM. Hereditary angioedema: the clinical syndrome and its management in the United States. Immunol Allergy Clin North Am 2006;26:653-68.
35. Zuraw BL. Clinical practice. Hereditary angioedema. N Engl J Med 2008;359:1027-36.
36. Figueroa J, Densen P. Infectious diseases associated with complement deficiencies. Clin Microbiol Rev 1991;4:369-95.
37. Thurman JM, Holers VM. The central role of the alternative complement pathway in human disease. J Immunol 2006;176:1305-10.

Immune responses to malignancies

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Immune responses to tumor-associated antigens (TAs) are often detectable in tumor-bearing hosts, but they fail to eliminate malignant cells or prevent the development of metastases. Patients with cancer generate robust immune responses to infectious agents (bacteria and viruses) perceived as a "danger signal" but only ineffective weak responses to TAs, which are considered as "self." This fundamental difference in responses to self versus nonself is further magnified by the ability of tumors to subvert the host immune system. Tumors induce dysfunction and apoptosis in $CD8^+$ antitumor effector cells and promote expansion of regulatory T cells, myeloid-derived suppressor cells, or both, which downregulate antitumor immunity, allowing tumors to escape from the host immune system. The tumor escape is mediated by several distinct molecular mechanisms. Recent insights into these mechanisms encourage expectations that a more effective control of tumor-induced immune dysfunction will be developed in the near future. Novel strategies for immunotherapy of cancer are aimed at the protection and survival of antitumor effector cells and also of central memory T cells in the tumor microenvironment. (J Allergy Clin Immunol 2010;125:S272-83.)

Key words: *Cancer, immunity, tumor escape, immune suppression, effector T cells*

Abbreviations used

Anx:	Annexin V
APC:	Antigen-presenting cell
APM:	Antigen-processing machinery
β2 m:	β_2-microglobulin
CTL:	Cytolytic T lymphocyte
DC:	Dendritic cell
FasL:	Fas ligand
FOXP3:	Forkhead box protein 3
iNOS:	Inducible nitric oxide synthase
MDSC:	Myeloid-derived suppressor cell
NK:	Natural killer
PD-1:	Programmed death 1
PD-L1:	Programmed death ligand 1
PGE_2:	Prostaglandin E_2
ROS:	Reactive oxygen species
STAT3:	Signal transducer and activator of transcription 3
TA:	Tumor-associated antigen
TAM:	Tumor-associated macrophage
TCR:	T-cell receptor
TIL:	Tumor-infiltrating lymphocyte
Treg:	Regulatory T
VEGF:	Vascular endothelial growth factor

Evidence accumulated over the last few years convincingly shows that the host immune system is involved in cancer development and progression, as well as control of metastasis. The presence of antitumor cellular responses, humoral responses, or both to tumor-associated antigens (TAs) has been observed in many, but not all, patients with cancer.[1,2] The evidence for such pre-existing antitumor immunity in patients with cancer confirms that the tumor-bearing host is capable of mounting an immune response to TAs. Tumor progression from a single transformed cell to a mass of malignant cells is a multistep process involving a series of genetic changes occurring in human subjects over a period of months or years and culminating in the established tumor.[3] During this period, neither the host immune system nor the developing tumor are idle: those newly emerging tumor cells that are recognized by the immune system are eliminated only to be replaced by genetic tumor variants resistant to immune intervention and giving rise to a heterogenous population of malignant cells found in any tumor. Tumors are genetically unstable, and the emergence of new genetic variants, which is responsible for the tumor heterogeneity, ensures that the tumor survives in the face of the host immune system. Only the tumor cells that manage to avoid recognition escape and survive, whereas those that are recognized by the immune system are eliminated as soon as they arise. The tumor development involves a prolonged series of checks and balances between the host attempting to curtail tumor growth and the tumor benefiting from genetic changes, altering its microenvironment and avoiding immune elimination. Thus the tumor becomes resistant to immune effector cells.

The interactions between the host and the tumor have been referred to as "immune surveillance," a concept that originated many years ago with F. M. Burnett and that introduced his vision of a vigilant host immune system able to spot, recognize, and eliminate tumor cells. A modern version of the immune surveillance theory not only emphasizes the ability of the host immune system to recognize and destroy tumor cells but also its contribution to "immune selection" of resistant tumor variants. Thus the "immune editing" hypothesis[2,4] has been advanced to suggest that by means of elimination of tumor cells sensitive to immune intervention, the host immune system edits for survival of tumors that become resistant to immune cells. An alternative hypothesis allows for the progressing tumor to develop immunosuppressive mechanisms that will thwart any attempt of immune tumor elimination and in effect will induce a state of tumor-specific tolerance.[5] In the first instance the immune system initiates the selection of resistant tumor variants, and in the second the tumor becomes a perpetrator of immune unresponsiveness. Central to the paradigms of immune selection or immune editing and

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immune suppression is the premise that the tumors acquiring new mutations are able to avoid immune intervention and are capable of both escaping and disabling the host immune system. Neither of the 2 hypotheses has been completely accepted today, and there are those who believe that tumors progress because of the genetic instability and others who favor tumor-specific tolerance of the immune system, which enables the tumor to take advantage of the tissue microenvironment regardless of the immune system and benefit from it. This controversy regarding the significance of the immune system in tumor development and progression underscores the complexity of interactions between the tumor and the immune cells. It surmises that these interactions might be bidirectional, are influenced by the local microenvironment, and not infrequently might result in demise not of the tumor but of tumor-reactive immune cells.

In this chapter the nature and components of the host immune response against tumors will be discussed, including the reasons for the failure of the immune system to contain tumor growth and metastasis. It is this latter aspect of the immunobiology of human malignancies that will be emphasized, largely because it directly affects cancer immunotherapy. A relatively recent realization that tumors have devised multiple and remarkably effective mechanisms for disarming the host immune system has opened a way for the introduction of novel therapeutic strategies aimed at eliminating tumor escape. If the tricks tumors use for protection from immune intervention by the host are responsible for their progression, then it could be surmised that a limited success of current immune therapies for cancer can be reversed by therapies that target the escape mechanisms, and because these escape mechanisms might be unique for each tumor rather than generalized, the future challenge will be to identify the "immunologic signature" of each tumor and then use selective therapies to eliminate the tricks and restore vigorous antitumor immunity.

TUMOR PROGRESSION AND THE HOST IMMUNE RESPONSE

There are several lines of evidence that point to an early, as well as late, involvement of the immune system in tumor development. Early tumor lesions, and even premalignant foci, such as melanocytic nevi, are frequently infiltrated with hematopoietic cells, including lymphocytes, macrophages, and occasionally granulocytes.[6,7] The presence of immune cells in the tumor at later stages of development (ie, the abundance of tumor-infiltrating lymphocytes [TILs]) has been associated with improved patient survival in several early studies (reviewed in Whiteside[8]). More recently, studies by Fridman's group performed a comprehensive multivariate analysis of cellular interactions in the tumor microenvironment based on the type, density, localization, and function of immune cells present within human colorectal cancer and demonstrated that immune reactivity at the tumor site influences clinical outcome.[9-11] Thus increased densities of T-cell infiltrates with a high proportion of $CD8^+$ T cells within primary colorectal carcinomas were associated with a significant protection against tumor recurrence.[11] Furthermore, the same group also showed that coexpression of genes mediating cytotoxicity and T_H1 adaptive immune responses accurately predicted survival in patients with colorectal carcinoma independently of the metastatic status.[12] In aggregate these multiparameter analyses of tumor-infiltrating cells *in situ* suggest that immune cells can and indeed often do play a role in tumor control but that both intrinsic and extrinsic factors in the tumor microenvironment alter the balance required for optimal control.[12]

In many patients with cancer, it is possible to expand in culture and *in vitro* test functions of tumor-specific cytolytic T lymphocytes (CTLs) from the peripheral blood or TILs.[8] This finding, which has been reproduced in many laboratories, suggests that precursors of such CTLs exist in the circulation or at the tumor site in patients with cancer and can be induced to proliferate when autologous dendritic cells (DCs) pulsed with relevant tumor epitopes and used as antigen-presenting cells (APCs). More recent experiments, using tetramers and flow cytometry, have directly demonstrated the presence of tumor peptide–specific T cells in the circulation of patients with cancer.[1,13,14] Furthermore, the frequency of such peptide-specific T cells appears to be higher in the circulation of patients with cancer than in healthy subjects.[15] Finally, the SEREX technology, based on the presence of tumor-specific antibodies in sera of patients with cancer, has been successfully used for tumor-antigen discovery in many laboratories.[16] These findings, as well as recent identification of numerous TAs that appear to be immunogenic in that they induce humoral immune responses, cellular immune responses, or both *in vitro* by using human immune cells and *in vivo* in animal models of tumor growth, strongly support the notion that the host immune system recognizes the presence of the tumor and responds to it by generating both local and systemic immune responses.

If the tumors are not ignored by the immune system, why do they progress? Several answers to this question can be considered. First, there is the old argument for the lack of a "danger signal"[17] in tumors akin to those presented by pathogens invading tissues during an infection. Recognition by DCs of pathogen-associated molecular patterns through the ubiquitous Toll-like receptors leads to efficient DC activation and maturation. It promotes generation of vigorous cellular and antibody responses to bacterial or viral antigens, presumably because the immune system perceives an infection as a danger signal[17] benefiting the host. However, functional Toll-like receptors are known to be expressed by many human solid tumors,[18] and recent data indicate that tumors use them to promote their own growth; for protection from spontaneous, immune-mediated, or drug-induced apoptosis; or both.[18,19]

Second, TAs are perceived by the immune system as "self" or "altered self" antigens, which evoke weak immune responses because tolerance prevents generation of immune responses to self. The only "unique" TAs are mutated antigens, and these are strongly immunogenic and elicit robust immune responses.[20] However, only a handful of such mutated TAs are known, and the vast majority of TAs are poorly immunogenic or simply tolerogenic. In this context cancer can be viewed as an autoimmune phenomenon in which tolerance to self prevents effective immune responses to TAs Patients with cancer who have not been treated with chemotherapy or radiotherapy generally have normal immune responses to viral or bacterial antigens, yet they are unable to respond to their own TAs. Except for late-stage disease, they generally have normal delayed-type hypersensitivity responses to recall antigens but are anergic to autologous TAs. Although tolerance to self is a detriment to the generation of antitumor responses in patients with cancer, another factor that exerts an overwhelming effect on these responses is the tumor microenvironment. Each tumor creates its own milieu characterized by the presence of immunosuppressive factors and by the excess of

TAs produced and released by the growing tumor. Evidence suggests that tumors produce a broad array of immunoinhibitory factors, which exert either local or systemic effects on the host antitumor immune responses.[5] Therefore it is not surprising that antitumor immunity might be weak, inefficient, or even absent in patients with cancer, depending on the nature of tumor-host interactions, as well as the robustness of regulatory mechanisms in control of immune tolerance.

Immune antitumor responses could be influenced by the gradual deterioration of the immune system with age.[21] The increased incidence of cancer present in the elderly might be due to immunosenescence (ie, progressive remodeling of the immune system with a reduced ability of immune cells to respond to activating stimuli and increased responsiveness to tolerogenic signals).[21] Immunosenescence can significantly interfere with the effectiveness of cancer immunotherapies, and it has been suggested that clinical trials testing immunopotentiating agents in patients with cancer should be conducted in elderly subjects.[21]

Recent multiparameter analyses of primary and metastatic human tumors (eg, colorectal carcinoma) recognize several major immune "coordination profiles," the presence of which is influenced by the balance between tumor escape and immune antitumor responses and that are subject to host-tumor cross-talk.[12] In this context it is important to consider differences between primary and metastatic tumors. Not only are metastatic tumors more immunosuppressive, but also they appear to be less readily recognized by TA-specific immune effector cells. The latter could be due to defects in the expression levels of antigen-processing machinery (APM) components, MHC molecules, or both in the tumor and its metastases.[22] Because different copy numbers of distinct trimolecular peptide–β_2-microglobulin (β2 m)–MHC complexes presented on the tumor surface might lead to differential T-cell recognition, this aspect of tumor–immune cell interactions is critical.[22,23] A recent comparison of primary renal cell carcinoma, renal cell carcinoma metastases, and normal renal tissue with respect to HLA ligand presentation and gene expression demonstrated a greater similarity between primary tumor and metastasis than between the tumor and normal tissue.[24] This observation provides a good rationale for peptide-based immunotherapy because it is likely to preferentially target the tumor and its metastases and not the normal tissue.

NATURAL VERSUS ADAPTIVE IMMUNE RESPONSES TO MALIGNANCIES

Antitumor immune responses can be innate (natural) or acquired (adaptive). Innate immunity is mediated by cells or soluble factors that naturally exist in tissues or body fluids and can interfere with tumor growth or survival. Among hematopoietic cells, macrophages, granulocytes, natural killer (NK) cells ($CD3^-CD56^+$), non–MHC-restricted T cells ($CD3^+CD56^-$), and $\gamma\delta$ T cells have the natural capability to eliminate tumor cell targets.[21] In addition, natural antibodies with specificities directed at surface components of tumor cells might be present in the sera of patients with cancer.[16] Other serum factors, including complement components, C-reactive protein, mannose-binding protein, and serum amyloid protein, also play a role in innate immunity.[25] Adaptive immune responses to tumors are mediated by $CD3^+$ T-cell receptor (TCR^+) T cells when they recognize tumor-derived peptides bound to self-MHC molecules expressed on APCs. Little is currently known about the molecular signals and cellular steps involved in directing APCs, such as DCs, to execute a tolerogenic versus immunogenic program in response to antigens. As indicated above, tumors can also serve as APCs, although low levels of MHC class I molecule expression, MHC class II molecule expression, or both on the surface of tumor cells makes this an inefficient process.[22] More likely, TAs are taken up by DCs present at the tumor site, processed, and cross-presented to T cells in the tumor-draining lymph nodes in the form of the trimolecular peptide–β2m–MHC complexes.[23] For adaptive immune response to occur, T cells expressing correct (cognate) TCRs have to be present. Recognition of the peptide and its binding to the variable domains of the TCR initiates signaling (signal 1) that leads to T-cell activation.[26] This requirement implies prior sensitization and a clonal expansion of memory T cells in response to a cognate tumor epitope (anamnestic or recall responses). Alternatively, precursor T cells expressing the TCR can be primed by the cognate peptide–MHC ligands presented on APCs, and the subsequent development of antitumor effector cells is viewed as a primary immune response. In either case costimulatory molecules (signal 2) are necessary for an immune response to proceed,[27] and once T-cell proliferation is initiated, appropriate cytokines (signal 3) become essential for sustaining the response.[28] Recent findings stress the key importance of signal 3 for the development of immune responses and for their contraction.[28] Like all immune responses, those that are TA specific do not go on forever but peak and then contract, restoring the preactivation balance. The precise mechanisms responsible for immune contraction are not yet defined, and regulatory T (Treg) cells, as well as other mechanisms, have been proposed to regulate immune reactivity, but it is clear that events in the environment play a dominant role in this respect.

Immune responses to malignant cells can be categorized as locoregional or systemic. *In situ* or local responses refer mainly to TILs, which accumulate in most human solid tumors and the role of which in tumor progression remains highly controversial. Long considered by some an effector arm of antitumor responses, TILs are viewed by others as victims of the tumor microenvironment because their effector functions are often impaired, presumably by tumor-derived factors.[29] A failure of local antitumor responses mediated by TILs is thought to contribute to tumor progression. Systemic immunity to tumors, as measured by delayed-type hypersensitivity responses or by various *ex vivo* assays of T-cell responses in the peripheral circulation of patients with cancer, are difficult to demonstrate, and TA-specific responses have been particularly elusive. Nevertheless, by using highly sensitive multicolor flow cytometry, it has been possible to detect and measure the frequency of TA-specific $CD8^+$ and $CD4^+$ T cells in the peripheral circulation of patients with cancer.[1] Although the response levels vary, TA-specific and nonspecific proliferative or cytotoxic responses of peripheral lymphocytes in patients with cancer appear to be at least partially impaired.[29-31] Data indicate that the same functional impairments seen in TILs are found in both circulating and lymph node lymphocytes of patients with cancer.[29,32] Thus it has been concluded that, in general, human tumors exert profound suppressive effects on both local and systemic antitumor immunity in these patients.

In contrast to the failure of antitumor immune responses to control tumor progression in human subjects, a large body of experimental evidence derived from preclinical animal models of cancer suggests that the immune system can prevent tumor growth or cause its rejection.[33] In the prevention setting

vaccination of animals with TAs plus adjuvant protects them from rechallenge with tumor,[34] whereas immunotherapy of established tumors with vaccines, cytokines, adoptively transferred immune cells, or immunomodulatory agents results in tumor rejection, provided the tumor is not in an advanced stage. Remarkably, this has been a consistent pattern seen with carcinogen-induced, virally induced, and spontaneously arising tumors in mice, suggesting a fundamental difference in immune responses to tumor antigens between mice and human subjects. Indeed, it appears that the difference might be due to appreciably greater immunogenicity of murine TAs, which in most cases are virus- or carcinogen-related epitopes and thus foreign rather than self-epitopes. Alternatively, the answer might be that experimental murine tumors are established, grow, progress, and are eliminated by therapy in the very short time required for the completion of the experiment, leaving no time for the development of tumor escape mechanisms. In contrast, human tumors are diagnosed and treated after many years of coexistence with the host. An introduction or establishment of the tumor in mice is a dramatic event that mobilizes host defenses in contrast to a silent coexistence of tumor cells with the immune system for many years in human subjects. To minimize this difference, transgenic murine models have been developed, allowing for ensured, genetically driven tumor development in a "spontaneous" environment.[35] Transgenic mice have been especially useful in the design of preventive cancer vaccines,[34] and information they provide is encouraging for the development of immunoprophylaxis of cancer in human subjects. Nevertheless, to date, it has been difficult to translate the positive results seen in mice to immunotherapy of established human tumors. It is plausible that numerous and varied mechanisms of escape developed by the latter during the prolonged residence and interactions with the host provide human tumors with advantages not afforded to murine tumors established in an experimental setting.

TUMOR ASSOCIATED ANTIGENS

Recent progress in the development of cancer vaccines has been greatly facilitated by the availability of well-defined TAs, many of which have been characterized in the last decade.[36] Most of these TAs are derived from self-proteins that are either mutated or otherwise differentially expressed in normal and tumor cells, as exemplified by oncogenes or oncofetal or cancer testis antigens. The major categories of TAs that have been often used as candidates for immune therapies are listed in Table I.[36,37] A recent report provides a much longer prioritized list of well-characterized cancer antigens best suited for use in cancer vaccines.[38] The list is based on criteria generated by a panel of experts convened by the National Cancer Institute[38] and is designed to assist investigators in the field of immunotherapy in the selection of the most promising TAs for further testing in clinical trials.

As already indicated, immune responses to TAs, even to those representing altered self-antigens, are detectable in tumor-bearing hosts, although in most cases no correlations between the presence of *in vitro* responses to TAs and prognosis have been documented. This is in contrast to numerous animal tumor models, which have provided strong evidence that in the presence of effective antitumor immunity, tumors fail to progress and established tumors regress.[39] Nevertheless, human cancer vaccine trials in patients with cancer have made use of many well-characterized TAs in the hope that their presentation on appropriately polarized DCs will overcome difficulties with the generation of a strong immune response in the therapeutic setting. The most recent reports of such clinical trials in patients with cancer indicate that multiple subcutaneous injections of an immunogenic tumor peptide, such as NY-ESO-1, plus a mix of 2 potent adjuvants, such as Montanide ISA-51 and CpG7909, can be effective in inducing sustained peptide-specific immune responses and significantly prolong survival, even in patients with advanced disease, including solid tumors other than melanoma.[40] These reports, demonstrating that antitumor, antivaccine, or both immune responses correspond to clinical outcome, suggest that the optimization of vaccination strategies is likely to overcome tumor-induced suppression and to restore the immune balance altered by cancer development.

IMMUNE CELLS IN THE TUMOR MICROENVIRONMENT

Immune cells that are most frequently found in the human microenvironment are lymphocytes, which are capable of mediating both innate and adaptive immunity, although monocytes, tumor-associated macrophages (TAMs), and DCs are also commonly seen.[41] Inflammatory cells present in the tumor are in intimate contact with tumor cells, stromal fibroblasts, extracellular matrix components, and blood vessels. Proinflammatory cytokines secreted by inflammatory cells can contribute to tumor progression, and soluble factors produced by the tumor in response to nonspecific or tumor-specific signals, such as prostaglandin E_2 (PGE_2), adenosine, or TGF-β, downregulate functions of immune cells. The tumor microenvironment is created by the tumor, and it is continuously shaped and dominated by the tumor, which directs all cellular and molecular events taking place in the surrounding tissue.

Immune cells recruited to the tumor include T cells ($CD3^+TCR^+$), which are by far the largest component of mononuclear tumor infiltrates[41] and have received the most attention. Although their accumulation in the tumor might be considered evidence of immune surveillance by the host, they are largely ineffective in arresting tumor growth, although they can proliferate and mediate antitumor cytotoxicity on their removal from the tumor bed and *ex vivo* IL-2 activation.[42]

Phenotypic and functional characteristics of human TILs are listed in Table II. More current data on the status of T cells found in human tumors suggest that their phenotypic and functional profile varies depending on the microenvironment created by the tumor and that this profile or "immune signature" can influence prognosis and disease outcome.[9,12] It appears that TILs obtained from advanced or metastatic lesions are more functionally impaired than those from early lesions, suggesting that tumor burden or the potential of a tumor to suppress immune cells might determine the functional status of infiltrating T cells. Among $CD4^+$ T cells present in the tumor, a subset of $CD4^+CD25^{high}$ forkhead box protein 3 (FOXP3)–positive Treg cells is expanded to constitute from 5% to 15% of CD4 T cells in the infiltrate. Their frequency is higher in the tumor than in the peripheral circulation.[43,44] These cells suppress functions of other immune cells in the microenvironment by mechanisms that might be cell contact dependent or might involve the production of inhibitory cytokines or adenosine.[43-46] Recently, a potent proinflammatory T-cell subset, IL-17–producing T_H17 cells, were observed among $CD4^+$ cells in patients with ovarian carcinoma. The presence of

TABLE I. Human TAs that are candidates for immune therapies*

TA category	Examples
Oncofetal	Oncofetal antigen/immature laminin receptor (OFA/iLRP)
	Glypican 3 (heparan sulfate protoglycan)
	α-Fetoprotein (AFP)
	Carcinoembryonic antigen (CEA)
Oncogenes	The RAS family: p53, Her2 neu
Cancer testis (CT) antigens:	MAGE-1
	BAGE
	GAGE
	NY-ESO-1/LAGE
	SAGE
	Other 35-40 CT antigens mapping to chromosome X (CT-X) or distributed throughout the genome (non-X CT)
Human melanoma antigens	MART-1/MELAN-A
	Gp100/pmel 17
	Tyrosinase
	Tyrosinase related proteins (TRP) 1 and 2
	Chondroitin sulfate proteoglycan (CSPG4)
Human glioma antigens	IL-13 receptor α2
	Eph A2
	Survivin
	EGFR variant III (EGFRvIII)
Head and neck cancer antigens	EGFR
	Human papilloma virus (HPV 16 or 18)
	Aldehyde dehydrogenase A1 (ALDHA1)
	CSPG4
Normal overexpressed or modified antigens	MUC-1
	Cyclin-B1
	Prostate-specific antigen (pSA)
	Prostate membrane-specific Ag (PMSA)

*The actual list of TAs available for immune therapies is much longer. The reader is referred to a more comprehensive recent listing of these antigens.[36,37]

these cells was significantly correlated to enhanced survival in these patients and was found to inversely correlate with the number of $FOXP3^+$ Treg cells.[47]

Macrophages ($CD14^+$) present in tumors are referred to as TAMs. Although normal macrophages uptake antigens and play an important role in control of infections, TAMs are reprogrammed to inhibit functions of immune cells through the release of inhibitory cytokines, such as IL-10, PGE_2, or reactive oxygen species (ROS).[48] It is hypothesized that reprogramming of TAMs occurs in the tumor microenvironment as a result of tumor-driven activation. Evidence has accumulated indicating that invasiveness of tumors, such as human primary colon carcinomas, is directly related to the number of TAMs detected in the tumor. In patients with invasive breast cancer, an increased TAM count is an independent predictor of reduced relapse-free survival, as well as reduced overall survival.[49] The available data support the active role of TAMs in tumor-induced immunosuppression on the one hand and in the promotion of tumor growth on the other. Furthermore, preliminary evidence suggests that the reciprocal differentiation of Treg and T_H17 cells from an uncommitted common $CD4^+$ precursor along either a suppressive or proinflammatory pathway, respectively, is biased by TAMs.[47] Thus TAMs appear to significantly contribute to shaping of the tumor microenvironment.

A subset of myeloid-derived cells equivalent to $CD11b^+/Gr1^+$ cells in mice, which are $CD34^+CD33^+CD13^+CD15^-$ and called myeloid-derived suppressor cells (MDSCs), accumulate in human tumors.[50] They are recruited from the bone marrow by means of tumor-derived soluble factors, such as GM-CSF, vascular endothelial growth factor (VEGF), and IL-10; migrate to lymph nodes, where DCs cross-prime T cells; and interfere with this process. They also migrate to tumors, become tumor-associated MDSCs, and inhibit immune cell functions through the production of arginase 1, an enzyme involved in the L-arginine metabolism. Arginase 1 synergizes with inducible nitric oxide synthase (iNOS) to increase superoxide and nitric oxide production, inhibiting lymphocyte responses by the induction of iNOS in surrounding cells.[51] Current data support the active role of MDSCs in tumor-induced immune suppression that contributes to functional dysfunction of immune cells in the tumor, as well as the peripheral circulation of patients with cancer.

DCs ($HLA\text{-}DR^+CD86^+CD80^+CD14^-$) are nature's best APCs. They are a common component of tumor immune infiltrates and are responsible for the uptake, processing, and cross-presentation of TAs to naive or memory T cells, thus playing a crucial role in the generation of tumor-specific effector T cells.[52] In addition, DCs control the induction of Treg cells. In patients with cancer, cellular interactions between antigen-presenting DCs and T cells lead to expansion and accumulation of Treg cells at the tumor site and in the periphery.[52] The DC-derived signals that determine the outcome of DC–T-cell interactions operate at the levels of (1) antigen presentation (signal 1); (2) display of costimulatory molecules (signal 2); and (3) the presence of immunomodulatory cytokines (signal 3). Stimuli that lead to upregulation of signals 1 and 2 in the absence of signal 3 might facilitate peripheral tolerance induction.[52] At the same time, newer evidence suggests that many conditions relevant to signal 1, such as antigen

TABLE II. Morphologic, phenotypic, and functional characteristics of TILs found in human solid tumors

Morphology: small to large lymphocytes
Phenotype: $CD3^+TCR\text{-}\alpha/\beta^+$ T cells; few (<5%) $CD3^-CD56^+$ NK cells
Mix of $CD4^+$ and $CD8^+$ cells; variable CD4/CD8 ratio
Largely $CD45RO^+CCR7^-$ memory T cells
Express activation markers (CD25, HLA-DR)
Nearly all are $CD95^+$
Accumulations of Treg cells ($CD4^+CD39$ + TGF-β^+) and $CD4^+IL\text{-}17^+$ T_H17 cells
Clonality: oligoclonal, as determined based on TcR Vβ gene expression
Specificity: autotumor-specific T cells detectable in some tumors at a low frequency
Functions: Low or absent ζ chain expression: inefficient TCR signaling
Suppressed nuclear factor κB activation
Decreased locomotion, proliferation, cytotoxicity
Cytokine profile: T_H2 type with IL-4, IL-5, and IL-13 production and no/little IL-2 or IFN-γ production; excess of IL-10 or TGF-β
In vitro response to IL-2 variable but more decreased in TILs recovered from metastatic rather than primary lesions
Increased levels of caspase-3 activity
Apoptosis of $CD8^+$ T cells (TUNEL+; Anx^+)

TUNEL, Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling.

dose, determine whether Treg or T_H2 effector (Teff) cells are induced, irrespective of the maturation state of DCs.[52] In addition, insights into the APM in DCs and evidence that some of the components of APM, including MHC class II molecules, might be downregulated or altered in patients with cancer,[23] suggest that Treg cell induction might be influenced not only by the nature and dose of the antigen but also by its processing and its presentation to T cells.

Tumor-associated DCs directly exposed to tumor cells, tumor-derived factors, or both have been shown to readily undergo apoptosis and to have impaired maturation.[53] Specifically, tumor-derived factors, such as gangliosides, were shown to inhibit DC generation and their function *in vitro*.[54] This suppressive effect of gangliosides on DCs was found to be mediated by tumor-derived VEGF, a known antidendropoietic factor.[53] The data on functional impairments of tumor-associated DCs have to be balanced by numerous reports in the literature, which suggest that the presence of DCs in tumors is associated with improved prognosis and prolonged patient survival, as well as a reduced incidence of recurrent or metastatic disease.[55] In contrast, patients with lesions reported to be scarcely infiltrated with DCs have a relatively poor prognosis.[56] Fewer DCs were observed in metastatic than in primary lesions. In one study it was shown that the number of DCs present in the tumor was by far the strongest independent predictor of overall survival, as well as disease-free survival and time to recurrence, in a large cohort ($n = 132$) of patients with oral carcinoma compared with such well-established prognostic factors as disease stage or lymph node involvement.[55] It appears that not only the number of DCs but also the presence of functionally unimpaired, normally signaling T cells in the tumor microenvironment are important for overall survival of patients with cancer.[55]

NK cells ($CD3^-CD56^+CD16^+$), which mediate innate immunity and contain both perforin-rich and granzyme-rich granules, are well equipped to mediate lysis of tumor cells. Although NK cells represent "the first line of defense" against pathogens,[57] most human tumor cells are resistant to perforin-mediated NK cell lysis, and NK cells are rarely found among TILs.[41] This is despite the fact that tumor cells often downregulate MHC antigen expression and are enriched in MICA and MICB molecules.[58] There might be several reasons for the paucity of NK cells in tumors, including the possibility that NK cells are present in premalignant or early lesions and absent from advanced tumors, which is consistent with their role in immune surveillance rather than killing of cancer cells at the tumor site.[41] More recent data suggest that the primary biologic role of NK cells in tumor-bearing hosts might not be the elimination of tumor targets but rather the facilitation of DC–T-cell interactions and driving the immune responses to TAs.[59] Because the tumor site is not likely to be the optimal milieu for this type of immune interaction, the paucity of NK cells in tumors might fit with their physiologic functions. The *in vivo* role of NK cells in antitumor immune defense is not yet clear, and work continues to define it further.

Polymorphonuclear leukocytes are infrequently seen in infiltrates of human solid tumors, with the exception of nests of eosinophils that might be present in association with tumor cells in some cases. In human tumors granulocytes, which are a major cellular component of many murine tumors, are rare, being largely replaced by TAMs or MDSCs. This could be explained by the fact that most inflammatory infiltrates into human tumors are chronic rather than acute, with granulocytes long gone by the time human tumors are diagnosed, biopsied, and examined.

B cells ($CD19^+$, $CD20^+$) are also rare in most human tumors, with the exception of breast cancer and melanoma.[6,60] The primary function of B cells is differentiation into antibody-producing plasma cells. Although TA-specific antibodies are frequently detected in the circulation of patients with cancer, these antibodies are made and secreted in the tumor-draining lymph nodes, spleen, or other lymphoid tissues. From these sites, IgG molecules can readily be transported through plasma or lymph to tissue sites. Therefore the presence of B cells or plasma cells in tumors is not expected *a priori*, although it might be that the ability to make antibodies *in situ* could be an important aspect of host defense.

Inflammatory infiltrates present in human tumors change in composition and intensity during tumor progression. The initial acute inflammation involving the recruitment and influx of antitumor effector cells is replaced by chronic inflammation in later stages of tumor progression. Tissue hypoxia plays a major role in shaping the nature of immune infiltrates in tumors. It is created by activation of hypoxia-responsive genes in tumor cells[61] and favors the influx of granulocytes and phagocytic macrophages, which depend on the glycolytic pathway for survival.[62] These cells take up and process dying tumor cells, producing an abundance of ROS. The subsequent reoxygenation of the microenvironment is accompanied by activation of the nuclear factor κB pathway in both tumor cells and infiltrating immune cells, leading to the excessive secretion of proinflammatory cytokines.[5] Responding to this nuclear factor κB–driven cascade of proinflammatory cytokines, the tumor and stromal cells produce a variety of soluble factors with wide-ranging biologic effects, including the promotion of tumor cell proliferation.[5] In the tumor microenvironment cellular expansion, differentiation, or activation, as well as cell migration, matrix remodeling, and blood vessel growth, are reprogrammed to benefit the tumor. Thus the nature of chronic inflammatory infiltrates and functions of the tumor-infiltrating immune cells depend on how aggressively a given tumor remodels its microenvironment.

IMMUNE EFFECTOR CELLS IN THE CIRCULATION OF PATIENTS WITH CANCER

In human subjects peripheral blood is the major source of cells for studies of their antitumor functions. T lymphocytes, NK cells, monocytes, DCs, and B cells and their subsets have all been extensively evaluated in the peripheral circulation of patients with cancer by using conventional phenotypic and functional *in vitro* assays. Results indicate that signaling abnormalities, functional impairments, and apoptosis seen in immune cells obtained from the tumor microenvironment are likewise present in peripheral blood cells of patients with cancer.[63,64] The finding of $CD8^+$ T-cell apoptosis in the circulation of these patients is perhaps the most convincing evidence that all is not well with immune effector cells in cancer.[65] The proportion of $CD8^+CD95^+$ T cells that bind Annexin V (Anx) and yet are 7-amino-actinomycin D negative ($7AAD^-$) or propidium iodide (PI) negative is significantly greater in the peripheral circulation of patients with cancer, including those with head and neck, breast, and ovarian carcinomas and melanoma, than in age- or sex-matched healthy donors.[65] As indicated in Table III,[66-68] T cells that undergo spontaneous apoptosis in the circulation of these patients are $CD3^+CD95^+$, bind Anx, and have increased levels of caspase-3 activity and decreased expression of the TCR-associated ζ chain.[63,69,70] Circulating $CD8^+$ T cells, especially the effector subpopulations ($CD8^+CD45RO^+CCR7^-CD27^-$ and $CD8^+CD28^-$), have a significantly greater propensity to undergo spontaneous apoptosis than $CD4^+$ T cells in patients with cancer. This could explain the functional deficits found in $CD8^+$ effector cells, such as the downregulation in expression of signaling molecules, specifically the ζ chain. The available data suggest that functional defects in T cells might be linked to their increased sensitivity to apoptosis and that the tumor participates in engineering spontaneous or activation-induced cell death of T cells.[65] The highest proportions of $Fas^+Anx^+CD8^+$ T cells are generally seen in a subset of patients with advanced active disease.[70] In patients with cancer, the vast majority of circulating $CD8^+$ T cells are $CD95^+$, and the Fas/Fas ligand (FasL) pathway contributes to their apoptosis because human solid tumors express FasL and export it to the periphery in the form of $FasL^+$ exosomes.[71,72] However, tumor-induced apoptosis of immune cells engaging death ligand/receptor interactions is only one of many mechanisms used by tumors to engineer an immune escape.[65] Based on increasing insights into these mechanisms, it is possible to speculate that the presence of the constellation of immune defects might allow for the identification of a subset of patients with cancer who have poor prognosis because their tumors create a particularly immunosuppressive environment.

Apoptosis of Fas^+, activated $CD8^+$ T cells in the circulation of patients with cancer leads to a rapid turnover of T lymphocytes, contributing to a loss of antitumor effector cells and an aberrant lymphocyte homeostasis.[66,73] Recent data indicate that circulating Vβ-restricted $CD8^+$ T cells and tumor peptide–specific tetramer–positive $CD8^+$ T cells are especially sensitive to apoptosis.[74] By using T-cell receptor excision circle (TREC) analysis, a PCR-based technique that allows for quantification of recent thymic emigrants in the peripheral circulation, it has been determined that patients with cancer had significantly fewer recent thymic emigrants than healthy age-matched donors.[67] The results suggest that the lymphocyte turnover is faster in patients with cancer than in healthy control subjects, either because the thymic output in patients is lower or the peripheral expansion of T cells is greater, diluting T-cell receptor excision circles and enhancing the maturation rate of naive T cells.[66,73] Such rapid turnover of T cells could have detrimental effects on antitumor responses. A loss of effector subpopulations of $CD8^+$ T cells, which appear to be targeted for apoptosis in patients with cancer, might severely compromise antitumor functions of the host and contribute to tumor progression.[73]

TABLE III. Characteristics of T lymphocytes in the peripheral circulation of patients with cancer*

Predominant phenotype	
T lymphocytes:	
% $CD3^+CD95^+Anx^+$ (increased vs NC)	
% $CD3^+CD25^+$ (increased vs NC)	
% $CD3^+HLA\text{-}DR^+$ (increased vs NC)	
$CD8^+$ subset:	% $CD8^+CD95^+Anx^+$ (increased vs NC)
$CD8^+$ naive:	% $CD8^+CD45RO^-CCR7^+$ (decreased vs NC)
$CD8^+$ central memory:	% $CD8^+CD45RO^+CCR7^+$ (decreased vs NC)
$CD8^+$ peripheral memory:	% $CD8^+CD45RO^+CCR7^-$ (increased vs NC)
$CD8^+$ effector cells:	% $CD8^+CD45RO^-CCR7^-$ (increased vs NC)
$CD4^+$ subset:	% $CD4^+CCR7^+$ (decreased vs NC)
$CD4^+$ naive:	% $CD4^+CD45RO^-CCR7^+$ (decreased vs NC)
$CD4^+$ memory cells:	% $CD4^+RO45RO^+CCR7^+$ (decreased vs NC)
$CD4^+$ Treg cells:	% $CD4^+CD25^+$ (increased proportions vs NC)
Clonality: Polyclonal with various restricted TCR Vβ specificities	
Specificity: TA-specific/tetramer$^+$ T cells detectable in many cases	
Functions	
Low ζ chain expression in T and NK cells: inefficient TCR signaling	
Decreased proliferation in response to anti-CD3 antibody, PMA/ionomycin, mitogens	
Decreased antitumor cytotoxicity and NK/lymphokine-activated killer activity	
Cytokine profile: highly variable	
Apoptosis of $CD8^+$ T cells and NK cells (Anx^+)	
Increased caspase-3 activity in T cells	
Increased lymphocyte turnover	

LAK, Lymphokine-activated killer; *NC*, healthy control subjects; *PMA*, phorbol 12-myristate 13-acetate.
*The percentage of positive cells in patients with cancer compared with healthy age- and sex-matched control subjects are from Kuss et al,[66] Kim et al,[67] and Kim et al.[68]

The clinical significance of spontaneous apoptosis of $CD8^+$ effector cells in patients with cancer is currently unknown. A search for surrogate markers of prognosis or a response to therapy in patients with cancer has led to further studies of $CD8^+$ T-cell apoptosis. The level of spontaneous apoptosis discriminates between patients with cancer and healthy control subjects but not between patients with active disease versus those who are NED after oncologic therapies.[67] However, expression of CCR7, which is also a differentiation marker for T cells, by $CD8^+$ T cells was observed to protect the $CD8^+$ effector cells from apoptosis because CCR7 signaling correlated with higher Bcl-2 expression but lower Bax and Fas expression and phosphoinositide 3-kinase pathway activation in $CD8^+$ T cells.[68] The frequency of circulating $CD8^+CCR7^+$ T cells now emerges as an immune biomarker that might be predictive of survival benefits in patients with cancer. Pending validation, this immunologic biomarker that is simply defined by flow cytometry could acquire substantial clinical usefulness in the future.

Another subset of antitumor effector cells, NK cells, representing 8% to 10% of lymphocytes in the peripheral circulation, has

been credited with the ability to eliminate tumor cells in the circulation and thus prevent establishment of distant metastases.[75] Recent data suggest that in addition to mediating perforin-mediated lysis, NK cells constitutively express several ligands of the TNF family and can therefore induce apoptosis in a broad variety of tumor cell targets.[76] This mechanism of tumor cell elimination might be of greater biologic importance than secretory, granule-mediated killing, largely because most tumor cells express receptors for the TNF family ligands and are sensitive to death by apoptosis.[76] NK cells, which are able to discriminate between normal and abnormal cells based on the presence and expression levels of MHC class I molecules, are considered to play a major role in early stages of tumor development. They express receptors that enable them to survey the target for the respective ligands. These receptors are of 2 types: killer inhibitory receptors, killer activating receptors, or both.[57] NK cell functions and their interactions with other cells or extracellular matrix molecules are regulated through these receptors and Fcγ receptors.[57] In the peripheral circulation of patients with cancer, NK cells, like $CD8^+$ T cells, can also be dysfunctional. On a per-cell basis, these NK cells mediate lower levels of cytotoxicity.[77] Furthermore, some studies suggest that NK cells are also sensitive to apoptosis.[78] Among circulating NK cells in patients with breast cancer, a subset of $CD56^{bright}CD16^{dim}$ NK cells, which represents about 95% of all NK cells and is responsible for effector functions, preferentially binds Anx and thus is primed for apoptosis.[79] These patients also had significantly lower NK activity than the age- and sex-matched healthy control subjects tested in parallel. These and other data suggest that endogenous circulating NK cells have the potential to play a role in tumor surveillance, but in the presence of the tumor, their antitumor functions are subverted, and no longer control metastasis dissemination. Once the tumor is established, it especially subverts the subsets of NK cells found at the sites of metastasis and those responsible for cytotoxic functions.

In addition to NK cells, another category of nonspecific effector cells, $CD3^+CD56^+$ NK/T cells, can potentially eliminate tumor targets. They represent a very minor subset of circulating lymphocytes in healthy subjects but have been reported to be expanded in patients with cancer, as well as tumor-bearing rodents.[80] NK/T cells are also a minor component of TILs. In the presence of IL-2, NK/T cells, like $CD3^-CD56^+$ NK cells, readily differentiate into lymphokine-activated killer cells containing numerous granzyme- and perforin-containing granules and are able to mediate tumor cell lysis.[77] Both NK and NK/T cells express receptors for IL-18 and thus are activated in the presence of this cytokine as well.

REGULATORY IMMUNE CELLS IN PATIENTS WITH CANCER

The presence in the circulation of patients with cancer of suppressor lymphocytes capable of downregulating functions of other immune cells was described many years ago.[81] Today such cells are phenotypically identified as $CD4^+CD25^{high}FOXP3^+$ T cells and referred to as Treg cells.[82] They can be isolated from PBMCs or tumor sites by means of immunoselection on magnetic beads coated with antibodies to surface antigens expressed on Treg cells, such as CD25 or CD39. In mice depletion of $CD4^+CD25^+$ T cells results in the development of autoimmunity, and in tumor-bearing animals it promotes immune responses to autologous tumor. In patients with cancer, tumor-associated lymphocytes are enriched in $CD3^+CD4^+CD25^{high}$ T cells.[83] On sorting by flow, these T cells have been shown to secrete TGF-β or IL-10 and to enzymatically cleave ATP to adenosine.[45,46] The mechanisms through which these T cells regulate antitumor immune responses are being intensively investigated, and because Treg cells come in different flavors (eg, natural Treg cells, inducible T_R1 cells, $CD39^+$ Treg cells, or cytotoxic T lymphocyte–associated antigen–positive Treg cells), these mechanisms vary, likely depending on the microenvironmental context. Similarly, the microenvironment influences the induction of Treg cells; for example, T_R1 cells are preferentially induced at the tumor site, which is rich in IL-10, TGF-β, and PGE_2, all of which have been shown to promote T_R1 cell generation.[43,44] The prognostic significance of Treg cells in patients with cancer has been controversial, with many reports linking their accumulations to poor prognosis, presumably as a result of suppressed antitumor immunity,[84] and others reporting better survival in the presence of increased Treg cell frequencies,[85] possibly because of their ability to suppress tumor-promoting mechanisms or induce tumor cell death. The controversy arises because in human subjects no definite identity marker for Treg cells exists, and their functional repertoire is broad and varied. Nevertheless, their responsibility for the contraction of immune responses is critical for health.

Another subset of $CD4^+$ T cells with an origin shared with Treg cells has recently been identified. Like Treg cells, $CD4^+$ T_H17-producing T cells originate from uncommitted $CD4^+$ T-cell precursors, and the participation of TGF-β in their differentiation links them to Treg cells.[86] However, T_H17 cells produce IL-17, IL-21, and IL-IL-22, promoting tissue inflammation, and require the presence of IL-6, as well as the transcription factors signal transducer and activator of transcription 3 (STAT3), RORγ, and RORα, for differentiation.[87] Although the presence of T_H17 cells has been documented in several human carcinomas,[86] their function in tumors remains controversial. Recent reports show that $CD4^+FOXP3^+CCR6^+$ Treg cells can produce IL-17 on activation and can inhibit proliferation of $CD4^+$ responder T cells,[87] confirming a relationship between Treg and T_H17 cells that can be modulated by cytokines in the tumor microenvironment. It also emphasizes the plasticity of T-suppressor and T-effector subsets of $CD4^+$ lymphocytes.

The second major subset of regulatory cells in cancer are MDSCs ($CD34^+CD33^+CD13^+CD11b^+CD15^-$).[50] Tumors recruit MDSCs from the bone marrow through tumor-derived soluble factors, such as GM-CSF, TGF-β, IL-10, and VEGF.[5] Immature myeloid cells migrate to lymph nodes, where DCs cross-prime T cells and interfere with this process, thus suppressing CTL generation. They also migrate to the tumor site and become MDSCs able to produce arginase I and promote iNOS activation.[5,51] MDSCs also produce high levels of ROS and indoleamine-2,3-dioxygenase, an enzyme involved in the catabolism of tryptophan, an essential amino acid for T-cell proliferation and differentiation.[88] In tumor-bearing mice MDSCs accumulate in the spleen, reaching a very high frequency and exerting potent immune suppression, thereby favoring tumor growth. GM-CSF, often used as an immune adjuvant,[89] is also a product of tumor cells, which recruits MDSCs from the bone marrow and is responsible for their accumulation in patients with cancer.[90] In patients with cancer, normal physiologic functions of GM-CSF and MDSCs are subverted by the tumor to promote its development.

The tumor uses a variety of mechanisms and produces various factors and enzymes that enable it to suppress the host antitumor

immune responses. Some of these factors are listed in Table IV. Among these factors, 2 have recently been in the limelight. B7-H1 is an immunoglobulin-like immunosuppressive molecule broadly expressed in tumor cells, which signals to its counterreceptor, programmed death 1 (PD-1), on T cells.[91] Signaling delivered to T cells through B7-H1 (programmed death ligand 1 [PD-L1]) inhibits their proliferation, cytokine production, and effector functions.[92] Also, triggering by the PD-L1$^+$ tumors of PD-1 on T cells increases tumor cell resistance to immune and drug-induced death,[91] demonstrating that cancer cells can use receptors on immune cells as signals to induce resistance to therapy. Blockade of PD-L1/PD-1 interactions promotes generation of TA-specific T cells and attenuates their inhibition by Treg cells.[93] Therefore PD-1 antagonists, which are expected to augment TA-specific immune responses, might be useful in therapy of cancer.[94] Levels of the cytokine IL-17 have been shown to be increased in the tumor microenvironment.[95] Adoptive transfer studies and examination of the tumor microenvironment suggest that CD4$^+$ T cells accumulating in the tumor are the main source of IL-17 and that the enhancement of tumor growth by IL-17 is mediated by its binding to IL-17 receptors expressed on tumor cells, initiating IL-6 production, which in turn activates oncogenic STAT3, upregulating prosurvival and proangiogenic genes.[95] Thus T_H17 seems to promote tumor growth, in part through activation of an IL-6/STAT3 pathway in tumor cells. These data are contradictory to the recently reported improved survival of those patients with ovarian cancer whose tumors contained large numbers of T_H17^+ TILs.[47] This discrepancy illustrates the difficulty of dissecting the role of T_H17 in human cancer and of interpreting environmental interactions occurring in different tumor types.

NEW INSIGHTS INTO ANTITUMOR IMMUNITY

The field of tumor immunity has long suffered from a misconception that cancer cells are ignored by the immune system and that tumors are passive targets for antitumor responses. It is now certain that growing tumors attract components of both innate and adaptive host immunity.[96] Although most TAs are self-antigens that are overexpressed or altered posttranscriptionally, immune responses to TAs, including those listed in Table I, are clearly made. A growing tumor releases TAs and produces numerous cytokines/chemokines, which attract immune cells, including DCs, to the tumor site and tumor-draining lymph nodes. These DCs take up TAs, maturing into IL-12–secreting cells, and process the TAs by using the APM components for the presentation to T cells as peptide–MHC class I–β2 m complexes. These T cells develop into T_H1-type CD8$^+$ CTLs (Fig 1). DCs can also take up and process another set of TAs through the MHC class II pathway, generating T_H1-type CD4$^+$ T_H cells that produce IFN-γ and IL-2. These cells help to expand the population of TA peptide–specific CTLs, which are capable of eliminating the tumor through cytotoxic molecules, perforin, and granzymes. T_H1-type help is essential for the generation of effective CTL responses. However, DCs taking up the same MHC class II–restricted TAs can also promote the development of Treg cells (Fig 1). Mechanisms involved in DC-mediated expansion of Treg cells, as opposed to T_H1 (effector) cells or T_H17 cells, are currently not understood, yet Treg cell accumulations at the tumor site and suppression by Treg cell of antitumor specific immunity appear to have adverse effects on the host's ability to eliminate

TABLE IV. Molecularly defined immunoinhibitory factors produced by human tumors*

TNF family ligands	Induce apoptosis through the TNF family receptors
FasL	Fas
TRAIL	TRAIL-R
TNF	TNF-R1
B7-H1 (PD1L)	Binds PD1 and inhibits lymphocyte and DC functions
Cytokines	
TGF-β	Inhibits lymphocyte proliferation and perforin and granzyme mRNA expression; promotes Treg cell expansion
IL-10	Inhibits cytokine production, including that of IL-12; promotes Treg cell expansion
GM-CSF	Promotes expansion of immunosuppressive tumor-associated macrophages; recruits MDSCs
IL-17	Largely produced by CD4$^+$ T cells in the tumor; binds to IL-17 receptor on tumor cells, initiating the IL-6/STAT3 cascade
Enzymes	
Indoleamine-2,3-dioxygenase (IDO)	Inhibits T-cell activation
Arginase I	Metabolizes L-arginine, another amino acid for essential T cell proliferation
iNOS	Produces immunosuppressive nitric oxide
COX2	Produces immunosuppressive PGE_2
Small molecules	
PGE_2	Inhibits leukocyte functions through increased cyclic AMP levels
Epinephrine	Inhibits leukocyte functions through increased cyclic AMP levels
Adenosine	Inhibits leukocyte functions through increased cyclic AMP levels
ROS	Inhibits leukocyte functions through superoxide generation
Viral-related products	
p15E (CKS-17, synthetic peptide)	Inhibits production of type I cytokines, upregulates IL-10 synthesis
EBI-3 (homologue of IL-12 p40)	Inhibits IL-12 production
Tumor-associated gangliosides	Inhibit IL-2–dependent lymphocyte proliferation, induce apoptotic signals, suppress nuclear factor κB activation, interfere with DC generation

FasL, Fas ligand; *TRAIL*, tumor necrosis factor-related apoptosis-inducing ligand.
*This partial listing of tumor-derived immunoinhibitory factors demonstrates the diversity of mechanisms that human tumors are known to have evolved to incapacitate the host immune system.

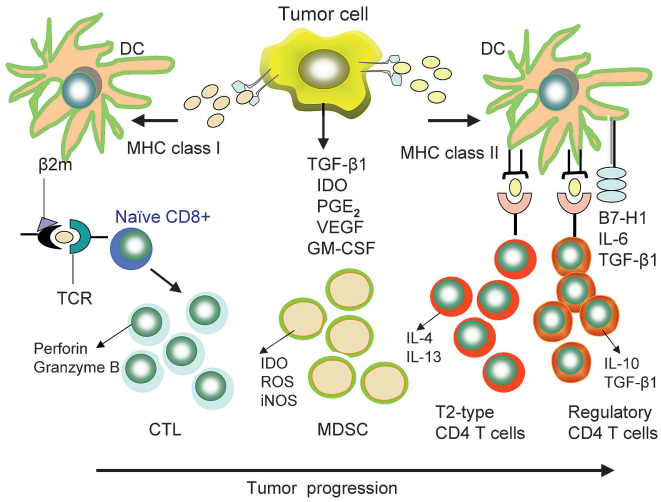

FIG 1. Effects of the tumor on immune cells. In the tumor microenvironment an excess of immunoinhibitory factors favors the generation and expansion of T_H2-type T cells and Treg cells ather than CTLs and T_H1-type effector cells. The downregulation of MHC molecules and defects in the APM components in DCs, as well as the immunosuppressive effects of accumulating MDSCs on DC maturation and function, contributes to the polarization of immune responses toward tolerance and away from immunity. The balance between stimulatory and suppressive responses shifts in favor of suppression as the tumor grows. Immune therapies are expected to shift this balance back to T_H1-type responses, which promote expansion of $CD4^+$ T_H1 cells producing IFN-γ and IL-2, as well as $CD8^+$ CTLs. *IDO*, Indoleamine 2,3-deoxygenase.

TABLE V. Potential strategies for the design of more effective antitumor therapies

Induce and sustain activity and survival of CTLs and of nonspecific antitumor effector cells: passive or active immunotherapy with antibodies, immune cells, or antitumor vaccines
Prevent immune suppression
Inhibit production or activity of tumor-derived suppressive factors
Inhibit generation or functions of Treg cells and MDSCs
Alter tumor microenvironment
Optimize lymphocyte/DC functions in the tumor microenvironment to enhance T_H1-type responses
Combine therapeutic antitumor vaccines with chemotherapy
Treat early disease or in an adjuvant setting

cancer and might influence prognosis.[84] In contrast, accumulations of $CD4^+$ T_H17^+ cells seem to predict a better survival in some cancers but in others correlate with tumor progression.[47] In patients with cancer, cellular interactions between TA-presenting DCs and T cells preferentially lead to expansion and accumulation Treg cells and MDSCs at the tumor site and in the periphery.[52] It appears that tumors have the capability to enhance the maturation of a distinct type of DC that does not promote the generation of TA-specific T_H1 cells but instead is programmed to induce Treg cells and to recruit MDSCs (Fig 1). The proinflammatory cytokines IL-6 and TNF-α produced by these DCs in combination with tumor-derived soluble immunoinhibitory factors appear to be important for shifting the balance of immune response from immunogenic to tolerogenic.

Thus signals delivered to T cells by DCs in the tumor microenvironment determine whether these T cells will develop into Treg or T_H1 cells. These signals might be influenced by (1) the dose and type of TA processed by DCs, (2) the DC maturation status because immature DCs are known to induce tolerance rather than immunity, (3) the expression of costimulatory molecules on DCs, and (4) the effects of cytokines produced by interacting DCs and T cells on the induction of Treg versus T_H1 cells.

At the time human tumors are diagnosed, the balance between immunogenic and tolerogenic signals delivered to immune cells is strongly skewed toward tolerance, mainly because of tumor-induced suppression. Therefore immune therapies administered in the minimal residual disease setting and designed to augment antitumor T_H1-type $CD4^+$ T cells and CTLs are expected to tip the balance in favor of immunostimulation and away from immunosuppression. For this reason, therapeutic antitumor vaccination strategies are considered a promising addition to conventional therapies for cancer. However, complexities of the tumor-induced immune suppression, which engages numerous molecular mechanisms, present a formidable challenge to antitumor therapies, including vaccines. Novel approaches targeting these mechanisms of immune suppression (Table V) are needed to improve the treatment of cancer.

CONCLUSIONS

The existing evidence for dysfunction and death of antitumor effector cells in tumor-bearing hosts introduces a new paradigm for immunotherapy of cancer. Although previous emphasis has been on activation of immune cells and upregulation of their antitumor functions, the current concept is to consider therapies that could block or reverse tumor escape, at the same time protecting immune cells from the influence of immunosuppressive factors present in the tumor microenvironment. These novel therapeutic strategies take advantage of the tremendous progress made recently in our basic understanding of interactions between the tumor and the host immune system. Current insights into these interactions suggest that combinations of conventional cancer therapies with newly designed DC-based vaccines and survival cytokines (eg, IL-2, IL-7, and IL-15) offer therapeutic benefits. Some of the other promising strategies under consideration for improvements in the effects of immune therapies are listed in Table V. It is expected that as molecular mechanisms used by tumors to avoid, bypass, or subvert the immune system of the host are becoming clear, novel and more effective antitumor therapies targeting these mechanisms will emerge in the near future.

REFERENCES

1. Romero P, Cerottini JC, Speiser DE. The human T cell response to melanoma antigens. Adv Immunol 2006;92:187-224.
2. Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. Nat Rev Immunol 2006;6:836-48.
3. Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, et al. Gene expression profiles in normal and cancer cells. Science 1997;276:1268-72.
4. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991-8.
5. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene 2008;27:5904-12.
6. Kornstein MJ, Brooks JS, Elder DE. Immunoperoxidase localization of lymphocyte subsets in the host responses to melanoma and nevi. Cancer Res 1983;43:2749-53.
7. Von Kleist S, Berling J, Bohle W, Wittekind C. Immunohistochemical analysis of lymphocyte subpopulations infiltrating breast carcinomas and benign lesions. Int J Cancer 1987;40:18-23.
8. Whiteside TL. Tumor infiltrating lymphocytes in human malignancies. Austin (TX): R.G. Landes Co 1993.
9. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960-4.

10. Galon J, Fridman W-H, Pages F. The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer Res 2007;67:1883-6.
11. Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, et al. Effector memory T cells, early metastasis and survival in colorectal cancer. N Engl J Med 2005;353:2654-66.
12. Camus M, Tosolini M, Mlecnik B, Pages F, Kirilovsky A, Berger A, et al. Coordination of intratumoral immune reaction and human colorectal cancer recurrence. Cancer Res 2009;69:2685-93.
13. Lee PP, Yee C, Savage PA, Fong L, Brockstedt D, Weber JS, et al. Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. Nat Med 1999;5:677-85.
14. Pittet MJ, Speiser DE, Lienard D, Valmore D, Guillaume P, Dutoit V, et al. Expansion and functional maturation of human tumor antigen-specific CD8 + T cells after vaccination with antigenic peptide. Clin Cancer Res 2001;7: 796s-803.
15. Hoffmann TK, Donnenberg AD, Finkelstein SD, Donnenberg VS, Friebe-Hoffmann F, Myers EN, et al. Frequencies of tetramer+ T cells specific for the wild-type sequence $p53_{264-272}$ peptide in the circulations of patients with head and neck cancer. Cancer Res 2002;62:3521-9.
16. Sahin U, Tureci O, Pfreundschuh M. Serologic identification of human tumor antigens. Curr Opin Immunol 1997;9:709-16.
17. Matzinger P. An innate sense of danger. Semin Immunol 1998;10:399-415.
18. Szczepanski MJ, Czystowska M, Szajnik M, Harasymczuk M, Boyiadzis M, Kruk-Zagajewska A, et al. Triggering of toll-like receptor 4 expressed on human head and neck squamous cell carcinoma promotes tumor development and protects the tumor from immune attack. Cancer Res 2009;69:3105-13.
19. Uematsu S, Akira S. Toll-like receptors and innate immunity. J Mol Med 2006;84: 712-25.
20. Hollstein M, Sidransky D, Vogelstein B, Harris C. p53 mutations in human cancers. Science 1991;253:49-53.
21. Provinciali M. Immunosenescence and cancer vaccines. Cancer Immunol Immunother 2009;58:1959-67.
22. Ferrone S, Whiteside TL. Tumor microenvironment and immune escape. Surg Oncol Clin North Am 2007;16:755-74.
23. Ferris RL, Whiteside TL, Ferrone S. Immune escape associated with functional defects in antigen processing machinery in head and neck cancer. Clin Cancer Res 2006;12:3890-5.
24. Stickel JS, Weinzieri AO, Hillen N, Drews O, Schuler MM, Hennenlotter J, et al. HLA ligand profile of primary renal cell carcinoma maintained in metastases. Cancer Immunol Immunother 2009;58:1407-17.
25. Fearon DT, Locksley RM. The instructive role of innate immunity in the acquired immune response. Science 1996;272:50-4.
26. Irving BA, Weiss A. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. Cell 1991;64:891-901.
27. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol 2005;23:515-48.
28. Kalinski P, Hilkens CM, Wierenga EA, Kapsenberg ML. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. Immunol Today 1999;20:561-7.
29. Whiteside TL. Immune suppression in cancer: effects on immune cells, mechanisms and future therapeutic interventions. Semin Cancer Biol 2006;16:3-15.
30. Reichert TE, Strauss L, Wagner EM, Gooding W, Whiteside TL. Signaling abnormalities and reduced proliferation of circulating and tumor-infiltrating lymphocytes in patients with oral carcinoma. Clin Cancer Res 2002;8:3137-45.
31. Uzzo RG, Clark PE, Rayman P, Bloom T, Rybicki L, Novick AC, et al. Alterations in NFκB activation in T lymphocytes of patients with renal cell carcinoma. J Natl Cancer Inst 1999;91:718-21.
32. Reichert TE, Rabinowich H, Johnson JT, Whiteside TL. Human immune cells in the tumor microenvironment: mechanisms responsible for signaling and functional defects. J Immunother 1998;21:295-306.
33. Cavallo F, Offringa R, van der Burg SH, Forni G, Melief CJ. Vaccination for treatment and prevention of cancer in animal models. Adv Immunol 2006;90:175-213.
34. Lollini PL, De Giovanni C, Pannellini T, Cavallo F, Forni G, Nanni P. Cancer immunoprevention. Future Oncol 2005;1:57-66.
35. Pannellini T, Forni G, Musiani P. Immunobiology of her-2/neu transgenic mice. Breast Dis 2004;20:33-42.
36. Parmiani G, De Filippo A, Novellino L, Castelli C. Unique human tumor antigens: immunobiology and use in clinical trials. J Immunol 2007;178:1975-9.
37. Finn OJ, Binder RJ, Brickner AG, Butterfield LH, Ferris RL, Kalinski P, et al. Human tumor antigens as targets of immunosurveillance and candidates for cancer vaccines. In: Gires O, Seliger B, editors. Tumor-associated antigens: identification, characterization, and clinical applications. Weinheim (Germany): Wiley-VCH; 2009. p. 23-43.
38. Cheever M, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a National Cancer Institute pilot project for the acceleration of translational research. Clin Cancer Res 2009;15:5323-37.
39. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol 2006;90:1-50.
40. Jager E, Karbach J, Gnjatic S, Neumann A, Bender A, Valmori D, et al. Recombinant vaccinia/fowlpox NY-ESO-1 vaccines induce both humoral and cellular NY-ESO-1-specific immune responses in cancer patients. Proc Natl Acad Sci U S A 2006;103:14453-8.
41. Whiteside TL. The local tumor microenvironment. In: Kaufmann H, Wolchok JD, editors. General principles of tumor immunotherapy: basic and clinical applications of tumor immunology. New York: Springer; 2007. p. 145-67.
42. June CH. Adoptive T cell therapy for cancer in the clinic. J Clin Invest 2007;117: 1466-76.
43. Bergmann C, Strauss L, Zeidler R, Lang S, Whiteside TL. Expansion of human T regulatory type 1 cells in the microenvironment of COX-2-overexpressing head and neck squamous cell carcinoma. Cancer Res 2007;67:8865-73.
44. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, et al. Regulatory CD4 + CD25 + T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. Cancer Res 2001;61:4766-72.
45. Strauss L, Bergmann C, Szczepanski M, Gooding W, Johnson TJ, Whiteside TL. A unique subset of CD4 + $CD25^{high}$FOXP3 + T cells secreting IL-10 and TGF-β1 mediates suppression in the tumor microenvironment. Clin Cancer Res 2007;13:4345-54.
46. Mandapathil M, Szczepanski MJ, Szajnik M, Ren J, Lenzner DE, Jackson EK, et al. Increased ectonucleotidase expression and activity in Treg of patients with head and neck cancer. Clin Cancer Res 2009;15:6348-57.
47. Kryczek I, Banerjee M, Cheng P, Vatan L, Szeliga W, Wei S, et al. Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor microenvironment. Blood 2009;114:1141-9.
48. Martinez O, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Front Biosci 2008;13:453-61.
49. Leek RD, Lewis CE, Whitehouse R, Geenall M, Clarke J, Harris AL. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res 1996;56:4625-9.
50. Serafini P, Borello I, Bronte V. Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. Semin Cancer Biol 2006;16:53-65.
51. Ochoa AC, Zea AH, Hernandez C, Rodríguez PC. Arginase, prostaglandins, and myeloid supressor cells in renal cell carcinoma. Clin Cancer Res 2007;13:721s-6s.
52. Diebold SS. Determination of T-cell fate by dendritic cells. Immunol Cell Biol 2008;86:389-97.
53. Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med 1996;2:1096-103.
54. Shurin GV, Shurin MR, Bykovskaja S, Shogan J, Lotze MT, Barksdale EM. Neuroblastoma-derived gangliosides inhibit dendritic cell generation and function. Cancer Res 2001;61:363-9.
55. Reichert TE, Scheuer C, Day R, Wagner W, Whiteside TL. The number of intratumoral dendritic cells and ζ-chain expression in T cells as prognostic and survival biomarkers in patients with oral carcinoma. Cancer 2001;91:2136-47.
56. Murphy GF, Radu A, Kaminer M, Berg D. Autologous melanoma vaccine induces inflammatory responses in melanoma metastases: relevance to immunologic regression and immunotherapy. J Invest Dermatol 1993;100(suppl):335S-41.
57. Lanier LL. Natural killer cell receptor signaling. Curr Opin Immunol 2003;15: 308-14.
58. Chang CC, Campoli M, Ferrone S. Classical and nonclassical HLA class I antigen and NK cell-activating ligand changes in malignant cells: current challenges and future direction. Adv Cancer Res 2005;93:189-234.
59. Kelly JM, Darcy PK, Markby JL, Godfrey DI, Takeda K, Yagitab H, et al. Induction of tumor-specific T cell memory by NK cell-mediated tumor rejection. Nat Immunol 2002;3:83-90.
60. Coronella JA, Spier C, Welch M, Trevor KT, Stopeck AT, Villar H, et al. Antigen-driven oligoclonal expansion of tumor-infiltrating B cells in infiltrating ductal carcinoma of the breast. J Immunol 2002;169:1829-36.
61. Denko NC, Fontana LA, Hudson KM, Sutphin PD, Raychaudhuri S, Altman R, et al. Investigating hypoxic tumor physiology through gene expression patterns. Oncogene 2003;22:5907-14.
62. Aller MA, Arias JL, Nava MP, Arias J. Posttraumatic inflammation is a complex response based on the pathological expression of the nervous, immune and endocrine function systems. Exp Biol Med 2004;229:170-81.
63. Dworacki G, Meidenbauer N, Kuss I, Hoffmann TK, Gooding MS, Lotze M, et al. Decrease ζ chain expression and apoptosis in CD3+ peripheral blood T lymphocytes of patients with melanoma. Clin Cancer Res 2001;7(suppl):947s-57.

64. Finke JH, Rayman P, George R, Tannenbaum CS, Kolenko V, Uzzo R, et al. Tumor-induced sensitivity to apoptosis in T cells from patients with renal cell carcinoma: role of nuclear factor kappa B suppression. Clin Cancer Res 2001;7(suppl):940s-6.
65. Whiteside TL. Tumor-induced death of immune cells: its mechanisms and consequences. Semin Cancer Biol 2001;12:43-50.
66. Kuss I, Schaefer C, Godfrey TE, Ferris RL, Harris J, Gooding W, et al. Recent thymic emigrants and subsets of naïve and memory T cells in the circulation of patients with head and neck cancer. Clin Immunol 2005;116:27-36.
67. Kim W-J, Tsukishiro T, Johnson JT, Whiteside TL. Expression of pro- and anti-apoptotic proteins in circulating CD8 + T cells of patients with squamous cell carcinoma of the head and neck (SCCHN). Clin Cancer Res 2004;10:5101-10.
68. Kim J-W, Ferris RL, Whiteside TL. Chemokine receptor 7 (CCR7) expression and protection of circulating CD8 + T lymphocytes from apoptosis. Clin Cancer Res 2005;11:7901-10.
69. Kuss I, Saito T, Johnson JT, Whiteside TL. Clinical significance of decreased ζ chain expression in peripheral blood lymphocytes of patients with head and neck cancer. Clin Cancer Res 1999;5:329-34.
70. Hoffmann TK, Dworacki G, Meidenbauer N, Gooding W, Johnson JT, Whiteside TL. Spontaneous apoptosis of circulating T lymphocytes in patients with head and neck cancer and its clinical importance. Clin Cancer Res 2002;8:2553-62.
71. Whiteside TL. The role of death receptor ligands in shaping tumor microenvironment. Immunol Invest 2007;36:25-46.
72. O'Connell J, O'Sullivan GD, Collins JK, Shanahan F. The Fas counterattack: Fas-mediated T cell killing by colon cancer cells expressing Fas ligand. J Exp Med 1996;184:1075-82.
73. Whiteside TL. Lymphocyte homeostasis and the antitumor immune response. Exp Rev Clin Immunol 2006;1:369-78.
74. Albers AE, Visus C, Tsukishiro T, Ferris RL, Gooding W, Whiteside TL, et al. Alterations in the T-cell receptor variable β gene-restricted profile of CD8 + T lymphocytes in the peripheral circulation of patients with squamous cell carcinoma of the head and neck. Clin Cancer Res 2006;12:2394-403.
75. Whiteside TL. Immune effector cells in the tumor microenvironment: their role in regulation of tumor progression. In: Yefenof E, editor. Innate and adaptive responses in the tumor microenvironment. New York: Springer; 2008. p. 1-34.
76. Vujanovic NL, Nagashima S, Herberman RB, Whiteside TL. Non-secretory apoptotic killing by human natural killer cells. J Immunol 1996;157:1117-26.
77. Whiteside TL, Vujanovic NL, Herberman RB. Natural killer cells and tumor therapy. Curr. Topics Microbiol Immunol 1998;230:221-44.
78. Hansson M, Asea A, Ericsson U, Hermodsson S, Hellstrand K. Induction of apoptosis in NK cells by monocyte-derived reactive oxygen metabolites. J Immunol 1996;156:42-7.
79. Whiteside TL. Apoptosis of immune cells in the tumor microenvironment and peripheral circulation of patients with cancer: implications for immunotherapy. Vaccine 2002;20:A46-51.
80. Smyth MF, Godfrey DI. NKT cells and tumor immunity—a double-edged sword. Nat Immunol 2000;1:459-60.
81. Gershon RK. A disquisition on suppressor T cells. Transpl Rev 1975;26:170-85.
82. Bluestone JA, Abbas AK. Natural versus adaptive regulatory T cells. Nat Rev 2003;3:253-7.
83. Bergmann C, Strauss L, Wang Y, Szczepanski MJ, Lang S, Johnson JT, et al. T regulatory type 1 cells (Tr1) in squamous cell carcinoma of the head and neck: mechanisms of suppression and expansion in advanced disease. Clin Cancer Res 2008;14:3706-15.
84. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma foster immune privilege and predicts reduced survival. Nat Med 2004;10:942-9.
85. Salama P, Phillips M, Grieu P, Morris M, Zeps N, Joseph D, et al. Tumor-infiltrating FOXP3 + T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol 2008;27:186-92.
86. Martin-Orozco N, Dong C. The IL-17/IL23 axis of inflammation in cancer: friend or foe? Curr Opin Investig Drugs 2009;10:543-9.
87. Voo KS, Wang Y-H, Santori FR, Boggiano C, Yi-H Wang, Arima K, et al. Identification of IL-17-producing FOXP+ regulatory T cells in humans. Proc Natl Acad Sci U S A 2009;106:4793-8.
88. Munn DH, Mellor AL. Indoleamine 2, 3-dioxygenase and tumor-induced tolerance. J Clin Invest 2007;117:1147-54.
89. Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting antitumor immunity. Proc Natl Acad Sci U S A 1993;90:3539-43.
90. Filipazzi F, Valenti R, Huber V, Pilla L, Canese P, Iero M, et al. Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. J Clin Oncol 2007;25:2546-53.
91. Azuma T, Yao S, Zhu G, Flies SJ, Chen L. B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. Blood 2008;111:3635-43.
92. Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. Cancer Immunol Immunother 2007;56:739-45.
93. Wang W, Lau R, Yu D, Zhu W, Korman A, Weber J. PD1 blockade reverses the suppression on melanoma antigen-specific CTL by CD4 + CD25hi regulatory T cells. Int Immunol 2009;21:1065-77.
94. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res 2005;65:1089-96.
95. Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H. IL-17 can promote tumor growth through an IL-6-Stat3 signalling pathway. J Exp Med 2009;206:1457-64.
96. Finn OJ. Cancer immunology. N Engl J Med 2008;358:2704-15.

Clinical laboratory assessment of immediate-type hypersensitivity

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Clinical laboratory analyses aid in the diagnosis and management of human allergic (IgE-dependent) diseases. Diagnosis of immediate-type hypersensitivity begins with a thorough clinical history and physical examination. Once symptoms compatible with an allergic disorder have been identified, a skin test, blood test, or both for allergen-specific IgE antibodies provide confirmation of sensitization, which strengthens the diagnosis. Skin testing provides a biologically relevant immediate-type hypersensitivity response with resultant wheal-and-flare reactions within 15 minutes of allergen application. Allergen-specific IgE antibody in serum is quantified by using 3 laboratory-based autoanalyzers (ImmunoCAP, Immulite, and HYTEC-288) and novel microarray and lateral-flow immunoassays. Technologic advances in serologic allergen-specific IgE measurements have involved increased automation, with enhanced reproducibility, greater quantification, lower analytic sensitivity, and component-supplemented extract-based allergen use. *In vivo* provocation tests involving inhalation, ingestion, or injection of allergens serve to clarify discordant history and skin- or blood-based measures of sensitization. Other diagnostic allergy laboratory analyses include total and free serum IgE measurement, precipitating IgG antibodies specific for organic dusts, mast cell tryptase, and indicator allergen analyses to assess indoor environments to promote patient-targeted allergen avoidance programs. A critique is provided on the predictive utility of serologic measures of specific IgE for food allergy and asthma. Reasons for the lack of clinical utility for food-specific IgG/IgG4 measurements in allergy diagnosis are examined. When the specific IgE measures are inconsistent with the clinical history, they should be confirmed by means of repeat and alternative method analysis. Ultimately, the patient's clinical history remains the principal arbiter that determines the final diagnosis of allergic disease. (J Allergy Clin Immunol 2010;125:S284-96.)

Key words: *Diagnosis, skin testing, RAST, IgE antibody, provocation testing*

Abbreviations used

BHR: Basophil histamine release
CI: Chronic urticaria
CLIA-88: Federal Clinical Laboratory Improvement Act of 1988
DBPCFC: Double-blind, placebo-controlled food challenge
ISAC: Immunosorbent Allergen Chip
PWV: *Polistes* species wasp venom
WHO: World Health Organization
YJV: Yellow jacket venom

The clinical laboratory plays an increasing role in the diagnosis and management of allergic disorders (immediate or type 1 hypersensitivity). The clinician begins the diagnostic process with a thorough clinical history and physical examination. Symptoms that suggest a diagnosis of asthma, allergic rhinitis and sinusitis, occupational asthma and allergy, food allergy, drug allergy, or an allergic disease of the skin are matched with suspected relevant allergen exposures. Once the clinician has a high degree of suspicion that the patient has a particular allergic disorder, *in vivo* (skin and provocation tests) and laboratory-based serologic analyses for IgE antibody are performed to strengthen the likelihood that the chosen allergy diagnosis is correct. A definitive diagnosis of allergic disease then permits a number of therapeutic interventions involving avoidance, pharmacotherapy, immunotherapy, or anti-IgE therapy to be instituted. Management of a patient with allergic disorders can also be facilitated with different laboratory analyses. This chapter examines clinical laboratory tests that aid in the diagnosis and management of patients with a disease associated with type 1 hypersensitivity.

IGE PROPERTIES

The reagin in serum that mediates the immediate-type wheal-and-flare reaction was identified as IgE in 1967.[1,2] The properties of human IgE are described in Table I. IgE (approximately 190,000 d) circulates as a monomer at a serum concentration that is highly age dependent. It constitutes approximately 0.0005% of the total serum immunoglobulins in adults.[3] Cord blood levels of IgE remain low (<2 kU/L [<4.8 μg/L]) because IgE does not cross the placental barrier in significant amounts. Mean serum IgE levels progressively increase in healthy children up to 10 to 15 years of age and then decrease from the second through eighth decades of life. By 14 years of age, total serum IgE levels of greater than 333 kU/L (800 μg/L) are considered abnormally increased and strongly associated with and suggestive of atopic disorders, such as allergic rhinitis, extrinsic asthma, and atopic dermatitis.[4,5]

Environmental antigen exposure can occur by means of inhalation, ingestion, or skin and parenteral contact. Once taken up by antigen-presenting cells, processed antigen is presented to helper T cells that secrete a number of cytokines that cause B-cell lymphocytes to proliferate and in some cases produce allergen-specific IgE antibody. IgE binds onto high-affinity Fcε receptors on the surface of a number of cells, particularly mast cells and basophils, creating a state of "sensitization" within the patient.

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Subsequent allergen exposure causes mast cell surface–bound IgE antibody to be cross-linked, leading to an increase in intracellular calcium levels and the release of both preformed mediators (eg, histamine and proteases) and newly synthesized lipid-derived mediators (eg, leukotrienes and prostaglandins). Allergic symptoms can subsequently occur as a result of mediator-induced physiologic and anatomic changes. The measurement of allergen-specific IgE antibody will thus be a principal focus of this chapter.

ALLERGENS

Several hundred allergenic proteins, (glycoproteins and lipoproteins), are extracted from well-defined (usually biological) sources, including weed, grass, and tree pollens and animal dander, molds, house dust mites, parasites, insect venoms, occupational allergens (eg, natural rubber latex), drugs, and foods.[6] These allergens elicit IgE antibody production when introduced into an immunocompetent and genetically predisposed host. Individual allergenic proteins can be identified by using IgE antibody–containing human serum in combination with a number of immunochemical assays that separate proteins based on their net charge (isoelectric focusing), size (Western blot analysis), and ability to bind antibody (competitive inhibition immunoassay). A compendium of the known clinically important allergens (together with their scientific names), purified major allergen components, and diagnostic codes is presented elsewhere.[6]

Many important allergenic proteins from dust mites, pollens, animal dander, insects (eg, cockroach and Hymenoptera venoms), molds, and foods have been cloned and sequenced, and recombinant proteins have been expressed during the past decade.[7,8] This has sparked a debate as to whether native allergens possess any unique advantages over their recombinant counterparts as diagnostic reagents and whether crude allergen mixtures should give way to the use of purified allergens in cocktails to detect IgE antibody in the skin and blood. Allergens extracted from natural sources are known to be heterogeneous, often containing many nonallergenic proteins. Moreover, different natural extracts vary in their allergen composition and potency, and they can be contaminated with allergens from other sources. Purified recombinant allergens are attractive because their availability in pure form simplifies reagent preparation and promotes reproducibility and standardization. However, allergic patients are known to respond differently to combinations of isoallergens that are essentially identical except for minor differences in their primary amino acid composition or substituted side chains. Thus a single recombinant allergen that does not represent all the isoallergen forms of that allergen might not be sufficiently "globally diagnostic" to be able to detect all clinically relevant IgE antibodies of that allergen specificity.

Extract-based reagents for both skin test and IgE antibody serology are here to stay for the foreseeable future because of their more comprehensive coverage of the allergenic repertoire of any particular specificity. However, the future use of certain purified recombinant allergens as diagnostic reagents in both *in vivo* and *in vitro* IgE antibody testing holds promise. A purified recombinant or native principal allergen of a particular specificity, such as Bet v 1 from birch pollen, can be a good indicator allergen for detecting sensitization to that specificity. However, it is not sufficiently comprehensive to replace the birch extract–based diagnostic reagent, especially for evaluation of subjects who produce IgE antibody to less predominant allergens that are present in the birch pollen extract. Additionally, the allergenic profile of any given specificity of an allergen-containing reagent, as produced by different manufacturers, is expected to vary in its protein composition, allergenic potency, and immunoreactivity, regardless of extensive cross-validation. One generic rule of allergy diagnostics has evolved from this, namely that despite clearance by regulatory agencies, such as the US Food and Drug Administration, each *in vivo* or *in vitro*-allergen extract–containing reagent should be expected to detect slightly different populations of IgE antibodies. Thus IgE antibody measurements generated with different skin test– or serum-based IgE antibody assay reagents are expected to produce reasonably clinically equivalent but not identical results.[6]

DIAGNOSTIC ALGORITHM FOR ALLERGIC DISEASE

The diagnosis of allergic disease begins with a thorough clinical history and physical examination.[9,10] The signs and symptoms associated with the various allergic disorders are extensively discussed in chapters 8-14. Once the history has been collected, one of several primary confirmatory tests for sensitization can be performed to detect allergen-specific IgE in the skin or blood. Because the history is viewed by many as the arbiter of the diagnostic test's performance, a subject with a positive clinical history for allergic disease and a positive skin or blood test result for IgE is considered to have a true-positive result (Table II). Ideally, all patients with a positive allergy history would have a positive allergen-specific IgE test result, and those with a negative history would have a negative allergen-specific IgE antibody test result. However, more realistically, some patients with allergic disease are classified as having false-negative IgE antibody test results, and others with no evident allergic disease are identified as having positive IgE antibody test results that would be considered false-positive results.

In vivo provocation tests are considered secondary-level confirmatory tests that are available when one needs to adjudicate the correctness of discordant clinical history and results from allergen-specific IgE antibody skin or serologic tests.[9-11] However, provocation tests are more difficult to perform in a reproducible manner than skin or blood tests for IgE antibodies, and they place the patient at some risk for a reaction because they involve a direct allergen challenge. Interpretation of their results can also be difficult because they often involve subjective end points that can be altered by observer and patient bias. In certain cases, such as food allergy, the *in vivo* provocation test (double-blind, placebo-controlled food challenge [DBPCFC]) has become the reference benchmark for identifying type 1 hypersensitivity to foods. The actual *in vivo* provocation test that is useful in the diagnostic workup of a patient ultimately depends on the nature of the disease process that is being investigated (eg, sting challenge for Hymenoptera venom allergy).

The presence of IgE antibodies is necessary but not sufficient for allergic disease expression. Allergen-specific IgE antibody might be detectable in the patient's skin or blood, and the patient might not have had any evident allergic symptoms after allergen exposure. Some health care workers with a positive immediate-type latex skin test result, IgE anti-natural rubber latex blood test result, or both experience no allergic symptoms when they are exposed to highly allergenic powdered latex examination gloves.[12] The relative strengths and limitations of *in vivo* and *in vitro* diagnostic tests and the principal technical reasons for false-negative and false-positive test results are discussed subsequently.

TABLE I. . Biological and chemical properties of human IgE and IgG antibodies

Property	IgE	IgG1	IgG2	IgG3	IgG4
Heavy (H) chain class	ϵ	γ1	γ2	γ3	γ4
H chain molecular weight (d)	70,000	50,000	50,000	60,000	50,000
H Chain carbohydrate %	18	3-4	3-4	3-4	3-4
H Chain no. of oligosaccharides	5	1	1	1	1
Light chain type	K and λ	K and λ	K and λ	K and λ	K and λ
Averaged immunoglobulin light chain K/L ratio		2.4	1.1	1.4	8.0
Molecular weight of secreted form (d)	190,000	150,000	150,000	160,000	150,000
H chain domain no.	5	4	4	4	4
Hinge (amino acids)	None	15	12	62	12
Interchain disulfide bonds per monomer	NA	2	4	11	2
pI range, mean (SD)	NA	8.6 (0.4)	7.4 (0.6)	8.3 (0.7)	7.2 (0.8)
Tail Piece	No	No	No	No	No
Allotypes	Em1	G1 m: a(1), x(2), f(3), z(17)	G2 m: n(23)	G3 m: b1(5), c3(6), b5(10),b0(11) b3(13),b4(14) s(15), t(16), g1(21)c5(24), u(26),v(27), g5(28)	G4 m Gm4a(i) Gm4b(i)
Distribution: % intravascular	50	45	45	45	45
Biological half-life (d)	1-5	21-24	21-24	7-8	21-24
Fractional catabolic rate (% intravascular pool catabolized per day)	71	7	7	17	7
Synthetic rate (mg/kg/d)	0.002	33	33	33	33
Total immunoglobulin in adult serum (%)	0.004	45-53	11-15	0.03-0.06	0.015-0.045
Approximate adult range (age 16-60 y) in serum g/L	0-0.0001 nonatopic subjects	5-12	2-6	0.5-1	0.2-1
Functional valency	2	2	2	2	1-2
Transplacental transfer	0	++	+	++	++
Binding to phagocytic cells		++	+	++	+
Binding to basophils and mast Cells	+++	?	?	?	?
Complement activation classical pathway	0 + alternate pathway	++	+	+++	0

NA, not available; *pI*, isoelectric point.
Modified from Tables I and II in Hamilton RG. Human immunoglobulins. In: O'Gorman MRG, Donnenberg AD, editors. Handbook of human immunology. 2nd ed. Boca Raton (FL): CRC Press; 2008.

DIAGNOSTIC SKIN TESTING

Skin testing is one of 2 primary confirmatory tests for allergen-specific IgE antibody that are used in the diagnosis of human allergic disease. An epicutaneous administration (previously referred to as a prick/puncture) or an intradermal injection can both be used to apply allergen in the form of an extract to the skin.[13]

Epicutaneous skin test

Performance of the epicutaneous skin test involves placing a drop of allergen, often in glycerinated saline, onto the surface of the skin. A variety of single-, dual-, and multiple-point standardized test devices are currently available to introduce the allergen into the epidermis.[13,14] Excess allergen is then removed with gauze or tissue paper, and any immediate reaction (wheal and erythema) is read at 15 to 20 minutes as it reaches a maximum diameter. Separate test sites need to be spaced sufficiently distant from each other to prevent overlapping of any erythema. False-positive results can occur as a result of bleeding or direct skin irritation by some extracts that might contain naturally occurring histamine. Dermographism which is a constitutional whealing tendency in which firm stroking of the skin can cause capillary and arteriolar dilatation and transudation of fluid causing edema, can also lead to a false-positive wheal and erythema and invalidation of the skin test result. False-negative results can occur as a result of prior consumption of antihistamines or other medications. A positive control comprising histamine (1.8 or 10 mg/mL) and a negative control of saline must be applied in parallel with the test allergen extracts to document validity and control for confounding problems associated with antihistamine premedication and dermographism.

Variability of epicutaneous skin test results can occur as a result of several factors.[13,14] These include the subject's biological responsiveness, the skin tester's skill, the general technique (needle and application method) used to perform the puncture, the reagents (stability, vehicle [eg, 50% glycerol], allergen concentration, and

TABLE II. Predictive value of diagnostic tests applied to populations without and with allergic disease

	Positive allergen-specific IgE test result	Negative allergen-specific IgE test result	Totals
Positive clinical history for allergic disease	True-positive allergic test result (TP)	False-negative allergen-specific IgE antibody test result (FN)	TP + FN
Negative clinical history for allergic disease	False-positive allergen-specific IgE antibody test result (FP)	True-negative test result in nonallergic subject (TN)	FP + TN
Totals	TP + FP	FN + TN	TP + FP + TN + FN

FN, Number of patients with allergic disease misclassified by a negative IgE antibody test result; *FP*, number of patients with no allergic disease misclassified by a positive IgE antibody test result; *TN*, number of patients with no allergic disease correctly identified with a negative IgE antibody test result; *TP*, number of patients with allergic disease correctly identified by a positive IgE antibody test result.
Diagnostic sensitivity of an IgE antibody test: Percentage positivity of an IgE antibody test result in patients with allergic disease = TP/[TP + FN] × 100.
Diagnostic specificity of an IgE antibody test: Percentage negativity of an IgE antibody test result in patients with no allergic disease = TN/[TN + FP] × 100.
Positive predictive value of an IgE antibody test: Percentage of patients with a positive IgE antibody test result who have allergic disease = TP/[TP + FP] × 100.
Negative predictive value of an IgE antibody test: Percentage of patients with a negative IgE antibody test result who have no allergic disease = TN/[TN + FN] × 100.
Efficiency of an IgE antibody test: Percentage of patients correctly classified as having allergic disease or not having allergic disease = [TP + TN]/[TP + FP + FN + TN] × 100.
Modified from Table 2C-3 in Galen RS, Peters T Jr. Analytic goals and clinical relevance of laboratory procedures. In: Tietz NW, editor. Textbook of clinical chemistry. Philadelphia: WB Saunders Co; 1986. p, 395-7.

purity), and the method used to delimit, measure, and report skin reactions. In one study dust mite contamination of dog dander extracts was shown to be the cause of false-positive epicutaneous skin test results in patients sensitized only to house dust mites.[15] The inoculum volume is another variable that can contribute to epicutaneous skin test variation. To examine this, Antico et al[16] performed 16 skin puncture tests (8 in each forearm) on 15 healthy volunteers (9 men and 6 women; age, 64 ± 4 years) using 50% glycerol-saline containing radioactive Tc_{99m} and a 1-mm acrylic copolymer, pyramidal-tipped, Morrow Brown needle (Alkaline Corp, Oakhurst, NJ). They measured a mean 16 nL of inoculum volume delivered to the skin (range, 0.42-82.25 nL) using a gamma camera. This high variability was shown to depend primarily on the characteristics of the subjects' skin and the reagents, whereas the skin tester's skill and technique were considered less significant sources of variability. The study concluded that variation in epicutaneous skin test results can only be reduced to certain limits by the standardization of the skin-testing technique and reagents.

Intradermal skin test

Intracutaneous (intradermal) administration of allergen (0.01-0.05 mL) can be accomplished by using a tuberculin syringe with a 26- to 27-gauge needle. A 2- to 3-mm-diameter bleb can be produced by injecting 0.02 mL. The skin test is then read at 15 to 20 minutes, when the wheal and erythema are considered maximal. A number of scoring schemes that have been used for skin testing are presented in Tables III through V. Subcutaneous injection of allergen can lead to a false-negative intradermal skin test result, whereas a minor change in the extract volume only minimally alters the wheal-and-flare diameters. The allergen concentration and the presence of naturally occurring histamine contamination of undialyzed extracts can markedly influence final intradermal skin test results. Rather than a single-dose injection, a skin test titration can be performed that involves the administration of the same volume (eg, 0.02 mL) of 3- or 10-fold serial dilutions of an extract into different sites in the skin. The purpose of skin test titration is to identify the concentration of an extract that produces a defined wheal or erythema diameter (eg, 8-mm wheal). The greater the patient's sensitivity to the allergen extract, the lower the concentration of allergen that is required to induce the predefined wheal or erythema diameter. The intradermal skin test requires approximately 1,000-fold lower concentrations of antigen than the epicutaneous skin test to produce a same-sized skin reaction.[17] Intradermal testing, when done as in clinical practice with an extract concentration of 1:500 or 1:1,000 versus 1:20 wt/vol for epicutaneous skin testing, is a "bigger dose" by approximately 100- to 1,000-fold because of the differential volumes and concentrations.

Adhesive cellulose tape can be applied over the wheal and erythema that has been previously outlined with a ballpoint pen to obtain a permanent record of the skin reaction. The maximal diameter and midpoint perpendicular diameter of the wheal and erythema are averaged. Alternatively, a midpoint diameter might be interpolated from the skin test titration reference curve in which the allergen dose is plotted against the wheal or erythema diameter. Erythema size is sometimes preferred over wheal size because the slope of the flare's regression line is reportedly steeper.[18] A strong relationship exists between the size of the intradermal erythema and the wheal, which is useful when evaluating reactions in dark skin, on which the erythema can be difficult to assess.

DIAGNOSTIC IMMUNOLOGY LABORATORY TESTS

Although the presence of allergen-specific IgE antibody is necessary but not sufficient for clinically manifested allergic disease, it has become the primary clinical laboratory measurement used in the diagnosis of human allergic disease. Most clinical laboratories offer a number of additional serologic tests that can be useful in selected circumstances for the diagnosis or management of patients with type 1 hypersensitivity. These measurements include total serum IgE, the Hymenoptera venom-specific IgE inhibition test, Hymenoptera venom-specific IgG, mast cell tryptase, eosinophil cationic protein, and precipitins for assessing hypersensitivity pneumonitis. Basophil histamine release (BHR), although rarely offered as a clinical test because of the requirement for fresh blood, can be a useful investigational or research tool to clarify discordant diagnostic test results, and thus it will also be examined in this section.

Allergen-specific IgE antibody

Table VI summarizes the analytes that are most commonly analyzed in the diagnostic immunology laboratory during the workup of an allergic patient. Of these, allergen-specific IgE antibody is the most important analyte in the diagnosis of type 1 hypersensitivity reactions. The Phadebas RAST (Pharmacia Diagnostics [currently Phadia], Uppsala, Sweden) was the first

TABLE III. Grading system for epicutaneous skin testing with histamine as a reference*

Grade or class	Wheal size
0	No discernible wheal
1+	<½ Histamine diameter
2+	≥½ Histamine and <histamine diameter
3+	= size of histamine wheal ± 1 mm
4+	>Histamine diameter and <2 × diameter
5+	>2 × Histamine control

*Prick/puncture histamine (1.8-10 mg/mL); intradermal histamine (100 μg/mL).

TABLE IV. Grading system for skin testing with wheal and erythema as criteria

Grade or class	Wheal and erythema size
0	No reaction or reach no different than negative control
1+	Erythema <21 mm
2+	Wheal <3 mm and erythema >21 mm
3+	Wheal >3 mm with surrounding erythema
4+	Wheal with pseudopods and surrounding erythema

Extracted from Sheldon J, Lovell R, Mathews K. A manual of clinical allergy. 2nd ed. Philadelphia: WB Saunders Company; 1967.

assay for the detection of allergen-specific IgE antibodies.[19] This early allergen-specific IgE antibody assay has evolved with many technologic advancements into 3 present-day autoanalyzer-based, allergen-specific IgE antibody assays that essentially mimic the RAST's solid-phase chemistry.[6] The ImmunoCAP by Phadia (UniCAP100, ImmunoCAP250) uses a cellulose sponge matrix in the form of a small cap as an allergosorbent on which allergen is covalently coupled. The Immulite System from Siemens (Berlin, Germany) uses a biotinylated allergen that is bound to an avidin solid phase. The HYTEC-288 system from Hycor/Agilent Technologies (Santa Clara, Calif) uses a cellulose wafer on which allergen is covalently coupled. All 3 systems are performed on autoanalyzers to maximize precision and minimize the turnaround time. They all use nonisotypically labeled anti-human IgE and are calibrated by means of interpolation of response data from a heterologous total serum IgE calibration curve that has been referenced to the World Health Organization (WHO) total IgE serum standard.[6]

Although convergence or harmonization of these technical factors has led to improved intermethod agreement among reported IgE antibody results, specific IgE antibody levels, as measured with different commercial assays, are still not interchangeable or identical. Differences remain in the specificity of the allergen-containing reagents used in the different assays.[20] Except for single-component drugs (eg, insulin, penicillin, and protamine) and recombinant or native component allergens, the allergen preparations used in IgE antibody assays remain mixtures of proteins that are prepared from biological extracts that differ in their composition between manufacturers because of several factors. The principal variables include the season in which the raw material is collected, the degree of difficulty in identifying a pure source of material, the presence of morphologically similar raw materials that might cross-contaminate, and differences in the extraction and final processing during allergen reagent production by the assay manufacturers. Fortunately, allergen extracts selected for use in allergosorbents undergo extensive quality control and documentation with isoelectrofocusing, SDS-PAGE, crossed immunoelectrophoresis, and immunoblotting methods.

TABLE V. Alternative skin test grading system for intradermal skin testing only involving wheal and erythema responses

Grade or class	Wheal size (mm)	Erythema size (mm)
0	<5	<5
+/−	5-10	5-10
1+	5-10	11-20
2+	5-10	21-30
3+	10-15	21-40
4+	>15 with pseudopods	41-50

Extracted from Norman PS. In: Middleton E, Ellis EF, Reed CE, editors. Allergy: principles and practice. 2nd ed. St Louis: Mosby; 1982.

Allergenic potency is assessed by using a soluble antigen inhibition format of the allergen-specific IgE assay. In this assay soluble allergen (typically in an extract) or buffer (sham control) are added to different aliquots of serum before the serum mixture is analyzed in the specific IgE assay. The percentage of inhibition is computed as a semiquantitative estimate of relative allergen potency. There are other issues with stability of allergen extracts during storage: heterogeneity of the human IgE antibody–containing sera used for quality control and different criteria for acceptance of the finished allergen-containing reagent by different manufacturers. Thus allergen-containing reagents from different manufacturers should thus be expected to detect different populations of IgE antibodies for any given allergen specificity.[6]

Several new IgE antibody technologies have emerged to enhance the allergen-specific IgE antibody data that are available to both the clinician and the patient. The microarray chip technology[21] has been commercialized in the form of the ImmunoCAP Immunosorbent Allergen Chip (ISAC) or Immuno Solid phase Allergen Chip (VBC Genomics-Phadia). It currently has 103 native/recombinant component allergens from 43 allergen sources that are dotted in triplicate onto glass slides. Twenty microliters of serum is pipetted onto the chip, and antibodies specific for the allergens attached to the chip surface bind during a 2-hour incubation period. After a buffer wash, bound IgE is detected with a fluorescently labeled anti-IgE. The chip is read in a fluorometer, and fluorescent signal units are interpolated into ISU or ISAC units as semiquantitative estimates of specific IgE antibody in the original serum. The analytic sensitivity of the ISAC varies as a function of the particular allergen specificity and is generally less than that of the ImmunoCAP system when the same allergens are coupled to sponge allergosorbent. This device has been providing clinical data to clinicians in Europe for several years but is not yet cleared by the US Food and Drug Administration for clinical use in the United States.

The unique clinical utility of the microarray system rests in its ability to identify the patient's IgE antibody cross-reactivity among structurally similar allergens from different biological substances. For instance, Bet v 1 from birch tree pollen has structurally similar homologues in the PR10 family that include allergenic proteins from alder tree pollen (Aln g 1), hazelnut pollen (Cor a 1), apple (Mal d 1), peach (Pru p 1), soybean (Gly m 4), peanut (Ara h 8), celery (Apr g 1), carrot (Dau c 1), and kiwi (Act d 8). A primary sensitivity to Bet v 1 might result in oral allergy symptoms after exposure to any of these other structurally similar (cross-reactive) allergenic molecules. The microarray also can assess cross-reactivity among other allergen families, such as the profilins, the lipid transfer proteins, the calcium-binding proteins, the tropomyosins, and the serum albumin family.[6]

TABLE VI. Analytes measured in the diagnostic allergy laboratory

Diagnosis
Allergen-specific IgE
Multiallergen-specific IgE screen (adult and pediatric forms)
Individual allergen specificities
Total serum IgE*
Free IgE (in serum of patients receiving omalizumab)
Precipitating antibodies specific for proteins in organic dusts
Tryptase (α and β; mast cell protease and used as a marker for mast cell–mediated anaphylaxis)
Other tests: Complete blood count and sputum examination for eosinophils and neutrophils
Management
Allergen-specific IgG (Hymenoptera)
Indoor aeroallergen quantitation in surface dust
Der p 1 and 2/Der f 1 and 2 (dust mite, *Dermatophagoides* species)
Fel d 1 (cat, *Felis domesticus*)
Can f 1 (dog, *Canis familiaris*)
Bla g 1/Bla g 2 (cockroach, *Blattela germanica*)
Mus m 1 (mouse, *Mus musculus*)
Rat n 1 (rat, *Rattus norvegicus*)
Cotinine (metabolite of nicotine measured in serum, urine, and sputum and used as a marker of smoke exposure)
Research analytes
IgE-specific autoantibodies
Eosinophil cationic protein
Mediators†,‡
Preformed biogenic amine: histamine
Newly formed: leukotriene C_4, prostaglandin D_2
Proteoglycans†
Heparin
Chondroitin sulfate E
Proteases†
Mast cell chymase
Mast cell carboxypeptidase
Cathepsin G
Fibroblast growth factor†
Cytokines
IFN-γ
TNF-α
IL-4, IL-5, IL-6, and IL-13‡

*Total serum IgE is the only one of these tests listed that is regulated under the CLIA-88.
†Primarily released from mast cells.
‡Primarily released from basophils.

Knowledge of the extent of IgE cross-reactivity among these structurally similar proteins provides unique information to the allergist as support to the clinical history in the diagnosis and management of the allergic patient.

A second trend in IgE antibody serology is the emergence of a point-of-care IgE assay in which a drop of blood from a finger prick is inserted into the sample well of a lateral-flow cassette. The ImmunoCAP Rapid (Phadia) allows antibody to flow with the fluid front across two nitrocellulose strips that have been impregnated with 5 lines each of extract-based aeroallergens (cat dander, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, Bermuda grass, short ragweed, oak tree, *Alternaria* species, timothy grass, elm tree, and dog dander). If IgE antibody is bound, it is detected with anti-IgE–colloidal gold that subsequently migrates up the same nitrocellulose strips after the addition of developing solution to the appropriate well. Use of the ImmunoCAP Rapid in a study of 215 children (1-14 years old) demonstrated effective (88.4% to 97.6%) correct identification of allergic sensitization in children with recurrent wheezing depending on the final color degree considered on the membrane.[22] This device has received US Food and Drug Administration clearance and is intended for use by primary care physicians who would then (in theory) refer their IgE-positive patients to an allergist for a comprehensive diagnostic workup. An additional point-of-care testing device for detection of 12 aeroallergen- or 12 food allergen–specific IgE antibodies from a finger-stick specimen is the FastCheck System from DST Diagnostiche & Technologies GmbH (Schwerin, Germany) that is available only in Europe.

Utility of quantitative measures of allergen-specific IgE antibody

The importance of quantitative IgE antibody measurements in blood can be illustrated by the results of a prospective study on food allergy.[23] One hundred children who were referred for food allergy evaluation provided sera that was tested for IgE antibodies to egg, milk, peanut, soy, wheat, and fish by using the ImmmunoCAP System. Diagnosis of food allergy was established in each child based on a history and an oral food challenge. The results of this study demonstrated that greater than 95% of food allergies were correctly identified by using previously established 95% predictive decision criteria with retrospective data.[24] An IgE antibody level was identified above which there was a defined probability of reacting to a food challenge. Using the ImmunoCAP, IgE antibody levels of egg (6 kUa/L), milk (32 kUa/L), peanut (15 kUa/L), and fish (20 kUa/L) could predict clinical reactivity (positive food challenge results) with greater than 95% certainty. The authors concluded that the need for oral food challenge could be reduced by about 50% by quantitatively measuring food-specific IgE antibody levels in serum and applying these 95% predictive decision criteria.

Quantitative estimates of serum IgE antibody might also facilitate the management of asthmatic patients who have pet epidermal and dust mite aeroallergens as triggers for their disease. By using specific IgE as a continuous variable, the risk of current wheeze and reduced lung function in children was shown to increase significantly with increasing summed measurements of dust mite-, cat-, and dog-specific IgE antibody.[25] These data identified subjects who were not only sensitized but who also could benefit from avoidance through environmental control measures.

Although the ability to quantify the level of select food allergen– and aeroallergen-specific IgE antibody in the blood has shown promise in facilitating the diagnosis and management of allergic disease, one must be careful in interpreting these reported positive, predictive IgE antibody decision points too literally. Using cow's milk–specific IgE as an illustration, Table VII[23,24,26-32] summarizes 8 published studies that report levels of IgE anti-milk in kUa/L, as measured by using the Phadia ImmunoCAP for positive predictive decision points.[24,25,27-32] The level of cow's milk–specific IgE antibody that allows one to predict a positive food challenge result with up to 95% confidence varies widely as a function of the age range and disease state of the children studied, the prevalence of cow's milk allergy in the population, the study design with either open or placebo-controlled food challenges, and the statistical method used to derive the predictive decision point. In addition, patient-dependent bias can occur in which IgE anti–cow's milk measurements alone by

TABLE VII. published positive predictive values of milk-specific IgE testing

Study	Population	Age (y)	No.	Prevalence of cows' milk allergy	Study design	Oral milk challenge	Statistical method for predictive decision point	Positive predictive decision point (%)/specific IgE (kUa/L)
Sampson and Ho[24]	With food allergy	5.2 (average)	196	49%	Retrospective	DBPCFC	2–by-2 tables	95%: 32 90%: 23
Sampson[23]	With suspected food allergy	3.8 (median)	100	66%	Prospective	DBPCFC	2–by-2 tables	95%: 15
Garcia-Ara et al[27]	With suspected cow's milk allergy	0.4 (average)	170	44%	Prospective	Open controlled	2–by-2 tables	95%: 5 90%: 2.5
Garcia-Ara et al[28]	With cow's milk allergy	0.4 (average), start	66	100%	Prospective follow-up	Open controlled	2–by-2 tables	95%: 2.7 for age 1.1-1.5 y 95%: 9 for age 1.6-2 y 95%: 24 for age 2.1-3 y 90%: 1.5 for age 1.1-1.5 y 90%: 6 for age 1.6-2 y 90%: 14 for age 2.1-3 y
Celik-Bilgili et al[29]	With suspected food allergy	1.1 (median)	501	*	Retrospective	DBPCFC or open	Logistic regression	90%: 88.8
van der Gugten et al[30]	With suspected cow's milk allergy??	3.0 (average)	213	44%	Retrospective	DBPCFC	2–by-2 tables logistic regression	95%: 52 90%: 26.8 95%: >100 90%: 66.4
Perry et al[31]	With food allergy, 77% allergic to >1 food	4.8 (median) at first challenge	391	*	Retrospective	Open	2–by-2 tables	50%: 2
Komata et al[32]	With suspected milk and egg allergy	1.3 (median)	969	*	Cross-sectional	Open (majority)	Logistic regression	95%: 5.8 for age <1 y 95%: 38.6 for age = 1 y 95%: 57.3 for age ≥2 y

*Not provided.

means of ImmunoCAP might underestimate by up to 2.4-fold the actual level of specific IgE that is measurable with the 5 milk component allergens (α-lactalbumin, β-lactoglobulin, casein, lactoferrin, and BSA) on individual allergosorbents.[26] Given these concerns with the published positive predictive decision points, one must be careful to interpret the serologic levels of IgE antibody within the context of the patient's clinical history.

Finally, the concentration, specific activity (specific/total IgE ratio), affinity (tightness of binding), and clonality (epitope specificity) of the IgE antibody response have all been shown to affect effector cell activation.[33] Higher levels of basophil activation occurred with higher overall concentrations of serum IgE anti–Der p 2, higher Der p 2–specific IgE/total IgE ratios, broader clonality (specificities or number of recognized epitopes), and higher IgE antibody affinities. Future design of serologic assays for IgE antibody will need to more effectively assess these 4 important humoral immune response parameters in the evaluation of patients for allergic disease.[34]

Performance of IgE antibody assays in the skin and blood

Comparison of the diagnostic performance (Table II) of any 2 *in vivo* tests, serologic tests, or both for allergen-specific IgE from peer-reviewed published data is difficult for several reasons. First, various investigators use different clinical criteria, test criteria, or both to define cases (subjects with disease). Second, study populations might vary widely within their disease category because of differences in the magnitude and frequency of their allergen exposures. Third, IgE antibody assay performance is highly dependent on the criterion that is used to define the positive threshold, which varies among clinical studies, especially for *in vivo* methods.

Table VIII summarizes the relative clinical utility of skin test and serologic assays for the assessment of systemic (venom and drug), food-related, and respiratory-related allergic diseases. Maximal clinical sensitivity is needed for evaluating patients with suspected venom and drug allergies because of the potential for life-threatening systemic reactions. In these cases the graded

TABLE VIII. Relative diagnostic utility of skin test and serologic measures of allergen-specific IgE antibody

	Allergen-specific IgE antibody	Epicutaneous (prick/puncture) skin test	Intradermal skin test
Systemic reactions Venom allergy Drug allergy	Complementary to intradermal skin test	Not sufficient	Preferred
Food allergy	Acceptable	Acceptable	Not needed (false-positive results)
Respiratory allergy	Acceptable	Acceptable	Usually not needed (false-positive results)

intradermal skin test, which errs on the side of false-positivity,[35,36] is preferred because the epicutaneous skin test is not sufficiently sensitive. By using dialyzed venom that removes irritating amines, the sensitivity of the intradermal venom skin test can be further enhanced.[37] When the intradermal skin test results are inconsistent with the clinical history, they should be repeated, and IgE antibody serology should be performed as a complementary test.

For food and respiratory allergy, IgE antibody as detected in the serum by using current autoanalyzer technology and in the skin by using the epicutaneous test are considered equivalent as confirmatory tests in terms of their sensitivity and accuracy.[35,38] The clinical use of intradermal testing with a single injection for foods or aeroallergens is contraindicated. Improved screening of patients for allergic disease can be achieved when epicutaneous skin test and serologic measurements of IgE antibody are used together.[39] Serologic IgE antibody assay results of greater than 0.35 kUa/L and epicutaneous skin test results larger than 3 to 4 mm have been most effectively correlated with the presence of allergic symptoms that are induced in allergen challenge studies. Serology has the advantage with complex allergen extracts, such as those derived from foods and molds, that it uses allergosorbents that have defined expiration dates and are quality controlled by using panels of human sera from subjects who are known to be clinically allergic to the specific target allergen.[6] In deciding which confirmatory diagnostic allergy test to use in clinical practice, the allergist needs to consider the test's relative sensitivity, inherent variability, the relationship between IgE antibody levels and disease expression, patient safety and comfort, timeliness, and cost.[6,36-41]

IgE screening assays

Occasionally patients provide a questionable or negative history for atopic disease or a history from which no one allergen specificity can be pinpointed with a reasonable certainty as the cause of allergic symptoms. The multiallergen IgE antibody screen is a single qualitative serologic assay that evaluates a patient's serum for the presence of IgE antibodies specific for a mixture of approximately 15 principal indoor and outdoor aeroallergens that are believed to account for a large majority of allergic respiratory disease.[6] A pediatric form of the multiallergen screening test can evaluate common food-specific IgE antibodies (eg, milk, egg, peanut, wheat, and soybean) in addition to IgE specific for common weed, grass, and tree pollens; molds; pet epidermal; and dust mite aeroallergens. A negative multiallergen screen result reduces the probability that IgE antibodies are involved in the patient's clinical problems to less than 5%. In a recent study one version of the pediatric multiallergen screen (Phadiatop, Phadia) correctly identified allergic sensitization in 97.6% of 215 children (ages 1-14 years) with recurrent wheezing.[22] These screening assays are possibly most useful in confirming the absence of significant atopic disease in subjects who are suspected of having an intrinsic or non–IgE-mediated respiratory, cutaneous, or gastrointestinal disease process. Such a test can minimize the need for multiple *in vivo* or serologic allergen-specific IgE measurements in patients with a low clinical probability of atopic disease. The use of this screening test in unselected populations is likely to generate many false-positive results because IgE antibody responses are much more frequent than symptomatic disease.

Total serum IgE

Total serum IgE measurement is currently the only diagnostic allergy test that is regulated in the United States under the Clinical Laboratory Improvement Act of 1988 (CLIA-88). These assays are either nephelometric or 2-site (capture and detection antibody), noncompetitive immunometric (labeled antibody) assays. The analytic sensitivity of the total serum IgE assays is 1 to 2 μg/L (1 kU/L is equivalent to 2.4 μg/L IgE). Intermethod agreement of commercially available IgE assays as assessed by using intermethod coefficients of variation are less than 10% for serum IgE levels of greater than 30 kU/L in a proficiency-based study.[42] Calibration of total serum IgE assays to the WHO IgE International Reference Preparation (WHO 75/502) has enhanced worldwide agreement.

The clinical utility of total serum IgE measurements in the diagnosis of allergic disease has always been limited by its age-dependent concentration and the wide overlap in concentrations in serum between atopic and nonatopic populations. The total serum IgE level must therefore be viewed always within the context of its nonatopic age-adjusted reference interval.[6] With the licensing of anti-IgE (Xolair [omalizumab]; Genentech, Inc, South San Francisco, Calif) therapy in 2003, there has been an increase in total IgE measurements because Xolair dosing requires knowledge of the patient's total serum IgE level. The increased use of Xolair has led to concern that some serum specimens are being analyzed for total serum IgE levels while containing Xolair, which can potentially interfere and reduce the assays' accuracy. In a proficiency survey–based study, total serum IgE levels, as measured by using ImmunoCAP, were shown to be minimally reduced (2.4% to 9.0%) by the presence of 50 to 200 molar excess of omalizumab to the level of serum IgE.[43] In contrast, other clinically used total serum assays showed marked reductions from 12.5% to 67.2% ($P < .001$), and the interference increased in proportion to the total serum IgE level in the serum. Counter to claims in the Xolair package insert, total serum IgE can be accurately measured by using the ImmunoCAP assay in the presence of therapeutic levels of Xolair. Clinical assays to measure free IgE or IgE that is not bound with therapeutically administered anti-IgE are in the developmental stage. Free IgE measurements should help the clinician with a problematic Xolair-treated patient to determine whether the dose of anti-IgE should be escalated to obtain greater clinical efficacy.

Proficiency testing for total and allergen-specific IgE antibody assays

The CLIA-88 requires that all federally licensed clinical laboratories participate in an external proficiency survey. One such diagnostic allergy survey is conducted by the College of American Pathologists.[42] The survey involves analysis of 5 or 6 challenge sera every 17 weeks (3 cycles per year) for total serum IgE and IgE antibody levels to 5 allergen specificities and a multiallergen screen. Results are impartially collated, and interlaboratory (intramethod) coefficients of variation are computed, critiqued, and then sent to both participating laboratories and credentialing agencies. Except for the occasional nonatopic serum, interlaboratory/intramethod and intermethod coefficients of variation for total serum IgE are routinely excellent at less than 15%.[42]

Allergen-specific IgE levels historically have been reported by different assays in nonequivalent arbitrary units or classes. Today, the 3 principal assays report allergen-specific IgE levels in more quantitative kUa/L units. Although differences continue to exist in the levels of reported allergen-specific IgE among the various IgE antibody assays, in general, the 3 principal assays correctly identify the IgE-negative (nonsensitized) subjects' sera from sera that are IgE antibody positive (sensitized) for most of the allergen specificities. It is the responsibility of the laboratory to indicate the method they use on their final report to the clinician. It is, however, the responsibility of the referring physician to ensure that the laboratory that performs IgE antibody testing is CLIA-88 certified and that they use a validated assay method and perform successfully on a diagnostic allergy proficiency survey.[6,44]

Venom competitive inhibition IgE antibody assay

One unique competitive inhibition form of the IgE antibody assay has a specific application to patients with Hymenoptera venom sensitivity. Of the medically important Hymenoptera, structural similarity exists between the vespid and *Polistes* species wasp allergens phospholipase A1/B (Ves g I and Pol a I) and hyaluronidase (Ves g II and Pol a II), which leads to IgE antibody cross-reactivity. A serologic venom inhibition assay is used to determine the most appropriate therapeutic composition of venoms for immunotherapy.[45] Patients with venom allergy who have a strong skin test response or high level of serum IgE antibody to yellow jacket venom (YJV) and a weak skin reactivity or low level of serum IgE antibody specific for *Polistes* species wasp venom (PWV) are candidates for this analysis. In the assay a patient's serum that contains YJV- and PWV-specific IgE antibodies is preincubated with soluble YJV (heterologous venom), PWV (homologous venom control), or buffer (no inhibition control). The mixtures are then incubated separately with PWV allergosorbent, and the assay is completed with the final addition of labeled anti-human IgE antibody. The amount of IgE anti-PWV bound to the PWV allergosorbent is measured, and greater than 95% inhibition of IgE anti-PWV binding with the addition of soluble YJV is considered complete cross-inhibition. Sera from 305 patients with Hymenoptera venom allergy with greater than 2 ng/mL of IgE antibody to YJV and PWV were evaluated to determine whether PWV should be included in the venom immunotherapy regimen together with yellow jacket or mixed vespid venom. The venom competitive inhibition assay identified one third (36.4%) of these subjects as having an exclusive YJV sensitivity. These subjects were candidates for exclusion of PWV from their immunotherapy regimen because their IgE anti-PWV was greater than 95% cross-inhibitable with soluble YJV.[45]

Hymenoptera venom–specific IgG

Allergen injections during immunotherapy are known to enhance the production of specific IgG "blocking" antibodies (Table I).[46] As a general rule, quantitative measurements of allergen-specific IgG (or IgG subclass) antibodies in studies of allergic rhinitis have not correlated well with improvement in clinical symptoms of individual patients receiving immunotherapy. However, clinically successful immunotherapy is almost always accompanied by high serum levels of allergen-specific IgG, particularly of the IgG4 subclass. One proposed application of allergen-specific IgG antibody measurements has been as an aid in documenting effective immunotherapy in patients with Hymenoptera venom sensitivity. In a prospective study Hymenoptera venom–specific IgG antibodies were monitored in the serum of 109 patients with venom allergy to examine whether their levels could provide an indication for the relative risk of a systemic reaction after a sting challenge in patients receiving venom immunotherapy.[47] Over a 4-year period, systemic symptoms occurred in 16% of 211 venom sting challenges in the group with less than 3 μg/mL venom-specific IgG antibody. This contrasted with a reaction rate of 1.6% in patients with venom IgG levels of greater than 3 μg/mL. The highest rate of allergic reactions (26%) occurred among patients who had both a venom-specific IgG antibody level of less than 3 μg/mL and less than 4 years of venom immunotherapy. The study concluded that quantitative venom-specific IgG antibody levels can be useful for individualizing the dose and frequency of injections to maximize its protective effects. The clinical utility of venom-specific IgG antibody measurements, however, appears to be restricted to the first 4 years of venom immunotherapy.

Food-specific IgG and IgG4 antibodies

Historically, IgG4 reaginic antibodies were believed to be diagnostic because monoclonal anti-human IgG4 could induce BHR from allergic donors cells.[48] In 1992, this issue was challenged by Lichtenstein et al,[49] who showed no histamine release (<10%) was detected from nonatopic donor cells after incubation with a panel of highly specific International Union of Immunological Societies–documented human IgG subclass–specific mAbs.[50] Moreover, 85% of these same cells released to anti-IgE. In contrast, the study confirmed that 75% of atopic donor basophils released greater than 10% of their histamine to 1 or more of the human anti-IgG subclass–specific mAbs and not only of the IgG4 subclass specificity. After a series of elaborate basophil-based lactic acid stripping and add-back experiments, it was shown that atopic subjects can possess basophil IgE receptor–bound IgG anti-IgE–IgE complexes, and cells from these subjects can be triggered by the addition of anti-IgG mAbs that cross-link the IgE receptors through this complex. This provided a rationale for why the presence or levels of IgG or IgG4 antibodies specific for food antigens have never shown a correlation with the diagnostic results of positive DBPCFCs. This also supports the European Academy of Allergy and Clinical Immunology Task Force recommendation[51] that food-specific IgG and IgG4 antibody responses are not useful diagnostic tools for assessing allergic disease or planning food-elimination diets. Further work on this issue is needed with modern IgG and IgG4 antibody autoanalyzers with sera from non–IgE-mediated food-sensitive subjects to confirm this recommendation and verify that allergen-specific IgG antibody levels are simply a reflection of the extent of a subject's environmental antigen exposure and not a marker for allergen sensitization.[52]

Precipitating IgG antibodies (precipitins)

Extrinsic allergic alveolitis, also referred to as hypersensitivity pneumonitis, is an inflammatory reaction in the lung interstitium and terminal bronchioles induced by chronic exposure to antigenic organic dusts (eg, molds and bird droppings). Although the lung-lesion histology indicates a cell-mediated pathology, most patients with hypersensitivity pneumonitis have high levels of precipitating IgG antibody to the offending antigens in their blood.[53,54] Some clinical laboratories still perform the double-diffusion (Ouchterlony) assay to detect precipitating antibodies to extracts of organic dusts. This involves inserting a crude antigen extract of the organic material (bird fecal material and mold) into one well and a control and the patient's serum (containing antibody) in 2 other closely spaced wells in a porous agarose gel. If diffusion of the antigen and antibody over 2 to 3 days in a moist chamber produces precipitating antibodies with lines of identity to the control antiserum, this can support the diagnosis of hypersensitivity pneumonitis. Precipitating antibodies, or precipitins, can be detected in the sera of nearly all patients who have active hypersensitivity pneumonitis, but they are also present in the sera of as many as 50% of asymptomatic subjects who have been exposed to the relevant organic dusts.[54] Immunoassays for IgG antibody to the appropriate organic dust antigens appear to be too sensitive and are viewed as less diagnostically useful. Precipitin assays are performed to organic dusts containing the thermophilic actinomyces (*Micropolyspora faeni*, *Thermoactinomyces vulgaris*, and *Thermoactinomyces candidus*), multiple antigens from *Aspergillus* species (*Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus*), pigeon serum, *Aureobasidum pullulans*, and fecal particles from parakeets and a variety of exotic household birds (eg, cockatiel and blue Amazon).

Mast cell tryptase

Mediators, which include prestored histamine and newly generated vasoactive mediators, are released from activated mast cells into surrounding soft tissue (Table VI). Mast cell tryptase (molecular weight 134,000 d), which is a serine esterase with 4 subunits, each with an enzymatically active site, is also released from an activated cell. When dissociated from heparin, tryptase rapidly degrades into its monomers and loses enzymatic activity. Human basophils contain 300- to 700-fold less tryptase than lung or skin mast cells, and therefore tryptase in serum is considered a marker of systemic mast cell activation.[55] The α-tryptase concentration in blood is a measure of the mast cell number, and it is estimated by subtracting β-tryptase levels from the total serum tryptase concentration. In contrast, β-tryptase levels in blood are considered a measure of mast cell activation.

Healthy nondiseased subjects have serum total tryptase levels that range from 1 to 10 ng/mL (average, 5 ng/mL). If baseline total serum tryptase levels exceed 20 ng/mL, systemic mastocytosis should be suspected. Serum β-tryptase levels of less than 1 ng/mL are observed in nondiseased subjects. β-Tryptase levels of greater than 1 ng/mL indicate mast cell activation. Blood samples should be collected from 0.5 to 4 hours after the initiation of a suspected mast cell–mediated systemic reaction for optimal results.[55,56] Peak β-tryptase levels of greater than 10 ng/mL in a postmortem serum suggest systemic anaphylaxis as one probable cause of death. An insect sting–induced β-tryptase level can peak at greater than 5 ng/mL by 30 to 60 minutes after the sting and then decrease with a biological half-life of approximately 2 hours.[57] Increased postmortem tryptase levels, however, have been observed in the absence of anaphylaxis,[58] thus reducing the utility of postmortem tryptase levels in placing the cause of death for some deceased Hymenoptera-sensitive subjects. Tryptase has also been measured in bronchoalveolar lavage fluid, nasal lavage fluid, tears, and skin chamber fluid; however, there are currently no clinical indications for such measurements.

BHR test

The BHR assay has been used to detect the presence of allergen-specific IgE on surface basophils by means of direct challenge or passive sensitization. When used as an alternative confirmatory diagnostic test for allergen-specific IgE antibody, BHR test results have highly correlated with results from skin testing and bronchoprovocation.[59] In the direct challenge BHR assay, peripheral blood leukocytes are isolated from whole blood by means of dextran sedimentation, washed, and incubated with allergen or anti-human IgE at varying concentrations (eg, 3- to 10-fold dilutions). In the passive sensitization BHR assay, basophils are stripped of their IgE by means of lactic acid elution and then incubated with serum containing IgE antibody and challenged with antigen. In either the direct or passively sensitized BHR assay, mediator release is complete within 30 minutes, and then histamine or leukotriene released into the supernatant is measured. The BHR dose-response curve typically consists of a characteristic bell-shaped curve with a linear ascending portion, which is maximal or peaks at the optimal cross-linking allergen dose, and a descending portion at higher than optimal allergen concentrations. The allergen concentration required to induce 50% histamine release can be used to define the relative sensitivity of the patient's basophils for a given allergen extract. At present, the BHR test is used primarily in research laboratories because of its need for fresh blood. It has been especially useful as an alternative assay for clarifying discrepancies between skin test and serologic IgE antibody test results.

The basophil has been examined as a possible indicator cell for assessing the autoimmune status of patients experiencing a form of chronic urticaria (CU).[60-63] Autoantibodies specific for IgE, FcεR1, or FcεRII can be present in 30% to 50% of patients with CU.[60] One clinical laboratory offers a CU index test in which highly selected donor basophils are incubated with serum from the patient with CU, and released histamine is quantified.[62] Other investigators dispute the validity of this assay and suggest that a primary basophil abnormality, unknown serologic factors, or both affecting basophils in patients with CU might be more clinically relevant to disease pathogenesis than the presence or level of FcεRI/II, IgE-reactive autoantibodies, or both.[63]

As a measure of basophil activation, flow cytometry has been used to quantify the level of basophil surface markers after exposure to allergen (CD63, CD203c), allergen exposure in the presence of IL-3 (CD63), and exposure to other degranulating stimuli, such as N-formyl-methionyl-leucyl-phenylalanine and ionophores. Although whole blood can be used for these analyses, conditions used to lyse contaminating red blood cells can interfere by directly stimulating basophil activation. Controversy remains as to whether an individual surface marker or the panel of activation markers should be analyzed to reflect basophil mediator release and how well the kinetics of change of each marker actually reflect basophil activation. Details of the BHR assay and flow cytometric detection of basophil activation surface markers are presented elsewhere.[64]

IN VIVO DIAGNOSTIC PROVOCATION TESTING

When discordance occurs between the clinical history and primary diagnostic confirmatory test results, one of several provocation tests might be performed.[13] Bronchial and nasal provocation challenges are techniques used to identify a relationship between an inhaled substance and a change in the patient's bronchial or nasal physiology. A DBPCFC is used to evaluate patients who have experienced food-induced gastrointestinal reactions (eg, nausea, colic, vomiting, and diarrhea) that can occur within minutes to hours after the consumption of selected foods. Each of these tests will be briefly discussed.

Bronchoprovocation studies involving the use of methacholine are particularly useful in the diagnosis of difficult cases of asthma.[65] In general, bronchoprovocation involves the administration of either methacholine or histamine by means of a calibrated nebulizer starting at doses of 0.05 to 0.1 mg/mL and doubling the concentration up to 10 to 25 mg/mL. Methacholine is an analog of acetylcholine that directly stimulates bronchial smooth muscle rather than inducing mast cell enzyme and mediator release. Alternatively, allergen extracts can be administered in increasing doses. Allergen, in contrast to methacholine, induces changes in pulmonary function as a direct result of mast cell activation in the lung. The clinical effect of the analyte exposure is monitored with pulmonary function tests after each dose. A positive response is usually defined as the concentration of agonist that results in a decrease in FEV_1 of 20% or more from the preprovocation baseline value, which must be greater than 70% of predicted value for valid interpretation. More extensive details regarding the methods and interpretation of bronchial challenges are presented elsewhere.[13,65]

Nasal provocation involves the controlled administration of buffer (human serum albumin–saline) or increasing concentrations of allergen into a washed nasal passage. A symptom score is collected (eg, number of sneezes induced) and/or the concentration of mast cell mediators or albumin released into nasal lavage fluids after each concentration of allergen indicates the relative sensitivity of a subject to the allergen in question. N-tosyl-L-arginine methyl ester [TAME] esterase and histamine are commonly monitored in nasal lavage fluid. Nasal airway resistance is a less satisfactory end point because of high intrinsic variation. Details of the procedure and applications can be found elsewhere.[66]

The DBPCFC involves the controlled ingestion of frequently eaten foods that are known to contain potent allergens. These foods typically include cow's milk (caseins, β-lactoglobulin, and α-lactalbumin), chicken egg white (ovalbumin, ovomucoid, and ovotransferrin), cereal grains (wheat, rye, barley, and oats), legumes (peanut, soybean, and white bean), fish, and seafood (shrimp, crabs, lobsters, and oysters). The DBPCFC begins with a strict elimination diet for the suspect foods for 7 to 14 days before the challenge. An equal number of randomly alternating food allergen and placebo challenges, starting with 125 to 500 mg of lyophilized food, are then administered to the patient in a fasting state, doubling the dose every 15 to 60 minutes. Clinical reactivity can be ruled out once 10 g of lyophilized food blinded in masking foods (eg, pudding and chili) or capsules is tolerated. Negative DBPCFC results must then be confirmed with an open feeding challenge under observation to rule out possible false-negative challenge results. Serum levels of food-specific IgE antibody can sometimes be used to exclude the need for a food challenge if the levels are sufficiently high to exceed reported 95% confidence limits for a positive food challenge result (Table VII).[23,24] An extensive discussion of the DBPCFC and variables influencing its outcome are presented elsewhere.[67]

INDOOR AEROALLERGEN TESTING

Avoidance by separating the allergic patient from the allergen source is possibly the least expensive and most effective mode of treatment for allergic disease, when it is achievable. Knowledge about the levels of allergen in an environment can support the decision to initiate expensive alterations of their home, school, or workplace to facilitate avoidance of indoor aeroallergens. Some clinical laboratories perform environmental allergen quantification in which an air sample or a surface dust specimen is collected with a vacuum from either the general indoor environment or individual rooms. An inexpensive air-sampling cassette or surface dust collector is attached to a vacuum, and a bulk dust specimen is collected. It is sent to a specially equipped laboratory, where it is processed through a 50-mesh metal sieve to exclude particles larger than 300 μm. Fine dust is then quantitatively extracted (eg, 100 mg per 2 mL of physiologic saline-albumin buffer). Soluble allergens, once extracted, are quantified with mAb-based immunoenzymetric assays or bead-based multiplex assays for dust mite–, pet epidermal–, rodent-, cockroach-, and mold-related indicator allergens. Currently, Der f 1 and 2 and Der p 1 and 2 are allergens that are excreted in fecal particles by dust mites (*D farinae* and *D pteronyssinus*). Fel d 1 and Can f 1 are allergens excreted by the sweat glands of the domestic cat *(Felis domesticus)* and dog *(Canis familiaris)*. Bla g 1/Bla g 2 allergens are released by the German cockroach *(Blatella germanica)*. Mus m 1 and Rat n 1 are allergens excreted into urine by the mouse *(Mus musculus)* and rat *(Rattus norvegicus)*. The level of these indoor allergens serve as "indicators" for environments that are contaminated with higher than desirable levels of allergens for sensitized subjects (especially children with atopic asthma). Indoor evaluation allows allergen-laden environments to be identified and cleaned in an attempt to facilitate avoidance of allergen exposure. Risk levels have been assigned for some of the allergens. Levels of Der p 1 allergen, Der f 1 allergen, or both of greater than 2,000 ng/g fine dust have been associated with an increased risk of allergic symptoms in sensitized subjects, whereas levels of greater than 10,000 ng/g of fine dust have been associated with an increased risk of sensitization. For other allergens, such as cockroach, mouse, and rat allergens, just the presence of detectable allergen can be an indicator of clinically relevant environmental contamination. Further details can be obtained elsewhere.[68]

The kingdom Fungi encompasses yeasts, molds, smuts, and mushrooms, which are plants without leaves, flowers, or roots that reproduce from spores (2-20 μm in diameter and 1-100 mm in length). Molds lack chlorophyll and vascular tissue and range in form from a single cell to a body mass of branched filamentous hyphae that spread into and feed off of dead organic matter or living organisms. Some molds produce allergen-laden spores that are generally invisible to the naked eye and are used in speciation of the mold by means of microscopic, immunologic, and molecular biological techniques.

Sampling for mold is unnecessary in cases in which visible mold growth or musty odors identify mold infestation. Alternatively, a bulk dust can be distributed on a microbiological culture plate containing media and antibiotics or inoculated with a swab or by being placed in a gravity sampler or a suction impactor.

Viable spores are enumerated 24, 48, and 72 hours later by means of macroscopic and microscopic assessment.[69] *Alternaria* and *Aspergillus* species allergens (Alt a 1 and Asp f 1) can be quantified in extracts of surface dust by using mAb-based immunoenzymetric assays; however, their utility is limited to environmental conditions in which molds secrete these allergens. Alternatively, an Environmental Relative Moldiness Index test involves PCR-based DNA analysis for the relative levels of 26 molds associated with water damage and 10 molds not associated with water damage. When levels of these 36 molds were measured by using DNA techniques in dust from 271 homes of asthmatic children, the Environmental Relative Moldiness Index level was more effective than a binary classification of homes as either moldy or nonmoldy based on onsite inspection in predicting the development of respiratory illness (wheeze, rhinitis, or both).[70]

OUTDOOR AEROALLERGEN TESTING

Most major cities across the United States have an aerobiology monitoring station with a collection device on a platform or roof top, typically 1 story off the ground (eg, 13 feet). Ideally it is in an open space distant from trees, which can bias the aeroallergen results. The Rotorod Sampler (Sampling Technologies, St Louis Park, Minn) is one widely used rotating-arm impactor that recovers airborne particles on 2 rapidly moving plastic collector rods.[71,72] It contains a pair of 1.59-mm-wide plastic rods that extend during rotation on a central arm at defined time intervals (eg, 10-60 seconds every 10 minutes). A thin layer of silicon grease that is coated on the leading edge of the rod (edge in the direction of rotation) impacts particles in the air, and they imbed in the grease. Every 24 hours, the rod is removed from the device, stained, and microscopically evaluated by a qualified technician for the number and types of pollens and mold spores (grains or spores per cubic meter of air sampled for the previous 24-hour period). The efficiency of particle collection on the Rotorod decreases with particle size (eg, 7-μm particle: 10% efficiency to 25-μm particle: 100% efficiency).[72] Fungal spores are smaller (diameter = 1 to >100 mm) than pollen grains (diameters = 20-70 μm). Newer devices, such as the Burkard Hirst Trap and Burkard SporeWatch (Burkard Mfg Co. Rickmansworth, Herts, England), are suction impactors that are more effective in detecting mold spores than the rotarod.[73] These devices also have the capacity to collect longitudinal samples over a 7-day period. Although pollen and spore counts are commonly transmitted to local weather stations and newspapers for public use, they are somewhat limited in their use because they describe the levels in the air over the previous 24 hours.

CONCLUSION

A number of analytic measurements are used to promote more accurate diagnosis and better management of allergic subjects. The clinician should remember that all *in vivo* and serologic analyses are subject to inherent variation and potential interference. Thus it is prudent to question the validity of any *in vivo* or laboratory test that is inconsistent with a carefully collected clinical history. One should repeat *in vivo* testing on a different day or perform serologic testing with a new blood specimen, a different laboratory, or both. Alternative tests might seem redundant, but they are useful in confirming observations because different methods (eg, skin test and serologic assays) measure different subsets of the IgE antibody response. Most importantly, let the clinical history drive the diagnosis. Maintain a healthy skepticism about diagnostic test results, and verify the quality control and validity of *in vivo* diagnostic reagents used and the performance standards of serologic assays and the laboratories that perform them.

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REFERENCES

1. Ishizaka K, Ishizaka T. Physiochemical properties of reaginic antibody. I. Association of reaginic activity with an immunoglobulin other than gamma A or gamma G globulin. J Allergy 1967;37:169-72.
2. Johansson SGO, Bennich H. Immunological studies of an atypical (myeloma) immunoglobulin. Immunology 1967;13:381-94.
3. Hamilton RG. Human immunoglobulins. In: O'Gorman MRG, Donnenberg AD, editors. Handbook of human immunology. 2nd ed. Boca Raton (FL): CRC Press; 2008. p. 63-106.
4. Barbee RA, Halomen M, Lebowitz M, Burrows B. Distribution of IgE in a community population sample: correlation with age, sex and allergen skin test reactivity. J Allergy Clin Immunol 1981;68:106-12.
5. Wittig HJ, Belloit J, DeFillippi I, Royal G. Age-related serum IgE levels in healthy subjects and in patients with allergic disease. J Allergy Clin Immunol 1980;66: 305-9.
6. Matsson P, Hamilton RG, Esch RE, Kleine-Tebbe J, Reeves JP, Renz H, et-al. Analytical performance characteristics and clinical utility of immunological assays for human immunoglobulin E antibodies of defined allergen specificities. Wayne, Pa: Clinical and Laboratory Standards Institute (CLSI); 2009. Approved guideline I/LA20
7. Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomes A. recombinant allergens for diagnosis and therapy of allergic disease. J Allergy Clin Immunol 2000;106:409-18.
8. Ballmer-Weber BK, Scheurer S, Fritsche P, Enrique E, Cistero-Bahima A, Haase T, et al. Component resolved diagnosis with recombinant allergens in patients with cherry allergy. J Allergy Clin Immunol 2002;110:167-73.
9. Hamilton RG. Assessment of human allergic diseases. In: Rich RR, Fleisher TA, Shearer WT, Schroeder HW, Frew AJ, Weyand CM, editors. Clinical immunology: principles and practice. 3rd ed. London: Elsevier; 2008. p. 1471-84.
10. Hamilton RG. Laboratory diagnosis and management of human allergic disease. In: Leung DYM, Sampson HA, Geha RS, Szefler SJ, editors. Pediatric allergy: principles and practice. 2nd ed London: Elsevier; 2009.
11. Kurtz MK, Hamilton RG, Adkinson NF Jr. Role and application of provocation in the diagnosis of occupational latex allergy. Ann Allergy Asthma Immunol 1999;83: 634-9.
12. Brown RH, Schauble JA, Hamilton RG. Prevalence of latex allergy in occupationally exposed healthcare workers: identification of sensitized but non-symptomatic latex allergic anesthesiologists. Anesthesiology 1998;89:292-9.
13. Damin DA, Peebles RS. In vivo diagnostic allergy testing. In: Detrick B, Hamilton RG, Folds JD, editors. Manual of molecular and clinical laboratory immunology. 7th ed. Washington (DC): American Society for Microbiology Press; 2006. p. 947-54.
14. Nelson HS, Lahr J, Buchmeier A, McCormick D. Evaluation of devices for skin prick testing. J Allergy Clin Immunol 1998;101:153-6.
15. van der Veen MJ, Mulder M, Witteman AM, van Ree R, Aalberse RC, Jansen HM, et al. False positive skin prick test responses to commercially available dog dander extracts caused by contamination with house dust mite *(Dermatophagoides pteronyssinus)* allergens. J Allergy Clin Immunol 1996;98:1028-34.
16. Antico A, Lima G, Arisi M, Ostan A, Morrica B. Assay of prick test inoculum volume. II. Average values and individual variability. Ann Allergy Asthma Immunol 2000;85:145-9.
17. Belin LG, Norman PS. Diagnostic tests in the skin and serum of workers sensitized to *Bacillus subtilis* enzymes. Clin Allergy 1977;7:55-68.
18. Turkeltaub PC, Rastogi SC, Baer H, Anderson MC, Norman PS. A standardized quantitative skin test assay for allergen potency and stability: Studies on the allergen dose-response curve and effect of wheal, erythema and patient selection on assay results. J Allergy Clin Immunol 1982;70:343-52.
19. Wide L, Bennich H, Johansson SGO. Diagnosis by an in vitro test for allergen specific IgE antibodies. Lancet 1967;2:1105-9.
20. Wood RA, Segall N, Ahlstedt S, Williams PB. Accuracy of IgE antibody laboratory results. Ann Allergy Asthma Immunol 2007;99:34-41.
21. Hiller R, Laffer S, Harwanegg C, Huber M. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. FASEB J 2002;16:414-6.

22. Diaz-Vazquez C, Torregrosa-Bertet MJ, Carvajal-Urueña I, Cano-Garcinuño A, Fos-Escrivà E, García-Gallego A, et al. Accuracy of ImmunoCAP(R) rapid in the diagnosis of allergic sensitization in children between 1 and 14 years with recurrent wheezing: the IReNE study. Pediatr Allergy Immunol 2009;20:601-9.
23. Sampson HA. Utility of food specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 2001;107:891-6.
24. Sampson HA, Ho DG. Relationship between food specific IgE concentrations and the risk of positive food challenges in children and adolescents. J Allergy Clin Immunol 1997;100:444-51.
25. Simpson A, Soderstrom L, Ahlstedt S, Murray CS, Woodcock A, Custovic A. IgE antibody quantification and the probability of wheeze in preschool children. J Allergy Clin Immunol 2005;116:744-9.
26. Bogdanovic J, Skripak JM, Matsui EC, Burkes AW, Wood RA, Hamilton RG. Agreement of total versus component milk specific IgE ImmunoCAP measurements [abstract]. J Allergy Clin Immunol 2009;123(suppl):S33.
27. Garcia-Ara C, Boyano-Martinez T, Diaz-Pena JM, Martin-Muñoz F, Reche-Frutos M, Martin-Esteban M. Specific IgE levels in the diagnosis of immediate hypersensitivity to cows' milk protein in the infant. J Allergy Clin Immunol 2001;107:185-90.
28. Garcia-Ara C, Boyano-Martinez T, Diaz-Pena JM, Martin-Muñoz F, Martin-Esteban M. Cow's milk-specific immunoglobulin E levels as predictors of clinical reactivity in the follow-up of the cow's milk allergy infants. Clin Exp Allergy 2004;34:866-70.
29. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocon M, Beyer K, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. Clin Exp Allergy 2005;35:268-73.
30. van der Gugten AC, den Otter M, Meijer Y, Pasmans SG, Knulst AC, Hoekstra MO. Usefulness of specific IgE levels in predicting cow's milk allergy. J Allergy Clin Immunol 2008;121:531-3.
31. Perry TT, Matsui EC, Conover-Walker MK, Wood RA. The relationship of allergen-specific IgE levels and oral food challenge outcome. J Allergy Clin Immunol 2004;114:144-9.
32. Komata T, Söderström L, Borres MP, Tachimoto H, Ebisawa M. The predictive relationship of food-specific IgE concentrations to challenge outcomes for egg and milk varies by patient age. J Allergy Clin Immunol 2007;119:1272-4.
33. Christensen LH, Holm J, Lund G, Riise E, Lund K. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. J Allergy Clin Immunol 2008;122:298-304.
34. Hamilton RG, Saito H. IgE antibody concentration, specific activity, clonality, and affinity measures from future diagnostic confirmatory tests. J Allergy Clin Immunol 2008;122:305-6.
35. Wood RA, Phipatanakul W, Hamilton RG, Eggleston PA. A comparison of prick skin tests, intradermal skin tests and the radioallergosorbent test in the diagnosis of cat allergy. J Allergy Clin Immunol 1999;103:773-9.
36. Golden DBK, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM. Insect sting allergy with negative venom skin tests. J Allergy Clin Immunol 2001; 107:897-901.
37. Golden DB, Kelly D, Hamilton RG, Wang NY, Kagey-Sobotka A. Dialyzed venom skin tests for identifying yellow jacket-allergic patients not detected using standard venom. Ann Allergy Asthma Immunol 2009;102:47-50.
38. Bernstein IL, Li JT, Bernstein DI, Hamilton RG, Spector SL, Tan R, Sicherer S, et al. Allergy diagnostic testing: an updated practice parameter. Ann Allergy Asthma Immunol 2008;100(suppl):S1–S148.
39. Rance F, Abbal M, Lauwers-Cances V. Improved screening for peanut allergy by the combined use of skin prick tests and specific IgE assays. J Allergy Clin Immunol 2002;109:1027-33.
40. Yunginger JW, Ahlstedt S, Eggleston PA, Homburger HA, Nelson HS, Ownby DR, et al. Quantitative IgE antibody assays in allergic diseases. J Allergy Clin Immunol 2000;105:1077-84.
41. McCann WA, Ownby DR. The reproducibility of the allergy skin test scoring and interpretation by board-certified/board eligible allergists. Ann Allergy Asthma Immunol 2002;89:368-71.
42. Hamilton RG. Proficiency survey based evaluation of clinical total and allergen-specific IgE assay performance. Arch Pathol Lab Med. In press. 2009.
43. Hamilton RG. Accuracy of Food and Drug Administration-cleared IgE antibody assays in the presence of anti-IgE (omalizumab). J Allergy Clin Immunol 2006; 117:759-66.
44. Hamilton RG. Responsibility for quality IgE antibody results rests ultimately with the referring physician. Ann Allergy Asthma Immunol 2001;86:353-4.
45. Hamilton RG, Wisenauer JA, Golden DB, Valentine MD, Adkinson NF Jr. Selection of Hymenoptera venoms for immunotherapy based on patient's IgE antibody cross-reactivity. J Allergy Clin Immunol 1993;92:651-9.
46. Lichtenstein LM, Holtzman NA, Burnett LS. A quantitative in vitro study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody. J Immunol 1968;101:317-21.
47. Golden DBK, Lawrence ID, Hamilton RG, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Clinical correlation of the venom specific IgG antibody level during maintenance venom immunotherapy. J Allergy Clin Immunol 1992;90:386-93.
48. Fagan DL, Slaughter CA, Capra JD, Sullivan TJ. Monoclonal antibodies to immunoglobulin G4 induced histamine release from human basophils in vitro. J Allergy Clin Immunol 1982;70:399-404.
49. Lichtenstein LM, Kagey-Sobotka A, White JM, Hamilton RG. Anti-human IgG causes basophil histamine release by acting on IgG-IgE complexes bound to IgE receptors. J Immunol 1992;148:3929-36.
50. Jefferis R, Reimer CB, Skvaril F, de Lange G, Ling NR, Lowe J, et al. Evaluation of monoclonal antibodies having specificity for human IgG subclasses: results of an IUIS/WHO collaborative study. Immunol Lett 1985;10:223-52.
51. Stapel SO, Asero R, Ballmer-Weber BK, Knol EF, Strobel S, Vieths S, et al. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report. Allergy 2008;63:793-6.
52. Hamilton RG. Relevance of (IgG anti-IgE)-IgE complexes, IgG subclass and modern IgG antibody autoanalyzers in the dying IgG reagin story. Allergy 2009;64:317-8.
53. Zacharisen MC, Schuleter DR, Kurup VP, Fink JN. The long-term outcome in acute, subacute and chronic forms of pigeon breeder's disease hypersensitivity pneumonitis. Ann Allergy Asthma Immunol 2002;88:175-82.
54. Fan LL. Hypersensitivity pneumonitis in children. Curr Opin Pediatr 2002;14: 323-6.
55. Duff Hogan A, Schwartz LB. Markers of mast cell degranulation. Methods 1997; 13:43-52.
56. Schwartz LB, Sakai K, Bradford TR, Ren S, Zweiman B, Worobec S, et al. The alpha form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. J Clin Invest 1995;96:2702-10.
57. Van der Linden PW, Hack CE, Poortman J, Vivie-Kipp YC, Struyvenberg A, van der Zwan JK. Insect sting challenge in 138 patients: relation between clinical severity of anaphylaxis and mast cell activation. J Allergy Clin Immunol 1992;90:110-8.
58. Randall B, Butts J, Halsey JF. Elevated postmortem tryptase in the absence of anaphylaxis. J Forensic Sci 1995;40:208-11.
59. Levy D, Osler A. Studies on the mechanisms of hypersensitivity phenomena. XIV. Passive sensitization in vitro of human leukocytes to ragweed pollen antigen. J Immunol 1966;97:203-12.
60. Kaplan AP, Greaves M. Pathogenesis of chronic urticaria. Clin Exp Allergy 2009; 39:777-87.
61. Saini SS. Basophil responsiveness in chronic urticaria. Curr Allergy Asthma Rep 2009;9:286-90.
62. Altrich ML, Halsey JF, Altman LC. Comparison of the in vivo autologous skin test with in vitro diagnostic tests for diagnosis of chronic autoimmune urticaria. Allergy Asthma Proc 2009;30:28-34.
63. Eckman JA, Hamilton RG, Saini SS. Independent evaluation of a commercial test for "autoimmune" urticaria in normal and chronic urticaria subjects. J Invest Dermatol 2009;129:1584-6.
64. Schroeder JT, Saini S. Assay method for measurement of mediator and markers of allergic inflammation. In: Detrick B, Hamilton RG, Folds JD, editors. Manual of molecular and clinical laboratory immunology. 7th ed. Washington (DC): American Society for Microbiology Press; 2006. p. 964-74.
65. Cockcroft DW. Bronchial challenge testing. In: Adkinson NF, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's allergy: principles and practice. 7th ed. London: Elsevier; 2009. p. 1295-308.
66. Togias A, Corren J, Wagenmann M. Nasal provocation testing. In: Adkinson NF, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's allergy: principles and practice. 7th ed. London: Elsevier; 2009. p. 1281-94.
67. Wood RA. Oral food challenge testing. In: Adkinson NF, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. Middleton's allergy: principles and practice. 7th ed. London: Elsevier; 2009. p. 1309-18.
68. Hamilton RG, Eggleston PA. Environmental allergen analyses. Methods 1997;13: 53-60.
69. Vesper S, McKinstry C, Ashley P, Haugland R, Yeatts K, Bradham K, et al. Quantitative PCR analysis of molds in the dust from homes of asthmatic children in North Carolina. J Environ Monit 2007;9:826-30.
70. Vesper SJ, McKinstry C, Haugland RA, Iossifova Y, Lemasters G, Levin L, et al. Relative moldiness index as predictor of childhood respiratory illness. J Expo Sci Environ Epidemiol 2007;17:88-94.
71. Solomon WR. Techniques of air sampling. In: Sheldon JM, Lovell RM, Mathews KP, editors. Manual of clinical allergy. 2nd ed. Philadelphia: WB Saunders; 1967.
72. Edmonds RL. Collection efficiency of Rotorod samplers for sampling fungal spores in the atmosphere. Plant Dis Reporter 1972;56:704-9.
73. Burge HP, Boise JR, Rutherford JA, Solomon WR. Comparative recoveries of airborne fungus spores by viable and non-viable modes of volumetric collection. Mycopathologia 1977;61:27-33.

Laboratory evaluation of primary immunodeficiencies

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Primary immunodeficiencies are congenital disorders caused by defects in different elements of the immune system. Affected patients usually present clinically with recurrent infections, severe infections, or both, as well as autoimmune phenomena that are associated with many of these disorders. Early diagnosis is essential for referral to specialized care centers and the prompt initiation of appropriate therapy. In this article the authors describe a general approach for the investigation of the most common primary immunodeficiencies, outlining the typical clinical symptoms and most appropriate laboratory investigations. (J Allergy Clin Immunol 2010;125:S297-305.)

Key words: *Primary immunodeficiency, laboratory assessment, immunologic diagnosis, immunity*

Abbreviations used

ALPS:	Autoimmune lymphoproliferative syndrome
APECED:	Autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy syndrome
DHR:	Dihydrorhodamine 123
FOXP3:	Forkhead box protein 3
HLH:	Hemophagocytic lymphohistiocytosis
IFNGR1:	IFN-γ receptor 1 gene
IFNGR2:	IFN-γ receptor 2 gene
IL12RB1:	IL-12 receptor β1 gene
IPEX:	Immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome
IRAK4:	IL-1 receptor–associated kinase 4
LAD:	Leukocyte adhesion deficiency
NEMO:	Nuclear factor κB essential modulator, also called IKK-γ
NK:	Natural killer
PID:	Primary immunodeficiency
SCID:	Severe combined immunodeficiency
STAT:	Signal transducer and activator of transcription
TCR:	T-cell receptor
TLR:	Toll-like receptor
TREC:	T-cell receptor excision circle
XLP:	X-linked lymphoproliferative syndrome

The clinical spectrum of characterized primary immunodeficiencies (PID) has expanded significantly over the past 2 decades, and the underlying genetic basis of the majority of primary immunodeficiencies (PIDs) also has been identified. The accurate diagnosis of patients with PIDs is critical for appropriate therapy and also affords the opportunity to provide appropriate genetic counseling to the patient and his or her family. In virtually all cases the clinical symptoms involve increased susceptibility to infection, and early diagnosis and therapy provides the greatest opportunity to prevent significant disease-associated morbidity. In this setting the laboratory serves as the primary source of diagnostic information used to define the immunologic defect. The optimal use of the laboratory for the diagnosis and characterization of PIDs is the focus of this chapter.

EVALUATING SUSPECTED ANTIBODY DEFICIENCY DISORDERS

When to suspect

The majority of patients with primary antibody deficiencies present with recurrent bacterial infections of the sinopulmonary tract, including recurrent otitis media, sinusitis, and pneumonia (Table I).[1,2] The most commonly isolated organism is *Streptococcus pneumoniae*, but *Haemophilus influenzae* (often untypeable), *Staphylococcus* and *Pseudomonas* species are also seen. Diarrhea affects up to 25% of these patients, often associated with *Giardia lamblia* infection. However, infections with rotavirus, enterovirus, *Campylobacter*, *Salmonella*, and *Shigella* species might also be found.[1] In addition, autoimmune manifestations are seen in up to 25% of these patients, with autoimmune hemolytic anemia and autoimmune thrombocytopenia being most commonly observed. Finally, granulomatous disease involving various organs with particular predilection for the lung might also be present, and in some patients this process can result in significant morbidity.[1]

PIDs that commonly manifest some degree of hypogammaglobulinemia include selective IgA deficiency, common variable immunodeficiency, and congenital agammaglobulinemias (both X-linked and autosomal recessive inheritance, Table II). Less common causes include agammaglobulinemia with thymoma (Good syndrome) and X-linked lymphoproliferative syndrome (XLP).[1] X-linked agammaglobulinemia should be suspected in all male patients with recurrent otitis and even a single episode of pneumonia, even if the family history is negative. This condition also might present with neutropenia and sepsis by *Pseudomonas* or *Staphylococcus*.[3] Occasionally, the ataxia-telangiectasia syndrome manifests with recurrent infections and upper respiratory tract symptoms associated with IgA deficiency before the onset of overt neurologic signs.[4] Concomitant bacterial sinopulmonary and opportunistic infections, including low pathogenic mycobacteria, should raise suspicion of a cellular defect that also affects antibody production, such as nuclear factor κB essential modulator (NEMO; also called IKK-γ) or CD40 ligand (CD154) deficiencies.[5,6] Selected complement deficiency and phagocytic defects might also have a clinical presentation similar to that of antibody deficiency and could be considered for investigation (Table II).

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TABLE I. Common pathogens and infection sites according to the underlying immune defect

Affected immunity arm	Typical site of infection	Common pathogens
B cells	Sinopulmonary tract, GI tract, joints, CNS	Pyogenic bacteria: streptococci, staphylococci, *Haemophilus influenzae* Enteroviruses: ECHO, polio *Mycoplasma* species
T cells	Sepsis, lung, GI tract, skin	Viruses: CMV, adenovirus, measles, molluscum Fungi: *Candida* and *Aspergillus* species, *Pneumocystis jiroveci* Pyogenic bacteria Protozoa: *Cryptosporidium* species
Phagocytes	Skin infections, lymphadenitis, liver, lung, bone, GI tract, gingivitis/periodontitis	Bacteria: staphylococci, *Serratia marcescens*, *Burkholderia cepacia*, *Klebsiella* species, *Escherichia coli*, *Salmonella* species, *Proteus* species Fungi: *Candida*, *Aspergillus*, and *Nocardia* species
Complement	Systemic infections, meningitis	Pyogenic bacteria: streptococci, *Haemophilus influenzae*, *Neisseria* species

GI, gastrointestinal; *CNS*, central nervous system; *ECHO*, echovirus; *CMV*, cytomegalovirus.

TABLE II. Differential diagnosis of antibody deficiencies and associated laboratory findings

Primary B-cell disorders
Common variable immunodeficiency: low IgG and IgA levels, variable IgM levels, usually normal B-cell numbers
Selective IgA deficiency: low IgA levels, normal IgG and IgM levels, normal B-cell numbers
Congenital agammaglobulinemia: low IgG, IgA, and IgM levels; undetectable or very low B-cell numbers (<2%)
Specific antibody deficiency: normal IgG, IgA, and IgM levels; normal B-cell numbers; defective antibody response to vaccination
Agammaglobulinemia with thymoma (Good syndrome): low IgG and IgA levels, variable IgM levels, low B-cell numbers
Combined cellular and humoral disorders
Hyper-IgM syndromes: low IgG and IgA levels, normal, low or high IgM levels, normal B-cell numbers
Ectodermal dysplasia with immunodeficiency syndrome (NEMO/IκBα deficiency): variable immunoglobulin levels, normal B-cell numbers
XLP: low IgG and IgA levels, variable IgM levels, typically normal B-cell numbers
Ataxia-telangiectasia syndrome: low IgA levels
Other causes to consider
Drug-induced hypogammaglobulinemia; sickle cell disease with secondary hyposplenism; primary asplenia; immunodeficiency, centromeric instability, facial anomalies syndrome; cystic fibrosis; complement component deficiency; myelodysplasia; chronic lymphocytic leukemia; multiple myeloma; dysmotile cilia syndrome; warts, hypogammaglobulinemia, immunodeficiency and myelokathexis (WHIM) syndrome

Laboratory evaluation

The initial clinical laboratory screening of antibody-mediated immune function can be accomplished by measuring the levels of the major immunoglobulin classes IgG, IgA, IgM, and IgE (Table III). The results must be compared with age-matched reference intervals (normal ranges) that are typically provided as 95% CIs. There are no rigid standards regarding the diagnosis of immunoglobulin deficiency, although an IgG value of less than 3 g/L (300 mg/dL) in an adolescent or adult, as well as values clearly below the age-appropriate reference (95% confidence interval) in a child should trigger further evaluation. An additional and readily available test is quantitation of IgG subclass levels. This test is most useful in evaluating an IgA-deficient patient with significant recurrent bacterial infections. However, in most settings, detection of an IgG subclass deficiency still requires documentation of an abnormality in specific antibody production before initiating therapy, making this test of more limited utility. Measurement of specific antibody responses is useful in confirming defective antibody production and is essential when the total immunoglobulin levels are only modestly decreased (or even normal) in the setting of recurrent bacterial infection. The simplest method is evaluation for spontaneous specific antibodies (eg, anti–blood group antibodies [isohemagglutinins]) and antibodies to previous immunizations or infections. The definitive method to evaluate *in vivo* antibody production involves immunizing a patient with protein antigens (eg, tetanus toxoid) and polysaccharide antigens (eg, Pneumovax, Merck & Co, Inc, Whitehouse Station, NJ) and assessing preimmunization and 3- to 4-week postimmunization antibody levels. Guidelines for normal responses, which are usually provided by the testing laboratory, typically consist of finding at least a 4-fold increase in antibody levels and/or protective antibody levels after immunization. An alternative method to access the humoral immune response that is specifically useful in patients already receiving immunoglobulin replacement therapy involves vaccination with a neoantigen, such as the bacteriophage Phi X174; however, this is only available in some specialized centers.[7]

Additional testing focuses on determining the presence or absence of B cells by using flow cytometry. This is particularly useful as a marker for congenital forms of agammaglobulinemia because this group of disorders typically is characterized by absent or extremely decreased circulating B-cell numbers based on the underlying defects that block B-cell development.[2] More recently, characterization of B-cell subsets, particularly directed at memory and immature B cells, has been put forward as a means of further characterizing patients with common variable immunodeficiency.[8] Studies that test *in vitro* B-cell signaling and immunoglobulin biosynthesis are generally performed only in research centers.

TABLE III. Evaluation of suspected antibody deficiency

Screening tests
Quantitative immunoglobulins
Specific antibody levels
Circulating specific antibody levels to prior vaccines and blood group antigens (isohemagglutinins)
Pre/postimmunization antibody levels
Protein antigens
Carbohydrate antigens
IgG subclasses
Secondary tests
B-cell immunophenotyping
In vitro functional studies
Tests to exclude rare and secondary causes
Thoracic computed tomography to exclude thymoma (particularly useful if patient is >50 years old with low B-cell numbers)
Intracellular flow cytometry or genetic evaluation for BTK (XLA) or SAP/XIAP (XLP)
Genetic evaluation of NEMO to rule out anhydrotic ectodermal dysplasia with immune deficiency
Fecal α_1-antitrypsin, urinary protein, serum albumin, absolute lymphocyte count to exclude gastrointestinal or urinary protein loss or lymphatic loss
HIV testing to exclude AIDS
Complement function (CH50, AP50) to exclude complement deficiency
Karyotype to exclude immunodeficiency, centromeric instability, facial anomalies syndrome
Sweat chloride or genetic evaluation to exclude cystic fibrosis

BTK, Bruton tyrosine kinase; *XLA*, X-linked agammaglobulinemia; *SAP/XIAP*, SLAM-associated protein/X-linked inhibitor of apoptosis.

TABLE IV. Most common T-cell and combined immunodeficiencies and distinctive features

SCID: failure to thrive, chronic diarrhea, oral thrush, recurrent or severe bacterial, viral and/or fungal infections
CD40 and CD40 ligand deficiency: recurrent sinopulmonary and opportunistic infections with low IgG and IgA levels and variable IgM levels
Wiskott-Aldrich syndrome: easy bruising, eczema, recurrent otitis media, diarrhea, thrombocytopenia with small platelets
DiGeorge syndrome: hypoparathyroidism, cardiac malformations, dysmorphic features, variable T- and B-cell defects
Anhydrotic/hypohidrotic ectodermal dysplasia with immunodeficiency (NEMO or IκBα deficiency): recurrent mycobacterial or pyogenic infections, with or without skin, hair, and nail abnormalities; poor fever responses
XLP: hypogammaglobulinemia, persistent or fatal EBV infection
Chronic mucocutaneous candidiasis: recurrent oroesophageal and skin *Candida* species infection

EVALUATING SUSPECTED T-CELL OR COMBINED T- AND B-CELL IMMUNODEFICIENCY DISORDERS

When to suspect

Patients affected by severe combined immunodeficiency (SCID) or other primary conditions with markedly abnormal T-cell function usually manifest failure to thrive and recurrent infections with opportunistic pathogens, such as *Candida albicans* (thrush), *Pneumocystis jiroveci*, or cytomegalovirus very early in life (Table I).[9] Other common findings are chronic diarrhea, recurrent bacterial infections affecting multiple sites, and persistent infections despite adequate conventional treatment. SCID is a pediatric emergency because early diagnosis can dramatically improve the clinical outcome. Skin rashes are common, particularly with specific T-cell disorders, including Omenn and Wiskott-Aldrich syndromes.[10] Other severe cellular or combined defects present with varied clinical symptoms, as listed briefly in Table IV.

Laboratory evaluation

Careful analysis of the white blood cell count and differential is of utmost importance when evaluating patients suspected of cellular immunodeficiency disorders. The absolute lymphocyte count must be compared with age-matched control ranges for proper interpretation. Severe lymphopenia in an infant (<3,000/mm^3) is a critical finding that should prompt immediate immunologic evaluation if confirmed on a repeat test. The caveat in using low T-cell number during infancy as the screen to detect defects in T-cell development is that this would not identify patients with Omenn syndrome. In this disorder normal or increased T-cell numbers are typically found in the face of profound cellular immunodeficiency caused by an oligoclonal expansion of T cells.[10] In addition, circulating T cells might also be seen in the face of a severe cellular immune defect as a result of maternal T-cell engraftment. The maternal T cells will consist of primarily memory $CD45RO^+$ cells (compared with naive $CD45RA^+$ T cells found in a healthy infant) that do not provide host protection.[11] Finally, transfusion of nonirradiated blood products in the setting of a severe cellular immune defect will result in circulating donor T cells that can produce graft-versus-host disease, a potentially fatal process. This scenario emphasizes the need to irradiate any blood product used in an infant with a suspected T-cell deficiency.

HIV infection has to be ruled out in all patients with symptoms of cellular immunodeficiency, and this typically requires testing for the presence of virus (ie, HIV viral load assay) rather than serologic testing for anti-HIV antibody (Table V).

After T-cell screening tests, the next step would be a directed assessment of cellular immunity (Table V). This includes immunophenotyping of T cells by means of flow cytometry together with *in vitro* functional testing (eg, proliferation and cytokine production assays).[12] The immunophenotyping for a patient suspected of having SCID not only helps to establish the diagnosis, but it can also point to the potential underlying genetic defect (Table VI).[12] It is important to carefully review the percentage and absolute numbers for all lymphocyte

TABLE V. Evaluation of suspected T-cell and combined immunodeficiency

Screening tests
HIV testing
Lymphocyte immunophenotyping
Delayed-type hypersensitivity skin testing
Secondary tests
T-cell proliferation (mitogens, alloantigens, recall antigens)
T-cell cytokine production
Flow cytometric evaluation of surface or intracellular proteins, such as CD40 ligand (CD154 on activated T cells), IL-2 receptor γ chain (CD132), MHC class I and II, IL-7 receptor α chain (CD127), CD3 chains, WASP
Enzyme assays: adenosine deaminase, PNP
FISH for 22q11 deletion
TREC numbers
TCR repertoire analysis
Mutation analysis

WASP, Wiskott-Aldrich syndrome protein; *PNP*, purine nucleoside phosphorylase; *FISH*, Fluorescence *in situ* hybridization; *TREC*, T-cell receptor excision circle.

subsets, comparing them with age-appropriate reference ranges. Typically, defects in cytokine signaling molecules result in a $T^-B^+NK^-$ phenotype, whereas mutations in DNA-editing proteins required for T- and B-cell receptor expression are associated with a $T^-B^-NK^+$ phenotype; severe metabolic defects usually are toxic for all lymphocyte types, resulting in a $T^-B^-NK^-$ phenotype (Table VI).

Other useful tests in special circumstances include fluorescence *in situ* hybridization for the 22q11 microdeletion found in the majority of patients with DiGeorge syndrome and specific enzyme assays to evaluate for adenosine deaminase and purine nucleoside phosphorylase (PNP) deficiencies.[13] Evaluation for intracellular Wiskott-Aldrich syndrome protein expression by means of flow cytometry can be performed in selected centers to screen for possible Wiskott-Aldrich syndrome.[14] Direct evaluation of T-cell function, as assessed by the proliferative response to mitogens, recall antigens, and/or alloantigens, is an important part of evaluating cellular immunity. The same sort of culture conditions can also be used to evaluate for cytokine production using the culture supernatant (alternatively, one can evaluate cytoplasmic cytokine expression using flow cytometry).[15]

Quantification of T-cell receptor excision circles (TRECs) and evaluation of the T-cell repertoire can be used for additional evaluation of immune status. TRECs are formed during the normal editing of the T-cell receptor (TCR) genes during T-cell differentiation and maturation within the thymus and persist within the cell as extragenomic circular pieces of DNA. TREC copies are diluted over time as the T cells proliferate after antigen encounter. Therefore naive T cells that have recently emigrated from the thymus will present relatively high TREC levels compared with those of aged, antigen-experienced T cells.[16] TREC evaluation (also $CD4^+CD45RA^+CD31^+$ T cells by flow cytometry) can be used as a diagnostic confirmation of low thymic output that would be found in DiGeorge syndrome or to monitor immune reconstitution after bone marrow transplantation. More recently, the quantification of TRECs on blood derived from the Guthrie card obtained from infants after delivery has been initiated as a neonatal screening tool for SCID (and other significant T-cell defects) in both Wisconsin and Massachusetts.[17] The finding of low TREC levels in neonates should prompt immediate follow-up with immunophenotyping by means of flow cytometry. A recent report from Wisconsin suggests that this test has a very low rate of false-positive or inconclusive results (approximately 0.00009% and 0.0017%, respectively).[18]

TABLE VI. Immunophenotypic findings and associated genetic defects in patients with SCID

Phenotype	Pathway affected and genetic defect(s)
$T^-B^+NK^-$	Cytokine signaling: IL-2 receptor γ, JAK3
$T^-B^-NK^+$	DNA editing: RAG1/2, Artemis, ligase 4, Cernunnos
$T^-B^-NK^-$	Metabolic defects: adenosine deaminase, AK2
$T^-B^+NK^+$	Cytokine signaling: IL-7 receptor α chain
$CD8^+CD4^-B^+NK^+$	Positive selection/signaling: MHC class II, p56lck
$CD4^+CD8^-B^+NK^+$	Signaling: ZAP70

JAK3, Janus kinase 3; *RAG*, recombination-activating gene; *AK2*, adenylate kinase 2; *ZAP70*, zeta-chain associated protein kinase, 70 kD.

Analysis of the T-cell repertoire can be useful in specific clinical situations. The T-cell repertoire in circulating T cells from healthy subjects includes expression of the majority of the 24 TCR Vβ chain families, which can be promptly assessed by flow cytometry.[19] Alternatively, evaluation of TCR Vβ CDR3 region diversity can be performed by PCR and is commonly referred to as spectratyping. The PCR-amplified product from each of these Vβ families normally demonstrates a Gaussian distribution of variously sized PCR products, each differing by 3 nucleotides. In settings in which there is an oligoclonal T-cell population, such as is found in patients with Omenn and atypical DiGeorge syndromes, a very limited number of Vβ families will be represented, with each demonstrating a very distorted (non-Gaussian) distribution.[19]

EVALUATING SUSPECTED PHAGOCYTE DYSFUNCTION SYNDROMES

When to suspect

The clinical features of neutrophil dysfunction (including neutropenia) usually include recurrent bacterial and fungal infections of the skin, lymph nodes, lung, liver, bone, and, in some cases, the periodontal tissue (Table I).[20] The clinical pattern of infection often can help to discriminate the underlying problem. Common phagocyte defects and accompanying laboratory findings are presented in Table VII. Patients with neutropenia and those with leukocyte adhesion deficiency (LAD) tend to have recurrent cellulitis, periodontal disease, otitis media, pneumonia, and rectal or gastrointestinal infections with diminished

TABLE VII. Differential diagnosis of phagocyte defects and associated laboratory findings

Chronic granulomatous disease: defective oxidative burst by means of DHR assay or NBT
Leukocyte adhesion defects
LAD1: low/absent CD18 and CD11 expression by means of flow cytometry; persistent leukocytosis
LAD2: Bombay phenotype; absent CD15 (Sialyl-Lewis X) expression
LAD3: mutation analysis only
Chediak-Higashi syndrome: giant lysosomal inclusion bodies observed on morphologic review of granulocytes (with partial albinism)
Griscelli syndrome type 2: neutropenia without inclusion bodies (with partial albinism)
Severe congenital neutropenia: persistent neutropenia; maturation arrest on bone marrow studies
Cyclic neutropenia: intermittent neutropenia requiring serial measurements
X-linked neutropenia: altered WASP expression by means of flow or mutation analysis
G6PD and MPO deficiency: abnormal functional enzymatic assay
Hyper-IgE syndrome: IgE level >2,000 IU/mL; low T_H17 cell numbers
Other disorders to be considered
Drug-induced neutropenia; autoimmune/alloimmune neutropenia; hypersplenism; chronic mucocutaneous candidiasis; TCII deficiency; hyper-IgM syndrome, XLA; Schwachman-Bodian-Diamond syndrome; warts, hypogammaglobulinemia, immunodeficiency and myelokathexis (WHIM) syndrome

NBT, Nitroblue tetrazolium; *WASP*, Wiskott-Aldrich syndrome protein; *G6PD*, glucose-6-phosphate dehydrogenase; *MPO*, myeloperoxidase; *XLA*, X-linked agammaglobulinemia.

TABLE VIII. Evaluation of suspected phagocyte defects

Absolute neutrophil count and morphologic analysis: congenital neutropenia syndromes and Chediak-Higashi syndrome
Oxidative burst by means of DHR or NBT assays: chronic granulomatous disease; rarely complete G6PD or MPO deficiency
CD18 (also CD11a, CD11b, and CD11c) expression by means of flow cytometry: LAD1
CD15 expression by means of flow cytometry: LAD2
Bombay phenotype: LAD2
Anti-neutrophil antibodies: autoimmune neutropenia
Bone marrow biopsy: exclude defective myeloid production in neutropenia syndromes
Chemotaxis/phagocytosis assays: limited utility

NBT, Nitroblue tetrazolium; *G6PD*, glucose-6-phosphate dehydrogenase; *MPO*, myeloperoxidase.

inflammation and lack of pus formation.[20] Although LAD is accompanied by a persistent granulocytosis, there is effectively a tissue neutropenia caused by the underlying adhesion defect that prevents the directed movement of these phagocytic cells to sites of infection. Delayed umbilical cord separation is commonly seen in patients with LAD; however, LAD is very rare, and most infants whose cords persist for up to 1 month are actually healthy. In patients with cyclic neutropenia, there are short periods of fever, mouth ulcers, and infections recurring at intervals of 18 to 21 days in concert with the decreased neutrophil count. Other more common instances of neutropenia include drug-induced and immune-mediated neutropenia.

In contrast, patients with chronic granulomatous disease have significant problems with liver and bone abscesses, as well as pneumonias with selected organisms, including *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia*, and *Nocardia* and *Aspergillus* species.[21] Furthermore, they tend to have a lower frequency of *Escherichia coli* and streptococcal species infections compared with patients with neutropenia or LAD.

Finally, patients with hyper-IgE syndrome present with recurrent skin abscesses and cavitary pneumonias caused by *S aureus* and other pyogenic bacteria and demonstrate chronic mucocutaneous candidiasis.[22] In addition, they typically demonstrate specific nonimmunologic findings, such as coarse facial features, scoliosis, hyperextensible joints, increased risk for bone fractures, and delayed or failed shedding of primary dentition.[23]

Laboratory evaluation

Screening studies directed at the evaluation of neutrophil function should start with a leukocyte count, differential, and morphologic review (Table VIII). The diagnosis of cyclic neutropenia requires multiple absolute neutrophil counts 2 to 3 times a week for at least 4 to 6 weeks.[24] A diagnosis of severe congenital neutropenia (Kostmann syndrome) is made with neutrophil counts of less than 0.5×10^9/L on several occasions.[24] Bone marrow analysis is useful to exclude insufficient production because of neoplasia or other causes and to document other abnormalities, such as the maturation arrest typical of Kostmann syndrome.

If neutropenia and morphologic abnormalities are ruled out, the evaluation should be directed at assays that provide functional information about neutrophils. LAD workup involves flow cytometric assessment of the neutrophil adhesion molecules CD11 and CD18, the expression of which is absent or decreased on neutrophils (and other leukocytes) from patients with LAD1.[25] CD15 (Sialyl-Lewis X) expression is absent on neutrophils from patients with LAD2.[26]

The neutrophil oxidative burst pathway can be screened with either the nitroblue tetrazolium tests or a flow cytometric assay (dihydrorhodamine 123 [DHR]), the results of both of which are abnormal in patients with chronic granulomatous disease, but the latter is a more sensitive test.[27]

The diagnosis of autosomal dominant and sporadic hyper-IgE syndrome has been associated with heterozygous pathogenic mutations in the gene encoding signal transducer and activator of transcription (STAT) 3.[28,29] A consistent feature in this disorder is a very increased IgE level (>2,000 IU/mL), and more recently, low to absent IL-17–producing T cells (T_H17) have been demonstrated.[30]

Finally, evaluation of neutrophil-directed movement (chemotaxis) can be performed *in vivo* by using the Rebuck skin window technique, as well as *in vitro* with a Boyden chamber or a soft agar system. Abnormalities of chemotaxis have been observed after

use of certain pharmacologic agents, as well as in patients with LAD, Chediak-Higashi syndrome, Pelger-Huet anomaly, and juvenile periodontitis. However, chemotactic tests are difficult to perform, very hard to standardize, and available in only a limited number of laboratories.

EVALUATING SUSPECTED NATURAL KILLER AND CYTOTOXIC T-CELL DEFECTS

When to suspect

Deficiency in natural killer (NK) cell function has been described in a limited number of patients with recurrent herpes virus family infections. Another category of NK and cytotoxic T-lymphocyte defects results in an uncontrolled inflammatory response initiated in association with certain specific infections that produces multiple organ damage (hemophagocytic lymphohistiocytosis [HLH]). One of these disorders is XLP, which is usually asymptomatic until the patient has an EBV infection and then leads to an uncontrolled lymphoproliferative disorder that is often fatal without aggressive treatment.[31] Importantly, approximately 30% of patients with XLP present with hypogammaglobulinemia without other symptoms. Bone marrow transplantation is the only long-term cure for XLP.[31]

The clinical manifestations of familial HLH are rather nonspecific, requiring a high suspicion index for early diagnosis.[32] They include persistent fever, hepatosplenomegaly, neurological symptoms (ataxia and seizures), lymphadenopathy, and skin rashes. Diagnosis mandates an immediate therapeutic response and prompt referral for bone marrow transplantation because this is currently the only curative approach. Disorders caused by defective intracellular vesicle trafficking, such as Chediak-Higashi syndrome and Griscelli syndrome type 2, also commonly manifest with a secondary lymphohistiocytic syndrome.[32]

Laboratory evaluation

Testing of NK cell function includes immunophenotyping NK cells by means of flow cytometry and assaying cytotoxicity with standard *in vitro* assays. Patients with XLP1 will demonstrate absent invariant-chain NK T cells in peripheral blood, as measured by $CD3^+V\alpha24^+V\beta11^+$ staining.[31] Additionally, intracellular flow cytometry can be used to evaluate for expression of SAP (SLAM-associated protein) and XIAP (X-linked inhibitor of apoptosis), the proteins defective in XLP1 and XLP2, respectively.[33,34] Absent protein would indicate disease, whereas normal expression could be the result of an abnormal protein that is not distinguished from the normal protein by means of antibody staining. Therefore this screening test would require further investigation directed at cell function when the protein is detected in a patient suspected of having XLP. HLH is commonly associated with cytopenias, including anemia and thrombocytopenia; increased liver function test results; hypofibrinogenemia; and hypertriglyceridemia.[32] High ferritin and circulating soluble CD25 levels are also typical and represent laboratory findings used to establish the diagnosis of HLH.[32] Low intracellular perforin expression, as determined by flow cytometry, can be used to diagnose HLH2, and decreased surface expression of CD107a (LAMP1, lysosomal-associated membrane protein 1) on NK cells after activation can predict the presence of mutations in MUNC13-4 and syntaxin 11.[35,36]

EVALUATING SUSPECTED DEFECTS INVOLVING THE ADAPTIVE-INNATE IMMUNITY INTERFACE

IL-12/23–IFN-γ pathways

An emerging concept in the field of PIDs is that monogenic disorders can cause recurrent severe infections involving 1 or a very restricted range of pathogens.[37] Recently, patients with severe invasive infections caused by low virulence or environmental *Mycobacteria* and *Salmonella* species have been found to harbor defects in genes encoding different components of the IL-12/23–IFN-γ pathway: the IFN-γ receptor 1 gene *(IFNGR1)*, the IFN-γ receptor 2 gene *(IFNGR2)*, the IL-12 receptor β1 gene *(IL12RB1)*, *IL12B*, and *STAT1*.[38] The 2 most prevalent genetic defects among this group involve *IL12RB1* and *IFNGR1*, typically resulting in absent cell-surface protein expression.[39] This can be readily assessed by using flow cytometry with monoclonal reagents specific for these 2 proteins.[25] In addition, there is an autosomal dominant defect affecting *IFNGR1* that results in overexpression of the protein, and this also can be detected with flow cytometry.[40] Screening for other defects in IFN-γ signaling (abnormalities in *IFNGR2* or *STAT1*) can be done by evaluating monocyte STAT1 phosphorylation (by means of flow cytometry or Western blotting) *ex vivo* in response to IFN-γ.[41] Defects in IL-12 production can be tested by evaluating IL-12 production in response to *ex vivo* stimulation of mononuclear cells with LPS and IFN-γ.

Toll-like receptor and NF-κB signaling defects

Recently, recurrent infections involving *S pneumoniae* and *Staphylococcus* species have been associated with defects involving molecules of the Toll-like receptor (TLR) pathway, including IL-1 receptor–associated kinase 4 (IRAK4), MYD88 (myeloid differentiation primary response gene 88), and NEMO.[42-44] One of the distinctive features of patients with IRAK4 and MYD88 mutations is the markedly diminished inflammatory response to infection with little or no fever and acute-phase reactants observed.[45] NEMO deficiency is a more complex X-linked recessive disorder with a wide-ranging clinical phenotype and varied degree of immunologic abnormalities.[5] Finally, susceptibility to herpes simplex encephalitis has been linked to mutations in the genes encoding the receptor, TLR3, and an accessory protein of the TLR pathway, unc-93 homolog (UNC-93B).[46,47] Additional defects in TLR function associated with specific clinical phenotypes are likely to be identified and represent an evolving field in clinical immunology. Currently, the evaluation of TLR function is confined to a limited number of centers that usually screen response by stimulating mononuclear cells with various TLR-specific ligands and measuring cytokine production. This can then be followed by direct sequencing of the suspected mutant gene or genes involved in the specific TLR signaling process. Recently, von Bernuth et al[48] described a simplified assay for the screening of TLR function that is reported to detect functional defects in the signaling process by using whole blood samples. This assay involves stimulation of leukocytes with a series of specific TLR ligands and then evaluating for CD62L shedding from granulocytes by using flow cytometry. In cells with intact TLR signaling pathways, CD62L is promptly shed from the cell surface in contrast to the failure of CD62L shedding in cells from patients with IRAK4 or UNC-93B deficiency. One caveat is that the sample has to be analyzed shortly after obtaining the blood sample to prevent interpretation problems resulting from spontaneous CD62L shedding.

TABLE IX. Main clinical and laboratory findings of immune dysregulation syndromes and causative genes

Disorder	Distinctive clinical findings	Key laboratory findings	Gene(s) involved
ALPS	Lymphadenopathy, splenomegaly, autoimmune hemolytic anemia and/or thrombocytopenia, high risk for lymphomas	↑ $CD3^+\alpha\beta$-TCR-$\alpha\beta^+CD4^-CD8^-$ cells, hypergammaglobulinemia, Coomb positive, ↑ plasma IL-10 levels, ↑ serum vitamin B12 levels, ↑ soluble Fas ligand levels	*FAS*, *FASL*, *CASP8*, *CASP10*, *NRAS*
IPEX	Early-life enteritis, dermatitis, autoimmune endocrinopathy (usually type 1 diabetes)	↑ IgE levels, diminished $FoxP3^+$ CD4 T-cell subpopulation	*FOXP3*
APECED	Adrenal insufficiency, hypothyroidism, chronic mucocutaneous candidiasis	Organ-specific autoantibodies	*AIRE*

FASL, Fas ligand; *CASP8*, caspase 8; *CASP10*, caspase-10, *NRAS*, neuroblastoma *RAS* viral oncogene homolog; *FOXP3*, forkhead box protein 3; *AIRE*, autoimmune regulator.

The identification of this new class of defects has also opened up potentially new therapeutic approaches, including the use of IFN-γ to augment antibiotics in selected patients with recurrent mycobacterial disease. In the case of herpes simplex encephalitis, the findings that patients with UNC-93B and TLR-3 defects have diminished virally induced type 1 interferon production suggests that supplementation of conventional antiviral therapy with IFN-α could be beneficial in terms of decreasing morbidity, but this study has yet to be undertaken.[49]

EVALUATING SUSPECTED COMPLEMENT DISORDERS

When to suspect

The clinical setting in which complement defects should be suspected depends on the site of the defect. Abnormalities in the early components of the classical complement pathway (C1, C4, and C2) typically manifest as systemic lupus erythematosus–like autoimmunity, but recurrent sinopulmonary infections are also seen, especially in C2 deficiency.[50] Defects in C3 produce a clinical phenotype that is indistinguishable from an antibody defect, although this complement deficiency is markedly less frequent than humoral immunodeficiencies.[51] Defects in the late components of complement producing defects in the generation of the membrane attack complex (C5-C9) present with increased susceptibility to infections with *Neisseria* species that might not manifest until adolescence or young adulthood.[51] Clinically, these patients manifest neisserial meningitis, sepsis, or gonococcal arthritis. Alternative complement pathway defects, including properdin, factor B and factor D deficiencies also present with severe neisserial and other bacterial infections. Factor H deficiency is associated with atypical (not associated with diarrhea) hemolytic uremic syndrome or glomerulonephritis and also with secondary C3 deficiency that can result in recurrent pyogenic infections.[51] Finally, C1 esterase inhibitor deficiency causes hereditary angioedema, whereas DAF (decay-accelerating factor) and CD59 defects are seen in patients with paroxysmal nocturnal hemoglobinuria.[51]

Laboratory evaluation

The best screening test for defects in the classical complement pathway is the total hemolytic complement activity (CH50) assay, whereas the AH50 assay screens for defects in the alternative complement pathway. Assuming correct handling of the serum sample (complement components are very labile), a classical complement component deficiency will result in virtual absence of hemolysis on CH50 testing in contrast to the markedly decreased but not absent results seen in diseases like systemic lupus erythematosus. A decreased AH50 test result suggests a deficiency in factor B, factor D, or properdin. A decrease in both CH50 and AH50 test results suggests deficiency in a shared complement component (from C3 to C9).

Selected component immunoassays are available in larger laboratories, whereas specific component functional testing is typically only available in a very limited number of specialized complement laboratories.

EVALUATING SUSPECTED IMMUNE DYSREGULATION DISORDERS

When to suspect

Under this category are included monogenic autoimmune disorders, such as the autoimmune lymphoproliferative syndrome (ALPS); the immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX); and the autoimmune, polyendocrinopathy, candidiasis, ectodermal dystrophy syndrome (APECED; Table IX). Patients with ALPS present early in life with persistent nonmalignant lymphadenopathy and splenomegaly commonly accompanied by immune thrombocytopenia, hemolytic anemia, or both.[52] Organ-specific or vasculitic-type autoimmunity is rarely seen in patients with ALPS. IPEX is an immunologic emergency and typically presents in the neonatal period with severe watery or bloody diarrhea, skin eczema, and type 1 diabetes.[53] An immediate diagnosis is mandatory because these children require aggressive immunosuppression to control the acute symptoms, and bone marrow transplantation is currently the only curative therapy that should be undertaken before islet cells are destroyed, if at all possible. Finally, APECED is characterized by endocrine organ–directed autoimmunity (adrenal insufficiency and hypothyroidism) and chronic mucocutaneous candidiasis.[54] Patients might also have type 1 diabetes, gonadal failure, pernicious anemia, autoimmune hepatitis, and cutaneous manifestations. This is usually not a life-threatening condition, and immunosuppression is usually not required, with specific therapy directed at the endocrine abnormalities.

Laboratory evaluation

The diagnosis of ALPS currently requires the presence of compatible clinical symptoms and the presence of a characteristic T-cell population on immunophenotyping that expresses CD3 and

αβ-TCR but does not express CD4 or CD8 markers, which are referred to as double-negative T cells (Table IX). Determination of this T-cell subpopulation requires the use of antibodies to αβ-TCR because most double-negative T cells in normal samples are $\gamma\delta$-TCR$^+$ and are not relevant for establishing a diagnosis of ALPS. Normal ranges for αβ double-negative T cells should be established for each laboratory. At the National Institutes of Health, more than 1% of the total lymphocyte population is considered abnormal in adults. Other supporting features include hypergammaglobulinemia and increased plasma IL-10, vitamin B12, and soluble Fas ligand levels (J.B.O. and T.A.F., unpublished observations).[55] In addition, for a diagnosis of certainty, one must demonstrate defective lymphocyte apoptosis *in vitro* or the presence of a mutation on *FAS*, *FASL* (FAS ligand), *CASP8* (caspase-8), *CASP10* (caspase-10), or *NRAS* (neuroblastoma RAS viral oncogene homolog).[56-61]

Screening for IPEX is based on demonstrating absent or diminished population of forkhead box protein 3 (Foxp3)–expressing CD4 T cells in the peripheral blood, as assessed by intracellular flow cytometry. Another common laboratory finding is a marked increase in IgE levels. The gold standard for diagnosis is the demonstration of mutations on the *FOXP3* gene. However, in approximately 50% of patients with clinical findings compatible with IPEX, no mutation is demonstrated (Troy Torgerson, personal communication). Diagnosis of APECED in the setting of a clinically consistent phenotype currently requires sequencing of the *AIRE* (autoimmune regulator) gene.

CONCLUSION

Laboratory testing serves as the critical approach necessary for evaluating immune function in the setting of a patient with a history of recurrent infections, unusual infections, or both. The appropriate and directed use of immune function testing provides not only critical diagnostic information but also directs decisions regarding the most appropriate therapy. The latter is crucial to limit disease-associated morbidity. The use of the laboratory in evaluating the immune system should not follow a shotgun approach but rather should be a focused evaluation using specific testing in an orderly process based on the clinical history.

REFERENCES

1. Yong PF, Chee R, Grimbacher B. Hypogammaglobulinaemia. Immunol Allergy Clin North Am 2008;28:691-713, vii.
2. Conley ME, Dobbs AK, Farmer DM, Kilic S, Paris K, Grigoriadou S, et al. Primary B cell immunodeficiencies: comparisons and contrasts. Annu Rev Immunol 2009;27:199-227.
3. Conley ME, Howard V. Clinical findings leading to the diagnosis of X-linked agammaglobulinemia. J Pediatr 2002;141:566-71.
4. Ersoy F, Berkel AI, Sanal O, Oktay H. Twenty-year follow-up of 160 patients with ataxia-telangiectasia. Turk J Pediatr 1991;33:205-15.
5. Hanson EP, Monaco-Shawver L, Solt LA, Madge LA, Banerjee PP, May MJ, et al. Hypomorphic nuclear factor-kappaB essential modulator mutation database and reconstitution system identifies phenotypic and immunologic diversity. J Allergy Clin Immunol 2008;122:1169-77, e1116.
6. Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. Science 1993;259:990-3.
7. Ochs HD, Buckley RH, Kobayashi RH, Kobayashi AL, Sorensen RU, Douglas SD, et al. Antibody responses to bacteriophage phi X174 in patients with adenosine deaminase deficiency. Blood 1992;80:1163-71.
8. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood 2008;111:77-85.
9. Notarangelo LD, Giliani S, Mazza C, Mella P, Savoldi G, Rodriguez-Perez C, et al. Of genes and phenotypes: the immunological and molecular spectrum of combined immune deficiency. Defects of the gamma(c)-JAK3 signaling pathway as a model. Immunol Rev 2000;178:39-48.
10. Villa A, Notarangelo LD, Roifman CM. Omenn syndrome: inflammation in leaky severe combined immunodeficiency. J Allergy Clin Immunol 2008;122:1082-6.
11. Muller SM, Ege M, Pottharst A, Schulz AS, Schwarz K, Friedrich W. Transplacentally acquired maternal T lymphocytes in severe combined immunodeficiency: a study of 121 patients. Blood 2001;98:1847-51.
12. Fleisher TA, Oliveira JB. Functional and molecular evaluation of lymphocytes. J Allergy Clin Immunol 2004;114:227-35.
13. Sullivan KE. Chromosome 22q11.2 deletion syndrome: DiGeorge syndrome/velocardiofacial syndrome. Immunol Allergy Clin North Am 2008;28:353-66.
14. Kawai S, Minegishi M, Ohashi Y, Sasahara Y, Kumaki S, Konno T, et al. Flow cytometric determination of intracytoplasmic Wiskott-Aldrich syndrome protein in peripheral blood lymphocyte subpopulations. J Immunol Methods 2002;260:195-205.
15. Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. J Immunol Methods 1995;188:117-28.
16. Douek, et al. Changes in thymic function with age and during the treament of HIV infection. Nature 1998;396:690-5.
17. Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. J Allergy Clin Immunol 2005;115:391-8.
18. Routes JM, Grossman WJ, Kumar A, Verbsky J, Brokopp C, Laessig RH, et al. Update of statewide newborn screening program for severe combined immunodeficiency (SCID) by T-cell receptor excision circles (TRECs). J Allergy Clin Immunol 2009;123(suppl):S68.
19. Pilch H, Hohn H, Freitag K, Neukirch C, Necker A, Haddad P, et al. Improved assessment of T-cell receptor (TCR) VB repertoire in clinical specimens: combination of TCR-CDR3 spectratyping with flow cytometry-based TCR VB frequency analysis. Clin Diagn Lab Immunol 2002;9:257-66.
20. Rosenzweig SD, Holland SM. Phagocyte immunodeficiencies and their infections. J Allergy Clin Immunol 2004;113:620-6.
21. Martire B, Rondelli R, Soresina A, Pignata C, Broccoletti T, Finocchi A, et al. Clinical features, long-term follow-up and outcome of a large cohort of patients with chronic granulomatous disease: an Italian multicenter study. Clin Immunol 2008;126:155-64.
22. Ozcan E, Notarangelo LD, Geha RS. Primary immune deficiencies with aberrant IgE production. J Allergy Clin Immunol 2008;122:1054-63.
23. Grimbacher B, Holland SM, Puck JM. Hyper-IgE syndromes. Immunol Rev 2005; 203:244-50.
24. Boztug K, Welte K, Zeidler C, Klein C. Congenital neutropenia syndromes. Immunol Allergy Clin North Am 2008;28:259-75, vii-viii.
25. Oliveira JB, Notarangelo LD, Fleisher TA. Applications of flow cytometry for the study of primary immune deficiencies. Curr Opin Allergy Clin Immunol 2008;8:499-509.
26. Etzioni A. Leukocyte adhesion deficiencies: molecular basis, clinical findings, and therapeutic options. Adv Exp Med Biol 2007;601:51-60.
27. Jirapongsananuruk O, Malech HL, Kuhns DB, Niemela JE, Brown MR, Anderson-Cohen M, et al. Diagnostic paradigm for evaluation of male patients with chronic granulomatous disease, based on the dihydrorhodamine 123 assay. J Allergy Clin Immunol 2003;111:374-9.
28. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med 2007;357:1608-19.
29. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature 2007;448:1058-62.
30. Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, et al. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 2008;452:773-6.
31. Nichols KE, Ma CS, Cannons JL, Schwartzberg PL, Tangye SG. Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. Immunol Rev 2005;203:180-99.
32. Filipovich AH. Hemophagocytic lymphohistiocytosis and other hemophagocytic disorders. Immunol Allergy Clin North Am 2008;28:293-313, viii.
33. Marsh RA, Villanueva J, Zhang K, Snow AL, Su HC, Madden L, et al. A rapid flow cytometric screening test for X-linked lymphoproliferative disease due to XIAP deficiency. Cytometry B Clin Cytom 2009;76:334-44.
34. Shinozaki K, Kanegane H, Matsukura H, Sumazaki R, Tsuchida M, Makita M, et al. Activation-dependent T cell expression of the X-linked lymphoproliferative disease gene product SLAM-associated protein and its assessment for patient detection. Int Immunol 2002;14:1215-23.
35. Marcenaro S, Gallo F, Martini S, Santoro A, Griffiths GM, Arico M, et al. Analysis of natural killer-cell function in familial hemophagocytic lymphohistiocytosis (FHL): defective CD107a surface expression heralds Munc13-4 defect and discriminates between genetic subtypes of the disease. Blood 2006;108:2316-23.
36. Kogawa K, Lee SM, Villanueva J, Marmer D, Sumegi J, Filipovich AH. Perforin expression in cytotoxic lymphocytes from patients with hemophagocytic lymphohistiocytosis and their family members. Blood 2002;99:61-6.

37. Casanova JL, Fieschi C, Zhang SY, Abel L. Revisiting human primary immunodeficiencies. J Intern Med 2008;264:115-27.
38. Al-Muhsen S, Casanova JL. The genetic heterogeneity of mendelian susceptibility to mycobacterial diseases. J Allergy Clin Immunol 2008;122:1043-52.
39. Filipe-Santos O, Bustamante J, Chapgier A, Vogt G, de Beaucoudrey L, Feinberg J, et al. Inborn errors of IL-12/23- and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. Semin Immunol 2006;18:347-61.
40. Jouanguy E, Lamhamedi-Cherradi S, Lammas D, Dorman SE, Fondaneche MC, Dupuis S, et al. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. Nat Genet 1999;21:370-8.
41. Fleisher TA, Dorman SE, Anderson JA, Vail M, Brown MR, Holland SM. Detection of intracellular phosphorylated STAT-1 by flow cytometry. Clin Immunol 1999;90:425-30.
42. von Bernuth H, Picard C, Jin Z, Pankla R, Xiao H, Ku CL, et al. Pyogenic bacterial infections in humans with MyD88 deficiency. Science 2008;321:691-6.
43. Picard C, von Bernuth H, Ku CL, Yang K, Puel A, Casanova JL. Inherited human IRAK-4 deficiency: an update. Immunol Res 2007;38:347-52.
44. Doffinger R, Smahi A, Bessia C, Geissmann F, Feinberg J, Durandy A, et al. X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. Nat Genet 2001;27:277-85.
45. von Bernuth H, Puel A, Ku CL, Yang K, Bustamante J, Chang HH, et al. Septicemia without sepsis: inherited disorders of nuclear factor-kappa B-mediated inflammation. Clin Infect Dis 2005;41(suppl 7):S436-9.
46. Zhang SY, Jouanguy E, Ugolini S, Smahi A, Elain G, Romero P, et al. TLR3 deficiency in patients with herpes simplex encephalitis. Science 2007;317:1522-7.
47. Casrouge A, Zhang SY, Eidenschenk C, Jouanguy E, Puel A, Yang K, et al. Herpes simplex virus encephalitis in human UNC-93B deficiency. Science 2006;314:308-12.
48. von Bernuth H, Ku CL, Rodriguez-Gallego C, Zhang S, Garty BZ, Marodi L, et al. A fast procedure for the detection of defects in Toll-like receptor signaling. Pediatrics 2006;118:2498-503.
49. Sancho-Shimizu V, Zhang SY, Abel L, Tardieu M, Rozenberg F, Jouanguy E, et al. Genetic susceptibility to herpes simplex virus 1 encephalitis in mice and humans. Curr Opin Allergy Clin Immunol 2007;7:495-505.
50. Truedsson L, Bengtsson AA, Sturfelt G. Complement deficiencies and systemic lupus erythematosus. Autoimmunity 2007;40:560-6.
51. Botto M, Kirschfink M, Macor P, Pickering MC, Wurzner R, Tedesco F. Complement in human diseases: Lessons from complement deficiencies. Mol Immunol 2009;46:2774-83.
52. Oliveira JB, Fleisher T. Autoimmune lymphoproliferative syndrome. Curr Opin Allergy Clin Immunol 2004;4:497-503.
53. Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked: forkhead box protein 3 mutations and lack of regulatory T cells. J Allergy Clin Immunol 2007;120:744-51.
54. Moraes-Vasconcelos D, Costa-Carvalho BT, Torgerson TR, Ochs HD. Primary immune deficiency disorders presenting as autoimmune diseases: IPEX and APECED. J Clin Immunol 2008;28(suppl 1):S11-9.
55. Magerus-Chatinet A, et al. FAS-L, IL-10, and double-negative CD4-CD8-TCR alpha/beta+ T cells are reliable markers of autoimmune lymphoproliferative syndrome (ALPS) associated with FAS loss of function. Blood 2009;113: 3027-30.
56. Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 1995;81:935-46.
57. Bi LL, Pan G, Atkinson TP, Zheng L, Dale JK, Makris C, et al. Dominant inhibition of Fas ligand-mediated apoptosis due to a heterozygous mutation associated with autoimmune lymphoproliferative syndrome (ALPS) Type Ib. BMC Med Genet 2007;8:41.
58. Chun HJ, Zheng L, Ahmad M, Wang J, Speirs CK, Siegel RM, et al. Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. Nature 2002;419:395-9.
59. Rieux-Laucat F, Le Deist F, Hivroz C, Roberts IA, Debatin KM, Fischer A, et al. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. Science 1995;268:1347-9.
60. Holzelova E, Vonarbourg C, Stolzenberg MC, Arkwright PD, Selz F, Prieur AM, et al. Autoimmune lymphoproliferative syndrome with somatic Fas mutations. N Engl J Med 2004;351:1409-18.
61. Wang J, Zheng L, Lobito A, Chan FK, Dale J, Sneller M, et al. Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. Cell 1999;98:47-58.

Allergen immunotherapy

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Specific immunotherapy (SIT) involves the administration of allergen extracts to achieve clinical tolerance of those allergens that cause symptoms in patients with allergic conditions. Immunotherapy is effective in patients with mild forms of allergic disease and also in those who do not respond well to standard drug therapy. Most SIT is given by means of injection, but there is increasing interest in performing SIT through the sublingual route. SIT remains the treatment of choice for patients with systemic allergic reactions to wasp and bee stings and should be considered as an option in patients with allergic rhinitis, asthma, or both. SIT can modify the course of allergic disease by reducing the risk of new allergic sensitizations and inhibiting the development of clinical asthma in children treated for allergic rhinitis. The precise mechanisms responsible for the beneficial effects of SIT remain a matter of research and debate. An effect on regulatory T cells seems most probable and is associated with switching of allergen-specific B cells toward IgG4 production. Few direct comparisons of SIT and drug therapy have been made. Existing data suggest that the effects of SIT take longer to develop, but once established, SIT achieves long-lasting relief of allergic symptoms, whereas the benefits of drugs only last as long as they are continued. (J Allergy Clin Immunol 2010;125:S306-13.)

Key words: *Immunotherapy, immunomodulation, rhinitis, asthma, T cell, B cell, IgE, IgG, sublingual*

In allergen specific immunotherapy (SIT) allergen extracts are given to patients with allergic conditions to modify or abolish their symptoms. The process is specific in that SIT targets those allergens identified by the patient and physician as responsible for symptoms. Although the precise mechanisms involved remain uncertain, there is a substantial body of clinical evidence and practice to support the use of SIT. Before deciding to use SIT, the patient's condition needs to be carefully assessed, with particular regard to allergic triggers. In addition, because the course of treatment is lengthy and relatively expensive, there must also be an assessment of the risks and costs compared with those of symptomatic treatment with antihistamines and topical corticosteroids.

Abbreviations used
EPD: Enzyme-potentiated desensitization
SIT: Specific immunotherapy
SLIT: Sublingual immunotherapy
VIT: Venom immunotherapy

Immunotherapy was first developed at St Mary's Hospital London at the end of the 19th century,[1] and many of the basic principles described by Noon and Freeman remain valid today. However, over the years, SIT has evolved in different ways in different centers and in different countries, leading to varied treatment regimens and distinct philosophic approaches to the therapy. Indeed, much of the early literature on SIT is striking for its clinical empiricism and the lack of the type of objective evidence that would be required if this technique were to be introduced nowadays. Unfortunately, this has allowed critics to level charges of unscientific practice against allergists, even though the same point could be made about a whole range of medical practice. In recent years, clinical trials conducted according to modern principles have confirmed the effectiveness of SIT and have validated several of the alternative regimens that have been tried over the years. However, there is still a range of clinical practice and a variety of strongly held opinion about the best way to perform SIT. In particular, American allergists tend to treat for all sensitivities identified as clinically relevant on skin testing using mixtures of extracts prepared from bulk vials, whereas in Europe patients are normally only treated with a single allergen, which is supplied direct from the manufacturer. Mixed allergen extracts are available and used in some parts of Europe but only as custom mixes from manufacturers. Another difference in clinical practice is that allergen extracts used in the United States are prepared in the allergist's office, whereas those used in Europe are usually supplied by the manufacturer in their final form. European extracts are dialyzed to remove low-molecular-weight components and standardized according to their ability to elicit a wheal. In the United States extracts might not be dialyzed; although ragweed and cat extracts are standardized in terms of major allergen content, most extracts are standardized by their ability to elicit erythema rather than wheal. However, at the end of the day, the basic aims and principles of SIT are similar worldwide: the differences are in the details.

Typically, patients receive a course of injections, starting with a very low dose of allergen and building up gradually until a plateau or maintenance dose is achieved. Maintenance injections are then given at 4- to 6-week intervals for 3 to 5 years. The updosing phase is generally given as a series of weekly injections, but several alternative induction regimens have been tried, some giving several doses on each day and then waiting a week before giving a further series of injections (cluster protocol), whereas others give the whole series of incremental injections in a single day (rush protocol). The main drawback to the rush protocol is the risk of adverse reactions, which are much more common than in conventional or cluster protocols. On the other hand, full

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protection against anaphylaxis induced by Hymenoptera stings can be attained in a few days compared with the 3 months required with the conventional regimen.

MECHANISMS OF IMMUNOTHERAPY

The primary reason for studying the mechanisms of SIT is to seek out the element or elements that are biologically important and hence devise new forms of immunotherapy that might improve efficacy, increase safety margins, shorten treatment courses, or achieve more durable results. Several mechanisms have been proposed to explain the beneficial effects of immunotherapy (Table I). Whether administered by means of injection or sublingually, SIT induces changes in T-cell and antibody responses. The challenge for clinical scientists has been to work out which of the observed changes drive the clinical benefit and which are just epiphenomena. Allergen-specific IgE levels increase temporarily during the initial phase of SIT but fall back to pretreatment levels during maintenance therapy.[2] The immediate wheal-and-flare response to skin testing usually reduces during the initial phases of SIT, but this effect is relatively small compared with the degree of clinical benefit. In contrast, the late-phase response to skin testing is virtually abolished after successful SIT. Similar patterns are observed for late-phase responses in the nose and airways.[3] SIT also induces allergen-specific IgG antibodies, particularly antibodies of the IgG4 subclass. At one time, it was believed that these antibodies might intercept the allergenic particles at the mucosal surface and "block" the allergic response. Current opinion is against this, partly because the increase in IgG levels follows rather than precedes the onset of clinical benefit and partly because many mast cells are on the mucosal surfaces and therefore meet allergen before antibodies can interpose themselves. Moreover, there is a poor correlation between the amount of allergen-specific IgG and clinical protection. In most studies the IgG level correlates better with the dose of allergen that has been given rather than with the degree of protection achieved. That said, there has been a recent resurgence of interest in a possible inhibitory role of specific IgG antibodies in grass pollen immunotherapy.[4] In particular, the time course of this effect raises the possibility of specific IgG antibodies interfering with IgE-dependent cytokine secretion from mast cells or facilitated antigen presentation to T cells.

SIT also induces changes in allergen-specific T-cell responses. In nasal and skin allergen challenge models, successful SIT is accompanied by a reduction in T-cell and eosinophil recruitment in response to allergen. In parallel, there is a shift in the balance of T_H1 and T_H2 cytokine expression in the allergen-challenged site. T_H2 cytokine expression is not affected, but there is an increased proportion of T cells expressing the T_H1 cytokines IL-2, IFN-γ, and IL-12.[5-7] After venom SIT, there is induction of allergen-specific $CD4^+$ regulatory T cells that express CD25, forkhead box protein 3, and IL-10, as well as a shift in T_H1/T_H2 balance.[8,9] Similar findings have also been reported after SIT with inhalant allergens.[10] IL-10 has a complex series of actions on the immune response, including downregulation of T cells and induction of allergen-specific IgG4 antibodies, which probably explains the IgG4 response to SIT. If the IL-10 effect on T cells is what matters, then the IgG4 response should perhaps be viewed as a surrogate marker of IL-10 induction rather than the beneficial mechanism of SIT.[11] Overall, it is clear that SIT has a modulatory effect on allergen-specific T cells, and it seems that this is why clinical and late-phase responses are attenuated without suppression of allergen-specific antibody levels or immediate allergic responses.

TABLE I. Possible mechanisms of immunotherapy

Reduction in specific IgE levels (long-term)
Induction of IgG (blocking) antibodies
Reduced recruitment of effector cells
Altered T-cell cytokine balance (shift to T_H1 from T_H2)
T-cell anergy
Induction of regulatory T cells

CLINICAL INDICATIONS

SIT for venom hypersensitivity

Anaphylaxis to Hymenoptera venom is relatively rare but can be fatal. Venom-specific IgE antibodies are found in 30% to 40% of all adults for a few months after a sting, but these usually disappear in a few months. This response is related to the total serum IgE level and the patient's IgE response to inhalant allergens. Some unlucky subjects react more vigorously with high concentrations of venom-specific antibodies, which can persist for many years without further exposure to stings. This group of patients are at risk of anaphylaxis to subsequent stings, and a small number die from anaphylaxis each year. Precise figures are hard to come by, but a figure of at least 40 deaths per year in the United States has been cited. Additional sting-related deaths may have occured in persons reported to have died of unknown cause.[12]

The purpose of venom immunotherapy (VIT) is 2-fold: to reduce the risk of fatality and to improve the patient's quality of life by allowing him or her to go out and work or play without worrying about the possibility of a serious allergic reaction. Given the relatively small number of fatalities, the main effect of VIT is on a person's quality of life. The decision to proceed with VIT is based on a careful assessment of the patient, as well as an understanding of the natural history of venom allergy.[13] Patients who have experienced systemic symptoms after a sting are at much greater risk of anaphylaxis on subsequent stings compared with patients who have only had large local reactions. The frequency of systemic reactions to stings in children and adults with a history of large local reactions is about 5% to 10%, whereas the risk in patients with a previous systemic reaction is between 30% and 70%. In general, children are less at risk of repeated systemic reactions, as are those with a history of milder reactions. With time, the risk of a systemic reaction decreases: by 10 years after a previous systemic response, the risk is about 15% compared with the general population's risk of 2% to 3%. Occupational and geographic factors that might affect the likelihood of future stings should also be considered. Bee stings are much more common in beekeepers, their families, and their neighbors. For most persons, wasp stings are sporadic, but they are an occupational hazard for bakers, greengrocers, gardeners, tree surgeons, for example. Other factors to consider are the potential risks of emergency treatment with epinephrine and the various medical contraindications to SIT (see below).

Desensitization with venom accelerates the rate at which the risk decreases and rapidly provides protection against field and laboratory stings. After completing VIT, there is a residual risk of systemic reactions of approximately 10%, but when reactions do occur to stings after VIT, they are typically mild. Patients who

receive VIT should be supplied with antiallergic medication for use in the event of a sting during or after therapy. Some allergists recommend providing injectable epinephrine during therapy, but this is not generally considered necessary once the patient has reached the maintenance dose of SIT.

SIT for allergic rhinitis

SIT is a useful treatment for allergic rhinitis, especially when the range of allergens responsible is narrow. As with all forms of SIT, it is important to select patients appropriately. The allergic basis of the rhinitis should be carefully assessed based on both history and skin or blood test results, and other causes of nasal symptoms should be excluded. Direct challenge tests to assess nasal sensitivity to allergen are not used in routine clinical practice but might be useful for assessing effectiveness in clinical trials. The most difficult group to assess are patients with persistent nonseasonal rhinitis, especially those who have small positive skin test responses to house dust mite or other perennial allergens. In this group it can be extremely difficult to determine whether the patient's symptoms are truly due to allergy or whether they have nonallergic rhinitis and just happen to be sensitized to an allergen that is not clinically relevant. This difficulty in determining clinical relevance contributes to the reported lower degree of efficacy in SIT trials with perennial allergens compared with SIT for seasonal allergies.

The effectiveness of SIT in patients with intermittent (seasonal) allergic rhinitis has been confirmed in many trials with grass, ragweed, and birch pollen extracts.[14] Importantly, SIT has been shown to be effective even in patients with severe seasonal rhinitis caused by grass pollen that is resistant to conventional drug therapy.[15] Importantly, this study showed that patients with multiple allergic sensitizations responded at least as well as those who were monosensitized to grass pollen.

The benefits of 1 year's treatment wear off quickly,[16] but there are good data showing that 3 years' therapy provides lasting benefit.[17] Less well-controlled data show that the effects of SIT can persist for many years after discontinuing therapy.[18] This contrasts with conventional drugs, the effects of which wear off very soon after discontinuing therapy. The benefits of SIT for perennial rhinitis are less than those for seasonal rhinitis. In part, this reflects the difficulty in determining the extent to which allergy is responsible for perennial symptoms. Sensitization to house dust mite is common and does not always cause symptoms. Conversely, there are other causes of perennial rhinitis, including vasomotor instability, infection, and aspirin sensitivity. Nevertheless, clinical trials have shown a definite benefit in appropriately selected subjects. Clearer evidence has been obtained in patients with rhinitis caused by pet allergy. Several studies have shown a marked improvement in tolerance of cat exposure after SIT, which was confirmed both on challenge tests and simulated natural exposure.[19]

As with any therapy, the risks and cost-effectiveness of SIT need to be assessed on a case-by-case basis. Current drug therapy for rhinitis can be very effective, but a significant minority of patients have suboptimal control of their symptoms.[20] Some patients with rhinitis experience nosebleeds from intranasal steroids or excessive drowsiness from their antihistamines; others find pharmacotherapy inconvenient or ineffective. Moreover, we are now more aware of the adverse effects of rhinitis on quality of life. SIT offers a useful option for these patients, as well as a logical approach to dealing with the underlying problem.

SIT for asthma

Immunotherapy has been widely used to treat allergic asthma, although the introduction of effective inhaled therapies has changed the general pattern of asthma care. Concern over adverse reactions, including a small number of fatalities, has led some countries (eg, the United Kingdom) to restrict the use of SIT for asthma treatment, although asthma remains a common indication for SIT in many parts of North America and continental Europe.[21,22]

Current drug therapies for asthma aim to suppress airways inflammation and relieve bronchospasm. None of these treatments are curative, and asthma recurs rapidly on ceasing treatment. Allergen avoidance helps in some patients with allergic asthma, but although extreme forms of allergen avoidance (eg, admission to the hospital and sending children to holiday homes at altitude) can improve asthma control, there is only limited evidence for benefit with the degree of allergen avoidance that can be achieved in suburban homes. There is thus the scope for improving asthma care and for identifying allergen-specific therapies. SIT offers the possibility of deviating the immune response away from the allergic pattern and toward a more protective or less damaging response. However, SIT remains controversial as a treatment for asthma because of the potential side effects.

The efficacy of SIT in adult asthma has been assessed in many trials over the last 65 years. The results of these studies have often been difficult to interpret, either because poor-quality allergen extracts were used or because of poor study design. Many trials were not placebo controlled; they were either open or single blind, and in most cases, only small numbers of patients were treated. A recently updated meta-analysis[22] identified 75 articles published between 1954 and 2001. Thirty-six of these were for mite allergy, 20 for pollen allergy, 10 for animal dander allergy, 2 for mold allergy, and 1 for latex allergy, and 6 used combinations of allergens. Concealment of allocation was clearly adequate in only 15 trials. A wide variety of different measurements were made, which makes it difficult to comment on the overall effectiveness of SIT. Symptom scores improved in the treated groups; it was necessary to treat 4 patients to prevent 1 from experiencing symptom exacerbation and to treat 5 patients to prevent 1 from needing an increase in medication use. SIT reduced the airways response to inhalation of specific allergen and also improved nonspecific bronchial reactivity.

Three double-blind, placebo-controlled studies have found that SIT has a beneficial effect in patients with grass pollen–induced asthma, as assessed by a reduction in asthma symptom and treatment scores. Active treatment led to a 60% to 75% reduction in symptom scores compared with those seen in placebo-treated patients. An important study of SIT for ragweed allergy found that patients who received active injections had an improvement in peak flow rates during the pollen season, as well as reduced hay fever symptoms and reduced sensitivity to laboratory challenge with ragweed pollen extracts.[23] In addition, the active group required much less antiasthma medication. However, the parallel economic analysis indicated that the cost savings in asthma drugs was less than the costs of SIT.

In asthmatic patients sensitive to cats, SIT reduces both the early asthmatic response to inhaled allergen and responses to simulated natural exposure in a "cat room." Interestingly, there was no protection against allergen-induced increases in

nonspecific bronchial hyperresponsiveness, despite the clear delay in onset of symptoms and an overall reduction in symptoms and peak flow recordings after exposure to cats. Others have found reductions in both specific and nonspecific bronchial reactivity after SIT for cat allergy (measured by using inhalation challenges with cat extract and histamine, respectively).[24]

The main drawback in using SIT to treat asthma is the risk of serious adverse reactions. The vast majority of fatal reactions to SIT have occurred in patients with asthma, and although asthma is not an absolute contraindication, it is clear that patients with unstable asthma should not be offered SIT, and caution should be exercised in anyone with an increased level of asthma symptoms or transiently reduced peak flow rates.

Comparison of SIT with other types of treatment for asthma

The majority of clinical trials of SIT for patients with asthma have compared SIT either with untreated historical control subjects or with a matched placebo-treated group. To date, the effectiveness of SIT in patients with asthma has rarely been compared with conventional management (avoidance measures and inhaled or oral antiasthma drugs). One recent study assessed SIT in asthmatic children receiving conventional drug therapy and found no additional benefit in patients who were already receiving optimal drug therapy.[25] There were some significant flaws in the design of this study, and further work of this type is urgently needed.

Effects on natural history of allergic disease

Children often start with a limited range of allergic sensitivities and progress over time to have IgE against a wider range of inhaled allergens. Treatment with SIT might limit this tendency to acquire new sensitizations,[26] although the clinical benefit of this preventive effect is not clear. A proportion of patients with allergic rhinitis develop asthma each year. This annual rate of progression has been estimated at 5% in college students,[27] but this is perhaps surprisingly an area of considerable ignorance. A number of long-term epidemiologic studies are now in progress under the auspices of the International Study of Asthma and Allergies in Childhood, and these should eventually shed light on the rate of progression at different ages and the extent of regional and international variation. It has been suggested that SIT might modify the natural history of asthma in children who are known to be atopic but have not yet developed asthma. Only limited data are available to support this proposition. In the key study a group of 205 children aged 6 to 14 years without previously diagnosed asthma were treated with SIT for birch or grass pollen allergy in an open randomized design. Three years after completing treatment, 45% of the untreated group had asthma, whereas only 26% of the treated group had asthma. These results have been sustained out to 7 years after completing therapy. Thus 4 children had to be treated to prevent 1 case of asthma, which makes this an extremely effective therapy.[28] SIT might also modify the progression of established asthma. An early open study with uncharacterized mixed allergen extracts supported this view, with about 70% of treated children losing their asthma after 4 years' therapy compared with about 19% of untreated control subjects, a result that was sustained up to the age of 16 years. The proportion of children whose asthma was severe at age 16 years was also much lower in the treated group.[29] By modern standards, this study was not well designed, and it needs repeating with modern SIT extracts in an up-to-date trial design.

In contrast, there is no current evidence that SIT influences the evolution of established asthma in adults. Studies that have investigated withdrawal of therapy have found rapid recurrence of asthma symptoms, although rhinitis symptoms seem to show much more sustained relief after SIT.[30]

Thus SIT is a valid but controversial treatment for asthma. Although it seems entirely logical to try to treat allergic disorders by specifically suppressing the immune response to the triggering agents, the critical issue is whether SIT in its present form is the best option for managing patients with asthma. To assess this properly would require comparisons of best current SIT versus best current drug therapy, with robust end points including symptoms, objective measures of lung function, evaluation of cost/benefit ratios, safety, and quality of life. *In vitro* and *in vivo* measures, such as skin test responses or allergen-specific IgG4 measurements, are not sufficiently specific or sensitive to serve as surrogates for clinical efficacy. To date, there have been relatively few well-controlled studies of SIT in asthmatic subjects, but there is increasing evidence that SIT is beneficial in patients with mite-induced and pollen-induced asthma. The clinical efficacy of SIT in adult asthmatic patients sensitive to cats or molds is less certain, and no comparative studies with conventional treatment have been performed. Further clinical trials are indicated, particularly in patients with mild-to-moderate childhood asthma and also in patients with atopic disease who have not yet had asthma but are at high risk of progression to asthma.

Safety of SIT

The most obvious risk of SIT is that of provoking a systemic allergic reaction. In the United Kingdom between 1957 and 1986, 26 fatal reactions caused by SIT were reported to the Committee on Safety of Medicines.[31] The indication for SIT was documented in 17 of the fatal cases, 16 of whom were in patients receiving SIT to treat their asthma. Similarly, in the American Academy of Allergy, Asthma & Immunology inquiry into SIT-associated deaths, asthma appeared to be the cause of death in most of the fatal cases.[32,33] In those cases in which asthma was not cited as a contributory factor, asthma status was not documented, whereas bronchospasm was a feature of the clinical course of the fatal anaphylactic reactions. The incidence of systemic reactions in patients receiving SIT for asthma varies between series and has been reported to range from 5% to 35%. The central issue in using safety as an end point is that we have to accept that all treatments carry risks. Where differential risks exist between therapies, a more risky therapy can only be justified if that therapy offers substantial additional benefit over the safer therapy. The science of assessing risk/benefit ratios is still in its infancy, and we have to recognize that even when faced with the same facts, different patients and agencies can come to widely varying risk assessments. However, where possible, we should take steps to minimize the risks.

Separately, there is some concern about the use of immunomodulatory treatments in patients with autoimmune disorders, immunodeficiency syndromes, or malignant disease. Although there is no hard evidence that SIT is actually harmful to these patients, some clinicians feel uncomfortable about manipulating the immune system in such patients, not least because of the risk that spontaneous and unrelated variations in the autoimmune

disorder or cancer might be blamed on SIT. However, provided the risks and benefits are weighed and discussed with the patient, SIT can be administered where the risk/benefit ratio is considered to be in favor of treatment. Other medical contraindications to SIT include the coexistence of significant cardiac disease that might be exacerbated by any adverse reactions to SIT. β-Blockers are also contraindicated in patients receiving SIT. Although they do not increase the risk of adverse reactions, they will prevent the patient from responding to the epinephrine that might be needed to treat adverse reactions to SIT. Where the indication for SIT is strong, alternatives to β-blockers should be used so that the SIT can be given safely. Some clinics advise avoiding angiotensin-converting enzyme inhibitors because they can accentuate angioedema (angiotensin receptor antagonists [sartans] do not share this property).

Alternative forms of immunotherapy

Alternative allergy practice covers 3 principal themes: the use of unconventional diagnostic tests to seek causative agents for diseases that everyone agrees are allergic in origin; the use of unconventional therapies to treat allergic disease; and the diagnosis and therapy of diseases that are not conventionally considered to involve allergic mechanisms. Alternative immunotherapy regimens fall into the second of these categories, but the other 2 areas fall outside the scope of this review.

Unconventional forms of immunotherapy include the use of topical immunotherapy, enzyme-potentiated desensitization (EPD), and homeopathic desensitization.

Topical immunotherapy. High-dose topical immunotherapy regimens were used in the first half of the 20th century but subsequently fell into disrepute. The last 20 years have seen a revival of interest in sublingual immunotherapy (SLIT). The precise mechanisms by which sublingual SIT works remain unclear. In mice locally administered allergen is taken up by mucosal dendritic cells and then presented to T cells together with IL-12, biasing the response toward a T_H1 profile and away from the pro-IgE T_H2 profile. It is less clear whether this mechanism can suppress established allergic responses. In contrast, the immunologic response to SLIT in human studies has been relatively modest. Some changes have been found in skin sensitivity, but most studies have not found any change in systemic parameters, such as specific IgE, specific IgG, or T-cell cytokine balance.

A body of evidence has accumulated from well-conducted clinical trials indicating that SLIT can be effective, with up to 30% to 40% reductions in symptom scores and rescue medication use in patients with seasonal allergic rhinitis.[34] Treatment regimens typically involve a rapid build-up phase followed by treatment given either daily or 3 times per week with rapidly dissolving tablets containing allergen extracts. Some preparations are supplied in liquid form, with a calibrated dropper. A recent meta-analysis of SLIT found 22 studies in which 979 patients received active therapy.[34] Although many of these studies were small and inconclusive, the combined results indicate that SLIT is indeed effective, with an estimated power of about two thirds that seen in comparable studies of injected SIT. Local side effects were common but well tolerated.

In the grass pollen tablet trials about half the patients experienced some local irritation with the first dose. This was minor and generally did not require a reduction in subsequent doses. About half of those with initial side effects had lost these by the eighth day of treatment; only 1 in 25 of all patients had continuing local side effects after 3 months treatment.[35] Systemic side effects were relatively rare, and none of the side effects were judged to be life-threatening. For perennial allergens, less trials data are available,[36] and only limited data are available in children, although the most recent studies have been encouraging.[37,38] Other forms of topical immunotherapy (oral and nasal) have limited efficacy but are associated with high levels of side effects.

SLIT is now being used routinely in some parts of Europe (especially Italy and France), but often the doses and regimens being prescribed are different from those used in the clinical trials. As performed in the published trials, SLIT involves giving 20 to 400 times the total dose that would be given in a course of injected SIT. There is no evidence that giving smaller doses sublingually has any clinical effect. Overall, SLIT is likely to widen the scope of SIT and bring in additional prescribers. As with all forms of immunotherapy, patient selection will be the key to ensuring that therapy is targeted to those who are likely to benefit from it.

Some areas of uncertainty remain. For example, the optimum duration and durability of therapy have not been defined. Recent clinical trials have confirmed that the benefits of SLIT persist for the first year after discontinuing treatment, but if they do persist, for how long do they persist? Based on experience with injected SIT, manufacturers recommend that SLIT should be continued for 3 years, although most clinical trials were short-term (6-12 months). For seasonal allergens, most open-label use in clinical practice has been intermittent, starting 2 to 3 months before the season and stopping at the end of the season. However, the manufacturer of the only licensed product recommends starting 4 months before the first grass pollen season and continuing throughout the year for 3 years. This has major implications for direct costs and cost-effectiveness,[39] and some supporting data would be welcome.

The relative efficacy of SLIT and injected SIT has not been determined. The only published comparative studies were far too small to produce meaningful results.[40,41] Based on the effect size seen in the meta-analyses,[14,34] it seems likely that SLIT has between 60% and 100% of the efficacy of injected SIT, although it is difficult to make a true comparison.

EPD. In EPD very small doses of allergens are given together with the enzyme β-glucuronidase. The allergen doses are approximately 0.1% of the doses used in conventional SIT, and side effects are apparently not encountered. The theory behind EPD is that the β-glucuronidase enables the allergen to gain access to the immune system more efficiently than is possible with conventional SIT. No convincing evidence has been published to support the efficacy of EPD.

Homeopathic desensitization. A detailed discussion of the principles underlying homeopathy lies outside the scope of this chapter. However, homeopathy espouses the concept that diseases can be treated with very small doses of substances that cause similar symptoms. Some homeopathic remedies are mimics of the disorder, whereas others use the actual material that triggers the disorder. Thus homeopathic remedies for hay fever bear some superficial similarity to SIT. A systematic review of homeopathy has concluded that homeopathy did appear to offer some benefit in patients with hay fever and cited trials of homeopathy in hay fever as an example of good practice in homeopathic research.[42] However, a more recent, carefully controlled study of homoeopathy for house dust mite allergy found no evidence of any benefit in patients with asthma.[43]

TABLE II. Possible new technologies for immunotherapy

Recombinant allergens
Hypoallergenic allergens (bioengineered recombinant molecules)
T-cell peptide vaccines
T_H1 immunostimulants (eg, mycobacteria and CpG)
Allergen-immunostimulant complexes
Anti-IgE

FUTURE DIRECTIONS

There is scope to improve conventional SIT (Table II). Possible avenues include the use of recombinant allergens, which would improve standardization of allergen vaccines and might allow fine tuning of vaccines for patients with unusual patterns of reactivity. Most allergic patients react to the same components of an allergen extract, the so-called major allergens, which are defined as those allergens recognized by more than 50% of sera from a pool of patients with clinically significant allergy to the material in question. However, not all patients recognize all major allergens, and some patients only recognize allergens that are not recognized by the majority of allergic patient sera. This latter group might not respond to standard extracts but might be better treated with a combination of allergens to which they are sensitive. Now that recombinant allergens for SIT are available, the range of sensitivities can be better characterized, and this might lead to patient-tailored vaccine products. Thus far, clinical trials have confirmed the efficacy of recombinant allergen cocktails but have not yet shown superiority to conventional vaccines.[44]

Novel forms of allergenic molecules can be created; for example, a recombinant trimer consisting of 3 covalently linked copies of the major birch pollen allergen Bet v 1 has been made. This trimer is much less allergenic, even though it contains the same B-cell and T-cell epitopes as the native molecule and induces T_H1 cytokine release and IgG antibodies analogous to the antibody response to standard SIT.[45] Folding variants and other modifications of the physical structure might also improve the safety of SIT.[46]

Because the epitopes recognized by IgE molecules are usually 3-dimensional, whereas T-cell epitopes are short linear peptide fragments of the antigen, it should be possible to use peptide fragments of allergens to modulate T cells without risking anaphylaxis. Two distinct approaches have been tested. Either large doses of natural sequence peptides are given, deceiving the T cell into high-dose tolerance,[47] or else an altered peptide ligand can be given. Both approaches require consideration of the MHC type of the subject undergoing treatment. By means of sequential alteration of *Dermatophagoides pteronyssinus* peptides, it is possible to suppress proliferation of T-cell clones recognizing native *D pteronyssinus* peptides, as well as suppressing their expression of CD40 ligand and their production of IL-4, IL-5, and IFN-γ. These anergic T cells do not provide help for B cells in class switching to IgE, and importantly, this anergy cannot be reversed by providing exogenous IL-4.[48]

In an animal model intranasal application of genetically produced hypoallergenic fragments of Bet v 1 produced mucosal tolerance, with significant reduction of IgE and IgG1 antibody responses, as well as reduced cytokine production *in vitro* (IL-5, IFN-γ, and IL-10). These reduced immunologic responses were accompanied by inhibition of the cutaneous and airway responses that were seen with the complete Bet v 1 allergen. The mechanisms of immunosuppression seemed to be different for the allergen fragments and the whole molecule in that tolerance induced with the whole Bet v 1 molecule was transferable with spleen cells, whereas that induced by the fragments was not.[49]

From epidemiologic and experimental studies, we know that vaccination with mycobacteria has antiallergic properties. In Japan early vaccination with BCG was associated with a substantial reduction in the risk of allergy,[50] although similar associations were not evident in Sweden.[51] In an animal model it has been shown that administration of BCG before or during sensitization to ovalbumin reduces the degree of airway eosinophilia that follows subsequent challenge with ovalbumin. This effect is not mediated through any direct effect on IgE production or blood eosinophil numbers but is mediated through IFN-γ and can be reversed by exogenous IL-5.[52]

Two new approaches using DNA vaccines are also undergoing serious consideration. The first of these is a general approach, using CpG oligodeoxynucleotides that mimic bacterial DNA and stimulate T_H1-type cytokine responses. In a murine model of asthma, preadministration of CpG oligodeoxynucleotides prevented both airways eosinophilia and bronchial hyperresponsiveness.[53] Moreover, these effects were sustained for at least 6 weeks after CpG oligodeoxynucleotide administration.[54] An alternative approach is to couple CpG oligodeoxynucleotides to the allergenic protein, which enhances immunogenicity in terms of eliciting a T_H1-type response to the allergen but reduces its allergenicity[55] and stimulates T_H1 cytokine expression in cultured human PBMCs.[56] Initial clinical trials confirmed that the hybrid vaccine elicits a T_H1-pattern response,[57] but subsequent trials have been inconclusive. A contrasting approach is to use allergen-specific naked DNA sequences as vaccines. This technology is still in its infancy, but preliminary data suggest that administering naked DNA leads to production of allergens from within the airways epithelial cells.[58,59] Because of the different handling pathways for endogenous and exogenous allergens, it seems that the endogenously produced allergen elicits a T_H1-type response, and if this can be reproduced in allergic human subjects, it is hoped that this might overcome the existing T_H2-pattern response and eliminate the allergy. However, the potential for generating a powerful T_H1-type response to ubiquitous agents means that this approach will require careful evaluation in animal models before it can be pursued in human subjects.

CONCLUSIONS

SIT has been used for more than a century and is clinically effective in patients with rhinitis or asthma whose symptoms are clearly driven by allergic triggers. Perhaps surprisingly, we are still unsure exactly how SIT works, but we do know that SIT induces regulatory T cells that dampen the response to allergen exposure in sensitized subjects. When used in appropriately selected patients, SIT is effective and safe, but care is needed to recognize and treat adverse reactions. As well as careful patient selection, appropriate training of allergists and SIT clinic support staff is essential. Future directions in SIT will include the development of better standardized vaccines and the use of recombinant allergens, both of which should improve the safety profile of SIT. In parallel, the development of allergen-independent immunomodulatory therapies might allow more general approaches to be developed, which would be particularly advantageous for those patients who are sensitized to multiple allergens.

REFERENCES

1. Freeman J. Vaccination against hay fever: report of results during the first three years. Lancet 1914;1:1178.
2. Creticos PS, Van Metre TE, Mardiney MR, Rosenberg GL, Norman PS, Adkinson NF. Dose-response of IgE and IgG antibodies during ragweed immunotherapy. J Allergy Clin Immunol 1984;73:94-104.
3. Iliopoulos O, Proud D, Adkinson NF, Creticos PS, Norman PS, Kagey-Sobotka A, et al. Effects of immunotherapy on the early, late and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. J Allergy Clin Immunol 1991;87:855-66.
4. Francis J, James L, Paraskevopoulos G, Wong C, Calderon MA, Durham SR, et al. Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. J Allergy Clin Immunol 2008;121: 1120-5, e2.
5. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, Kay AB, et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T-lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing mRNA for interferon-gamma. J Allergy Clin Immunol 1996;97:1356-65.
6. McHugh SM, Deighton J, Stewart AG, Lachmann PJ, Ewan PW. Bee venom immunotherapy induces a shift in cytokine responses from a Th2 to a Th1 dominant pattern: comparison of rush and conventional immunotherapy. Clin Exp Allergy 1995;25:828-38.
7. Ebner C, Siemann U, Bohle B, Willheim M, Wiedermann U, Schenk S, et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from Th2 to Th1 in T-cell clones specific for Phl p1, a major grass pollen allergen. Clin Exp Allergy 1997;27:1007-15.
8. Jutel M, Akdis M, Blaser K, Akdis CA. Mechanisms of allergen specific immunotherapy—T-cell tolerance and more. Allergy 2006;61:796-807.
9. Nasser SM, Ying S, Meng Q, Kay AB, Ewan PW. IL-10 levels increase in cutaneous biopsies of patients undergoing wasp venom immunotherapy. Eur J Immunol 2001;31:3704-13.
10. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of IL-10 in specific immunotherapy. J Clin Invest 1998;102:98-106.
11. Bellinghausen I, Knop J, Saloga J. The role of IL-10 in the regulation of allergic immune responses. Int Arch Allergy Appl Immunol 2001;126:97-101.
12. Graft DF. Insect sting allergy. Med Clin North Am 2006;90:211-32.
13. Golden DB, Marsh DG, Freidhoff LR, Kwiterovich KA, Addison B, Kagey-Sobotka A, et al. Natural history of hymenoptera venom sensitivity in adults. J Allergy Clin Immunol 1997;100:760-6.
14. Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. Cochrane Database Syst Rev 2007; (1):CD001936.
15. Frew AJ, Powell RM, Corrigan CJ, Durham SR. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006;117:319-25.
16. Naclerio RM, Proud D, Moylan B, Balcer S, Freidhoff L, Kagey-Sobotka A, et al. A double blind study of the discontinuation of ragweed immunotherapy. J Allergy Clin Immunol 1997;100:293-300.
17. Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Nobel W, et al. Long-term clinical efficacy of grass pollen immunotherapy. N Engl J Med 1999; 341:468-75.
18. Eng PA, Borer-Reinhold M, Heijnen IA, Gnehm HP. Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. Allergy 2006;61:198-201.
19. Varney VA, Edwards J, Tabbah K, Brewster H, Mavroleon G, Frew AJ. Clinical efficacy of specific immunotherapy to cat dander: a double blind placebo controlled trial. Clin Exp Allergy 1997;27:860-7.
20. White PJ, Smith HE, Baker N, Davis W, Frew AJ. Symptom control in patients with hay fever in UK general practice: how well are we doing and is there a need for allergen immunotherapy? Clin Exp Allergy 1998;28:266-70.
21. Bousquet J, Lockey RF, Malling HJ. WHO position paper. Allergen immunotherapy: therapeutic vaccines for allergic disease. Allergy 1998;53(suppl):1-42.
22. Abramson M, Puy R, Weiner J. Immunotherapy in asthma: an updated systematic review. Allergy 1999;54:1022-41.
23. Creticos PS, Reed CE, Norman PS, Khoury J, Adkinson NF, Buncher CR, et al. Ragweed immunotherapy in adult asthma. N Engl J Med 1996;334:501-6.
24. Lilja G, Sundin B, Graff-Lonnevig V, Hedlin G, Heilborn H, Norrlind K, et al. Immunotherapy with partially purified and standardised animal dander extracts. IV. Effects of 2 years of treatment. J Allergy Clin Immunol 1989;83:37-44.
25. Adkinson NF, Eggleston PA, Eney D, Goldstein EO, Schuberth KC, Bacon JR, et al. A controlled trial of immunotherapy for asthma in allergic children. N Engl J Med 1997;336:324-31.
26. Des Roches A, Paradis L, Menardo JL, Bouges S, Daures JP, Bousquet J. Immunotherapy with a standardised *Dermatophagoides pteronyssinus* extract. VI. Specific immunotherapy prevents the onset of new sensitisations in children. J Allergy Clin Immunol 1997;99:450-3.
27. Horak F. Manifestation of allergic rhinitis in latent sensitised patients. A prospective study. Arch Otorhinolaryngol 1985;242:242-9.
28. Niggemann B, Jacobsen L, Dreborg S, Ferdousi HA, Halken S, Host A, et al. Five-year follow-up on the PAT study: specific immunotherapy and long-term prevention of asthma in children. Allergy 2006;61:855-9.
29. Johnstone DE, Dutton A. The value of hyposensitization therapy for bronchial asthma in children. A 14 year study. Pediatrics 1968;42:793-802.
30. Shaikh WA. Immunotherapy vs inhaled budesonide in bronchial asthma: an open parallel comparative trial. Clin Exp Allergy 1997;27:1279-84.
31. Committee on the Safety of Medicines. CSM update: immunotherapy. BMJ 1986; 293:948.
32. Stewart GE, Lockey RF. Systemic reactions from allergen immunotherapy. J Allergy Clin Immunol 1992;90:567-78.
33. Bernstein DI, Wanner M, Borish L, Liss GM. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990-2001. J Allergy Clin Immunol 2004; 113:1129-36.
34. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005;60:4-12.
35. Dahl R, Kapp A, Colombo G, de Monchy JG, Rak S, Emminger W, et al. Efficacy and safety of sublingual immunotherapy with once daily grass allergen tablets for seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006;118:434-40.
36. Alvarez-Cuesta E, Berges-Gimeno P, Mancebo EG, Fernandez-Caldas E, Cuesta-Herranz J, Casanovas M. Sublingual immunotherapy with a standardised cat dander extract: evaluation of efficacy in a double-blind placebo-controlled study [published erratum in Allergy 2007;62:1100]. Allergy 2007;62:810-7.
37. Di Rienzo V, Marcucci F, Puccinelli P, Parmiani S, Frati F, Sensi L, et al. Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: a 10 year prospective study. Clin Exp Allergy 2003;33:206-10.
38. Bufe A, Ziegler-Kirbach E, Stoeckmann E, Heidemann P, Gehlhar K, Holland-Letz T, et al. Efficacy of sublingual swallow immunotherapy in children with severe grass pollen allergic symptoms: a double-blind placebo-controlled study. Allergy 2004;59:498-504.
39. Bachert C, Vestenbaek U, Christensen J, Griffiths UK, Poulsen PB. Cost-effectiveness of grass allergen tablet (GRAZAX) for the prevention of seasonal grass-pollen-induced rhinoconjunctivitis—a Northern European perspective. Clin Exp Allergy 2007;37:772-9.
40. Khinchi MS, Poulsen LK, Carat F, Andre C, Hansen AB, Malling HJ. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. Allergy 2004;59:45-53.
41. Mauroa M, Russelloa M, Incorvaiab C, Gazzolaa GB, Di Carac G, Frati F. Comparison of efficacy, safety and immunologic effects of subcutaneous and sublingual immunotherapy in birch pollinosis: a randomized study. Allerg Immunol (Paris) 2007;39:119-22.
42. Reilly DT, Taylor MA, McSharry C, Aitchison T. Is homeopathy a placebo response? Controlled trial of homeopathic potency with pollen in hay fever as a model. Lancet 1986;2:881-6.
43. Lewith GT, Watkins AD, Hyland ME, Shaw S, Broomfield JA, Dolan G, et al. Use of ultramolecular potencies of allergen to treat asthmatic people allergic to house dust mite: double blind randomised controlled clinical trial. BMJ 2002;324:520.
44. Pauli G, Larsen TH, Rak S, Horak F, Pastorello E, Valenta R, et al. Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. J Allergy Clin Immunol 2008;122:951-60.
45. Valenta R, Niederberger V. Recombinant allergens for immunotherapy. J Allergy Clin Immunol 2007;119:826-30.
46. Purohit A, Niederberger V, Kronqvist M, Horak F, Gronneberg R, Suck R, et al. Clinical effects of immunotherapy with genetically modified recombinant birch pollen Bet v1 derivatives. Clin Exp Allergy 2008;38:1514-25.
47. O'Hehir RE, Yssel H, Verma S, de Vries JE, Spits H, Lamb JR. Clonal analysis of differential lymphokine production in peptide and superantigen-induced T-cell anergy. Int Immunol 1991;3:819-26.
48. Fasler S, Aversa G, de Vries JE, Yssel H. Antagonistic peptides specifically inhibit proliferation, cytokine production, CD40L expression and help for IgE synthesis by Der p 1-specific human T-cell clones. J Allergy Clin Immunol 1998;101:521-30.
49. Wiedermann U, Herz U, Baier K, Vrtala S, Neuhaus-Steinmetz U, Bohle B, et al. Intranasal treatment with a recombinant hypoallergenic derivative of the major birch pollen allergen Bet v 1 prevents allergic sensitization and airway inflammation in mice. Int Arch Allergy Immunol 2001;126:68-77.
50. Shirakawa T, Enomoto T, Shimazu SI, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. Science 1997;275:77-9.

51. Strannegaard IL, Larsson LO, Wennergren G, Strannegaard O. Prevalence of allergy in children in relation to prior BCG vaccination and infection with atypical mycobacteria. Allergy 1998;53:249-54.
52. Erb KJ, Holloway JW, Sobeck A, Moll H, Le Gros G. Infection of mice with *Mycobacterium bovis*-BCG suppresses allergen-induced airways eosinophilia. J Exp Med 1998;187:561-9.
53. Kline JN, Waldschmidt TJ, Businga TR, Lemish JE, Weinstock JV, Thorne PS, et al. Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. J Immunol 1998;160:2555-9.
54. Sur S, Wild JS, Choudury BK, Sur N, Alam R, Klinman DM. Long-term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligodeoxynucleotides. J Immunol 1999;162:6284-93.
55. Tighe H, Takabayashi K, Schwartz D, van Nest G, Tuck S, Eiden JJ, et al. Conjugation of immunostimulatory DNA to the short ragweed allergen Amb a 1 enhances its immunogenicity and reduces its allergenicity. J Allergy Clin Immunol 2000;106:124-34.
56. Marshall JD, Abtahi S, Eiden JJ, Tuck S, Milley R, Haycock F, et al. Immunostimulatory sequence DNA linked to the Amb a 1 allergen promotes Th1 cytokine expression while downregulating Th2 cytokine expression in PBMCs from human patients with ragweed allergy. J Allergy Clin Immunol 2001;108:191-7.
57. Creticos PS, Eiden JJ, Broide D, Balcer-Whaley SL, Schroeder JT, Khattignavong A, et al. Immunotherapy with immunostimulatory oligonucleotides linked to purified ragweed Amb a 1 allergen: effects on antibody production, nasal allergen provocation and ragweed seasonal rhinitis. J Allergy Clin Immunol 2002;109:743-4.
58. Hsu CH, Chua KY, Tao MH, Lai YL, Wu HD, Huang SK, et al. Immunoprophylaxis of allergen-induced IgE synthesis and airway hyperresponsiveness in vivo by genetic immunisation. Nat Med 1996;2:540-4.
59. Hartl A, Kiesslich J, Weiss R, Bernhaupt A, Mostbock S, Scheiblhofer S, et al. Immune responses after immunisation with plasmid DNA encoding Bet v 1, the major allergen of birch pollen. J Allergy Clin Immunol 1999;103:107-13.

Immunomodulator therapy: Monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins

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The immune system consists of a diverse array of immunocompetent cells and inflammatory mediators that exist in complex networks. These components interact through cascades and feedback circuits, maintaining physiologic inflammation (eg, tissue repair) and immunosurveillance. In various autoimmune and allergic diseases, a foreign antigen or autoantigen might upset this fine balance, leading to dysregulated immunity, persistent inflammation, and ultimately pathologic sequelae. In recent years, there has been tremendous progress delineating the specific components of the immune system that contribute to various aspects of normal immunity and specific disease states. With this greater understanding of pathogenesis coupled with advances in biotechnology, many immunomodulatory agents commonly called "biologic agents" have been introduced into the clinic for the treatment of various conditions, including immune globulins and cytokines. The 2 most common classes of approved biologic agents are mAbs and fusion proteins with exquisite specificity. These agents have the potential both to optimize outcomes through more thorough modulation of specific parts of the dysregulated immune response and to minimize toxicity compared with less specific methods of immunosuppression. (J Allergy Clin Immunol 2010;125:S314-23.)

Key words: *Monoclonal antibodies, fusion proteins, immunoglobulins, cytokines, autoimmunity*

Abbreviations used

AS: Ankylosing spondylitis
CHF: Congestive heart failure
CTLA-4: Cytotoxic T lymphocyte–associated antigen 4
DMARD: Disease-modifying antirheumatic drug
FDA: US Food and Drug Administration
ICAM: Intercellular adhesion molecule
IL-1Ra: IL-1 receptor antagonist
IVIG: Intravenous immunoglobulin
LFA: Lymphocyte function–associated antigen
MS: Multiple sclerosis
PML: Progressive multifocal leukoencephalopathy
PsA: Psoriatic arthritis
RA: Rheumatoid arthritis
SCIG: Subcutaneous immunoglobulin
SLE: Systemic lupus erythematosus

Biologic agents can work through several mechanisms. The simplest would be inhibition of the function of a target molecule by binding to it, thereby preventing ligation with its counter-receptor and downstream effects. Potential targets include (1) lineage- or activation status–specific molecules on B cells, T cells, and other immunocompetent cells; (2) soluble inflammatory mediators, such as cytokines, chemokines, complement proteins, enzymes, and immunoglobulin molecules; and (3) surface receptors for these mediators. Biologic agents can alter cell populations by engaging effector functions, including the complement cascade and antibody-dependent cellular cytotoxicity; of note, many mAbs and fusion proteins possess functional IgG Fc pieces. Cell depletion can also be induced by apoptosis subsequent to ligation of appropriate targets. Small-molecular-weight immunomodulators, such as glucocorticoids, are reviewed in Chapter 16.

MONOCLONAL ANTIBODIES

Monoclonal antibodies to human targets can be generated either in other species, such as mice, or through recombinant engineering (Fig 1). With chimeric mAbs, the variable region of a murine mAb is fused to the Fc piece of a human IgG molecule. The resulting construct is approximately one quarter murine. For humanized mAbs, only the complementarity determining regions from the original murine mAb are retained, resulting in a construct that is approximately 95% human. There are a number of approaches to create human mAbs to human targets, including immunizing human/severe combined immunodeficient murine chimeras, using EBV-transformed human B cells, and repertoire cloning, in which target antigen is used to capture human complementarity determining regions generated from vast human cDNA libraries, with the mAb then generated from there. Proteins such as mAbs can have residues of polyethylene glycol added. This process, called pegylation, enhances the half-life of the native protein by reducing its renal and cellular clearance after administration. Although even fully human proteins can be immunogenic, in general, the more human a construct, the less immunogenic. Pegylation might further reduce antigenicity and immunogenicity of the native protein. Immunogenicity can develop to molecules with amino acid sequences identical to human sequences related to factors such as differences in patterns of glycosylation. In addition, immunogenicity to mAbs can be anti-idiotypic. Other factors affecting immunogenicity include route of administration (intravenous vs subcutaneous), treatment paradigm (continuous vs intermittent), and concurrent use of immunosuppressive therapy.

Standard nomenclature for mAbs identifies their source with the last 4 or 5 letters: -omab, murine; -ximab, chimeric; -zumab,

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humanized; and -umab, human (Fig 1). The middle part of the name reflects the disease indication for which the mAb was initially intended: -lim- for immune and inflammatory diseases, -cir- for cardiovascular disorders, and -tu- for tumors or neoplastic conditions. The first 3 or 4 letters can be chosen by the sponsor/developer. A number of mAbs have been approved for human use; this chapter will focus on several key mAbs used in the treatment of autoimmune conditions.

FUSION RECEPTORS

Fusion proteins are typically composed of the extracellular domains of native transmembrane proteins, such as cell-surface receptors, linked to another molecule. In most cases the linker that has been used has been the Fc portion of human immunoglobulin, which enhances the pharmacokinetic properties of the construct. The Fc portion of the fusion receptor can be engineered to be functional or not. As their primary mechanism of action, fusion receptors competitively inhibit the binding of a ligand to its specific counterreceptor and thereby prevent downstream effects.

AGENTS THAT INHIBIT PROINFLAMMATORY CYTOKINES

In patients with autoimmune diseases, imbalances in the cytokine cascade can help the initiation and propagation of the immune driven inflammation. In several inflammatory arthritides, including rheumatoid arthritis (RA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS), the proinflammatory cytokine TNF-α has been shown to play a central role in inflammatory reactions and has proved to be an especially attractive target for biologic agents. Among its sundry activities, TNF-α activates various cell types, promotes accumulation of immunocompetent cells at sites of inflammation by means of activation of the vascular endothelium and upregulation of adhesion molecules, and stimulates synthesis of other proinflammatory cytokines (eg, IL-1, IL-6, and GM-CSF), chemokines (eg, IL-8), and other mediators. IL-1 also stimulates production of other proinflammatory cytokines, angiogenic factors, and endothelial adhesion molecules. Both TNF-α and IL-1 mediate bone and cartilage destruction through activation of osteoclasts (eg, receptor activator for nuclear factor κB ligand and macrophage colony-stimulating factor) and macrophages to release destructive mediators (eg matrix metalloproteinases, collagenase, and prostaglandins). IL-6 is a regulatory cytokine involved in T- and B-cell activation, osteoclast differentiation/activation, and other activities relevant to the pathogenesis of RA. Other immunomodulatory cytokines considered of significance in the treatment of infectious diseases and malignancies include interferon type I (α and β), IFN-γ, IL-2, and IL-7.

TNF inhibitors: Therapeutic uses

There are 5 currently available TNF inhibitors: infliximab, a chimeric anti–TNF-α mAb initially approved in 1998; etanercept, a recombinant soluble p75 TNF receptor (CD120b)–IgG Fc fusion protein initially approved in 1998; adalimumab, a human anti–TNF-α mAb initially approved in 2002; certolizumab pegol, a pegylated Fab′ fragment of a human anti–TNF-α antibody initially approved in 2008; and golimumab, a human anti–TNF-α mAb initially approved in 2009 (Table I). Although not all 5 TNF inhibitors are approved for the following conditions, TNF inhibitors are most commonly used for the treatment of RA, PsA, AS, Crohn disease, juvenile idiopathic arthritis, and psoriasis.

All 5 TNF inhibitors have been shown to substantially improve the signs and symptoms of disease, functional status, and quality of life and slow radiographic progression in patients with established RA.[1-8] Several studies have demonstrated an even greater clinical and radiographic response and the probability of disease remission among patients with early RA.[9-11] Interestingly, the inhibition of radiographic progression of disease seemed to be dissociated from clinical efficacy, as measured with the typically used composite scoring measures, such as the American College of Rheumatology 20% improvement criteria. Thus some patients who did not achieve an American College of Rheumatology 20% improvement criteria response still experienced inhibition of radiographic damage.[2,12] Although they can be administered as monotherapy, all TNF inhibitors appeared to be more effective when used in combination with disease-modifying antirheumatic drugs (DMARDs), commonly methotrexate. Combination therapy with methotrexate has beneficial pharmacokinetic effects for some TNF inhibitors in addition to clinical synergy for the treatment of RA.

Etanercept and adalimumab have been approved for the treatment of juvenile idiopathic arthritis.[13,14] Children who received TNF inhibitors either with or without methotrexate had better clinical outcomes, as measured by using the American College of Rheumatology Pediatric 30% (ACR Pedi 30) response, which represents a 30% or greater improvement in the signs and symptoms of juvenile idiopathic arthritis.

PsA is characterized by the association of inflammatory arthritis with skin psoriasis. The treatment of patients with PsA requires consideration of peripheral arthritis, axial arthritis, skin and nail involvement, dactylitis, and enthesitis. TNF-α levels are notably increased in biopsy samples of skin and synovial tissues from patients with PsA, providing a rationale for the use of TNF inhibitors in the treatment of PsA and psoriasis. TNF inhibitors have been shown to be highly effective in improving the signs and symptoms of arthritis and increasing functional status and quality of life among patients with PsA. Similar to the effect seen in patients with RA, TNF inhibitors also attenuated the progression of radiographic joint damage.[15-18] Moreover, dramatic improvements in the symptoms of skin psoriasis were achieved, as were improvements in the extra-articular involvement characteristics of PsA, such as dactylitis and enthesitis. Improvement in skin psoriasis with TNF inhibitor therapy has likewise been noted in patients without arthritis. Although improvements in joints and skin often occur in parallel, there might be discordance between dermatologic and articular outcomes in individual patients, suggesting potential heterogeneity to pathophysiologic mechanisms underlying different clinical manifestations.

Until the advent of TNF inhibitors, nonsteroidal anti-inflammatory drugs were the only agents shown to alleviate axial symptoms related to AS. In recent years, TNF inhibitors have demonstrated their ability to substantially decrease signs and symptoms of spinal inflammation.[19-23] Paralleling data from patients with RA, TNF inhibitors provided rapid clinical improvement, often as early as 2 weeks. Patients with increased acute-phase reactants at study entry or with evidence for spinal inflammation on magnetic resonance imaging tended to respond more favorably to TNF inhibitors. Because methotrexate is not an effective therapy for spinal inflammation in patients with AS, it has not been used in studies of the TNF inhibitors. A goal in

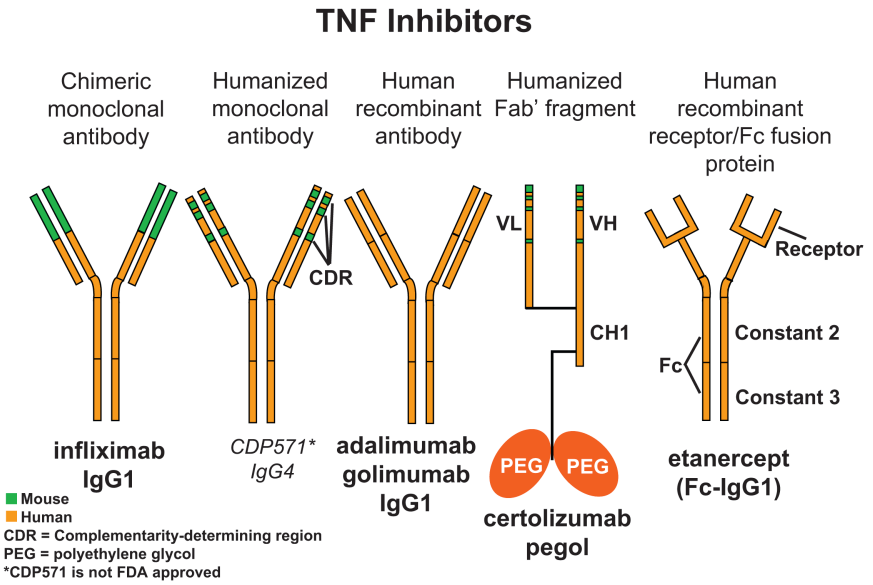

FIG 1. Structure and nomenclature of TNF inhibitors.

TABLE I. Characteristics of biologic agents: Dosing, half-life, and indications

Agent	Typical adult dosing	Mode of delivery	Half-life
Cytokine inhibitors			
Etanercept	25 mg biweekly or 50 mg every week	SQ	4-5 d
Infliximab	3-10 mg/kg q4-8 wk	IV	8-9.5 d
Adalimumab	40 mg every other week	SQ	12-14 d
Golimumab	50 mg every month	SQ	19-27 d
Certolizumab	200-400 mg every 2-4 wk	SQ	12-14 d
Anakinra	100 mg every day	SQ	4-6 h
Rilonacept	320 mg then 160 mg every week	SQ	6-8 d
Tocilizumab	4-8 mg/kg every 4 weeks	IV	12 d
T-cell modulators			
Abatacept	RA: 10 mg/kg every 4 weeks	IV	14.7 d
Alefacept	15 mg IM every week × 12 wk	IM	270 h
B-cell modulators			
Rituximab	1,000 mg every 2 wk × 2 doses	IV	60-170 h
Adhesion cell modulators			
Natalizumab	300 mg every 4 wk	IV	11 d

SQ, Subcutaneous; *IV*, intravenous; *IM*, intramuscular.

treating AS would be to stop the progression of spinal ankylosis. Despite their ability to attenuate spinal inflammation on a sensitive imaging modality, such as magnetic resonance imaging, TNF inhibitors have not seemed to be able to affect radiographic progression when compared with historical control of TNF inhibitor–naive patients with AS.[24]

Levels of TNF-α are increased in the mucosa of inflamed intestines and thought to exert deleterious effects relevant to the pathophysiology of inflammatory bowel disease (Crohn disease and ulcerative colitis). Treatments with TNF inhibitor mAbs have shown improvement in both clinical and endoscopic luminal fistulas and bowel mucosal inflammation associated with Crohn disease.[25-30] To date, etanercept has not been shown to be effective in inflammatory bowel disease.[29] Initially, treatment of Crohn disease with TNF inhibitors was reserved for the most severe, refractory fistulizing disease as a single course. After the success in this group of patients, repeated treatments and more chronic dosing regimens are being used. Intermittent use of infliximab, which is commonly used in the treatment of Crohn disease, has been associated with a greater propensity for the development of antibodies to infliximab and can be attenuated by the concomitant use of immunosuppressive agents, such as corticosteroids, azathioprine, methotrexate, and 6-mercaptopurine.[31] The use of infliximab in combination with immunosuppressive agents (eg, methotrexate and azathioprine) has been shown to enhance efficacy and decrease immunogenicity.[27] TNF inhibitor mAbs are also being studied and used in the treatment of ulcerative colitis.

Several studies, mostly anecdotal and in patients with RA, have demonstrated that switching from one TNF inhibitor to another can be effective and restore clinical response in patients who have lost therapeutic efficacy with the first.[32] Although the success of TNF inhibitors in these autoimmune conditions has been remarkable, it is worth noting that almost uniformly, treatment failed to induce long-term treatment-free remission or immunologic tolerance. Thus maintenance of clinical response required continuous therapy. Also, TNF inhibitors have not proved effective in other

conditions, including several wherein there was pathophysiologic evidence for a role for this cytokine in the disease process. Among autoimmune conditions, TNF inhibitor therapy has been notably ineffective to date in patients with Sjögren syndrome and several forms of vasculitis, including Wegener granulomatosis and polymyalgia rheumatica/temporal arteritis. With regard to congestive heart failure (CHF), data from animal models of ischemic cardiomyopathy implicated TNF as a key mediator of deteriorating cardiac function and hence an attractive target. However, TNF inhibitors have failed to improve symptoms in patients with CHF in clinical trials and sometimes resulted in worsened clinical outcome. Although limited, there were studies on TNF inhibitors that showed negative results in patients with multiple sclerosis (MS), along with anecdotal reports of the development or worsening of demyelinating symptoms among patients with RA treated with these agents. TNF inhibitors are still being actively investigated in a variety of other diseases.

TNF inhibitors: Safety considerations

In general, TNF inhibitors have been well tolerated in clinical trials. *In vitro* studies suggested that TNF inhibitors selectively decrease proinflammatory cytokine levels while preserving both the humoral and cell-mediated arms of the immune response. However, a number of relevant safety issues regarding the use of TNF inhibitors have emerged in postmarketing pharmacovigilance assessments.[33,34] Adverse events associated with TNF inhibitors can be broadly classified as target/class related or agent related. Target-related adverse events include those potentially attributable to the immunosuppression inherent in blocking a key component of the immune system, such as an inflammatory cytokine; this would include increased susceptibility to infections and malignancies. In addition, specific inhibition of TNF might predispose patients to increased susceptibility to tuberculosis, autoantibody production, hepatotoxicity, demyelinating disease, and clinical worsening of CHF. Agent-related adverse events, such as allergic reactions and antigenicity, are idiosyncratic reactions that relate to the particular agent used.

Safety data from clinical trials and registries have shown a small but consistent increase in infections among TNF inhibitor–treated patients compared with those treated with DMARDs, most commonly methotrexate. Generally, the risk of serious infections was not substantially greater, with relative risks ranging from 0 to 2. The risk of infection with TNF inhibitors increased significantly when combined with other biologic agents. For example, combination therapy with the TNF inhibitor etanercept and the IL-1 receptor antagonist (IL-1Ra) anakinra resulted in a higher rate of serious infections in patients with RA, despite the failure to achieve any additive clinical benefit. Data, particularly from pharmacovigilance, have noted a number of opportunistic infections (eg, listeriosis, histoplasmosis, and coccidioidomycosis) among those patients treated with TNF inhibitors. Because of the increased baseline risk of infection among patients with RA, without a control group, it is difficult to ascertain the excess infection risk specifically attributable to TNF inhibitors in these patients. Another potential sequela of immunosuppression is malignancy. With a few notable exceptions, the bulk of the data to date do not support an increased risk of solid tumors related to TNF inhibitor therapy. However, greater numbers of hematologic malignancies, particularly non-Hodgkin lymphoma, have been observed in some registries. Complicating the assessment of the risk attributable to therapy is the increased baseline risk of lymphoma among patients with RA, especially among those with higher disease activity. This introduces bias toward observing cases among patients treated with TNF inhibitors as the most severe, and patients with active RA are often the most common type of patients treated. The relative effect of dose and duration of therapy and host factors, such as comorbidities, relevant genetic polymorphisms, and concomitant medications, on the risk of infections and malignancy remains incompletely defined. Because of potential immunosuppression, vaccination with live vaccines is not recommended while patients are receiving TNF inhibitors.

In addition, inhibition of TNF might predispose patients to a variety of untoward effects that seem to be specific to inhibition of the TNF molecule. There are a fair amount of animal and *ex vivo* data supporting the important role played by TNF in controlling tuberculosis. In contrast to typical presentation of acute tuberculosis as pneumonia, about half of the cases of tuberculosis related to TNF inhibitors presented as extrapulmonary or disseminated tuberculosis. The majority of these tuberculosis cases appear to be reactivation of latent tuberculosis, with infection occurring within the first few months of therapy; however, newly acquired cases have been well described. The incidence of cases might be greater with the mAb TNF inhibitors than with the fusion protein inhibitor. Fortunately, screening for latent tuberculosis before initiating TNF inhibitor therapy has been an effective strategy, with a reduction in incidence of new tuberculosis cases by approximately 85%. Latent tuberculosis can be screened by using either a tuberculin skin test with purified protein derivative or *ex vivo* tests that quantify IFN-γ release from sensitized lymphocytes in blood incubated with tuberculosis antigens. Treatment with TNF inhibitors has also been associated with development of autoantibodies. Although the mechanism of this is unknown, it does not appear to result from inhibition of TNF itself, perhaps through induction of apoptosis. The autoantibodies typically generated include the antinuclear antibody (which develops in about half of patients with RA treated with TNF inhibitors), antibodies to double-stranded DNA (which develop in approximately 10% to 15% of patients treated with TNF inhibitors), and anticardiolipin antibodies. Although rare, progression to a lupus-like illness can occur in patients treated with TNF inhibitors. Also, mild-to-moderate increases in liver function test results (generally <3 times the upper limit of normal) have been observed with TNF inhibitors. Many of these cases were confounded by concomitant use of potentially hepatotoxic drugs and underlying medical conditions. However, in light of the occurrence of liver failure of unidentifiable cause in several cases, clinicians should be aware of these rare events and consider monitoring liver function tests. Lastly, several cases of MS and other demyelinating conditions have been identified among patients treated with TNF inhibitors, although the true effect of TNF inhibitors on the development of MS remains undefined.

Despite their shared ability to inhibit TNF, there are some notable differences between the 5 approved TNF inhibitors. Infliximab, adalimumab, golimumab, and certolizumab are IgG1 mAbs that are specific for TNF-α; etanercept is a fusion protein of the type II TNF receptor and binds both TNF-α and lymphotoxin α (also known as TNF-β). The clinical relevance of this distinction is unknown. In addition, the binding characteristics of the mAbs and the fusion protein differ slightly. Although all agents bind soluble TNF with high affinity, the mAbs have slightly

higher affinity for membrane-bound TNF, presumably related to the physical constraints of the binding domains of the soluble TNF receptor, compared with that of mAbs. Whether these differences might account for the variability in efficacy and safety remains to be seen. The successful introduction of certolizumab pegol would suggest that the ultimate mechanism of action of TNF inhibitors does not appear to require Fc fragment–related activities.

IL-1 inhibitors: Anakinra and rilonacept

IL-1 is synthesized as an inactive precursor. On cleavage by IL-1β–converting enzyme, it activates a variety of cells that can then release mediators destructive to bone and cartilage. In the RA synovium, although there is an increase in the naturally occurring IL-1Ra that prevents the binding of IL-1 to its receptor, the levels are apparently insufficient to counteract the effects of IL-1.

Anakinra, approved in 2001 for the treatment of RA, is a recombinant IL-1ra that differs from the endogenous IL-1Ra by a single amino acid addition at the amino terminus (Table I). Compared with the TNF inhibitors, the clinical responses achieved by anakinra are generally more modest; this, combined with cost and the need for daily injections, has led to its relatively infrequent use in the treatment of RA. However, it has been gaining renewed interest and has been shown to be effective in the treatment of cryopyrin-associated periodic syndromes, including familial cold autoinflammatory syndrome and Muckle-Wells syndrome.[35] These rare autosomal dominant disorders, characterized by a gain-of-function mutation in the cryopyrin gene (CIASI, NLRP3), are associated with oversecretion of IL-1β, rash, arthralgia, and fever. Rilonacept (previously known as IL-1–Trap), which was approved in 2008 for the treatment of cryopyrin-associated periodic syndromes, is a fusion protein comprised of the extracellular domain of the IL-1 accessory protein and IL-1 receptor type 1 attached to the Fc portion of IgG1. Rilonacept binds to IL-1α and IL-1β with high affinity (Table I) and was generally well tolerated, with injection site responses being the most common adverse events.[36] Physicians should remain vigilant about infections with any IL-1 inhibitor. Studies evaluating the role of anakinra and rilonacept in other diseases associated with IL-1 oversecretion, such as chronic gout and adult-onset Still disease, are ongoing, with promising early results.

IL-6 inhibitors: Tocilizumab

Tocilizumab is a humanized anti–IL-6 receptor mAb that binds to both soluble and membrane-bound IL-6 receptor (Table I). Tocilizumab has been shown to improve the signs and symptoms of disease and functional status and slow radiographic progression in patients with RA. The clinical improvement was rapid and evident within the first 2 weeks of treatment.[37-40] Although it can be administered as monotherapy, tocilizumab appears to be more effective when used in combination with methotrexate.

In clinical studies tocilizumab was associated with a slightly higher rate of infections, mainly respiratory and gastrointestinal tract infections. Transient decreases in neutrophil counts, increases in serum lipid levels (total cholesterol, high-density lipoprotein, and low-density lipoprotein), and increases in liver function test results have been observed with tocilizumab. The potential long-term implications of these laboratory abnormalities have not been fully defined.

CYTOKINES

IFN-α

IFN-α is produced by the cells of the immune system in response to the presence of double-stranded RNA viruses, inducing cell activation of macrophages and natural killer cells and enhancing antigen presentation. Both IFN-α2a and IFN-α2b have been used therapeutically with similar results. IFN-α is used in combination with ribavirin in the treatment of hepatitis C viral infection,[41] reducing viremia and providing protection against the development of chronic liver disease and cryoglobulin-associated vasculitis. Side effects can be significant, with up to 68% of patients presenting with psychiatric symptoms, such as depression, irritability, and insomnia. It has also been used to improve survival in patients with advanced renal cancer, although with a modest increase of 2.6 months, achieving a median survival of 11 months.[42] Other uses are in the management of melanoma, hepatitis B infection, and systemic vasculitis.

IFN-β

IFN-β is produced in fibroblasts and is 45% identical to IFN-α, sharing similar antiviral activity against double-stranded RNA viruses. Clinically, it has been used in the treatment of MS because of its additional anti-inflammatory effect.[43] IFN-β slows progression of disease, reducing the percentage of patients with disability from 35% to 22% after 2 years of treatment. Common adverse effects are depression and suicidal ideation, flu-like symptoms, and increase of liver enzyme levels.

IFN-γ

IFN-γ is produced by leukocytes to induce macrophage activation and increase oxidative burst. It is clinically used to enhance immunity in patients with chronic granulomatous disease, in which it has been shown to help by reducing the frequency of infections up to 67% when used in combination with antibacterial and antifungal prophylaxis.[44] IFN-γ is administered subcutaneously at 50 $\mu g/m^2$ 3 times a week. Potential side effects include fever, hypotension, and flu-like symptoms. In patients with congenital osteopetrosis, IFN-γ slows disease progression. It is also used on a trial basis in some patients with the rare occurrence of deficiency of the IFN-γ/IL-12 axis caused by a deficiency of either of these cytokines, expecting that the administration of this cytokine would reduce the patient's susceptibility to severe mycobacterial disease.

IL-2

Recombinant IL-2 has been approved by the US Food and Drug Administration (FDA) for the treatment of metastatic renal cancer[42] and malignant melanoma.[45] IL-2 promotes the activation of T cells and natural killer cells, enhancing their antitumor activity. It also induces the differentiation of regulatory T cells, which are of significance to the control of inflammatory responses. The administration of IL-2 to patients with HIV[46] has resulted in an increase in $CD4^+$ T-cell counts and, when used in combination with highly active anti-retroviral treatment drugs, did not increase HIV viremia and reduced the occurrence of AIDS-defining infections. Side effects are dose related and include hypotension, flu-like symptoms, behavioral changes, and renal impairment.

IL-7

Because of its biologic activity in the homeostasis of T cells, which includes the expansion of naive and memory T cells in the setting of lymphopenia, IL-7 has been suggested as an adjuvant in the treatment of HIV infection and in lymphopenia after chemotherapy. Reports of its administration in HIV-infected patients showed less significant side effects than IL-2 treatment, with sustained dose-dependent expansion of T cells.[47]

AGENTS THAT INHIBIT T CELLS

There is a large body of evidence suggesting autoreactive T cells, especially $CD4^+$ T_H1 T cells, serve a key role in orchestrating the immune-driven inflammatory responses in patients with autoimmune diseases, such as RA, Crohn disease, PsA, and psoriasis. Productive $CD4^+$ T-cell responses require 2 signals: binding of specific antigen-associated MHC class II molecule to the T-cell receptor complex and a second signal from costimulatory molecules. If T cells do not receive the second signal, then tolerance or ignorance of the antigen ensues, and a productive immune response is not generated. Among the most important costimulatory molecules is CD28, which binds CD80 and CD86. CD28 and its natural inhibitor, cytotoxic T lymphocyte–associated antigen 4 (CTLA-4; CD152), are present on T cells and bind to CD80 and CD86 on antigen-presenting cells. CD28 ligation results in stimulation of T cells, whereas CTLA-4 serves an inhibitory role. CTLA-4, which binds CD80 and CD86 with substantially higher affinity than CD28, inhibits the stimulatory effects of CD28 by competitively binding to CD80 and CD86.

Daclizumab and basiliximab

These 2 therapeutic antibodies are directed against CD25, the protein α component of the IL-2 receptor.[48] Their therapeutic effect is the block of IL-2 binding in T and B cells, inhibiting their activation and the development of an immune response and inducing anergy. They are indicated for the prevention of organ transplant rejection, particularly kidney grafts, and have been suggested for the management of autoimmune disorders. For this purpose, the humanized antibody daclizumab is in phase II trials for the treatment of MS and has been shown to decrease the frequency of relapses. These agents induce a state of immunosuppression, which results in an increased frequency of urinary tract infections and respiratory tract infections; however, opportunistic infections have not been observed. Other side effects are paresthesias, transient increased in liver enzyme and bilirubin levels, and skin rash.

Abatacept

Abatacept, approved in 2005 for the treatment of RA, is a soluble protein consisting of the extracellular domain of CTLA-4 linked to the Fc portion of IgG1 (Table I). Abatacept has been shown to improve the signs and symptoms of disease, functional status, and quality of life and slow radiographic progression in patients with RA.[49,50] Abatacept was well tolerated in clinical trials, with a slight increase in the incidence of infections, especially among those with underlying chronic obstructive pulmonary disease. In one study abatacept appeared to have efficacy comparable with that of a TNF inhibitor in patients with RA receiving methotrexate.[51] As with other biologic agents, live vaccines should be avoided when receiving abatacept. A safety study assessing the combination of abatacept and TNF inhibitor therapy observed a higher incidence of serious adverse effects, including infections, at 1-year follow-up compared with that seen in those receiving monotherapy.[52] Given similar findings of increased infections with TNF inhibitors and IL-1ra combination therapy, combination therapy with abatacept and other biologic agents is also discouraged.

Alefacept

Alefacept, approved in 2003 for the treatment of chronic plaque psoriasis, is a fusion protein of a soluble form of the extracellular domain of lymphocyte function–associated antigen (LFA) 3 attached to the Fc portion of an IgG1 molecule. It binds $CD2^+$ T cells and is thought to improve symptoms of psoriasis by inducing memory T-cell apoptosis, inhibiting inflammatory gene expression, and preventing T-cell migration into psoriatic plaques. The interaction of LFA-3 on antigen-presenting cells and CD2 on T cells is thought to be important in T-cell activation and in the development of cells into memory T cells. Alefacept, either as monotherapy or in combination with other psoriasis therapy (eg, methotrexate), has been shown to be effective for skin psoriasis.[53,54] T-cell depletion related to therapy did not correlate or predict the response rate during treatment or follow-up. Despite its effectiveness in the treatment of psoriasis, alefacept appears to be only modestly effective for PsA.[55] With the availability and effectiveness of TNF-I in the treatment of PsA, alefacept is therefore rarely used for the treatment of PsA.

Anti-p40 agents

Another approach to modulating the function of T cells in autoimmune and inflammatory diseases targets cytokines relevant to the development of certain T-cell subsets. IL-12, a cytokine central to the development of T_H1 T cells, and IL-23, a cytokine that helps sustain T_H17 T cells, share a common p40 subunit.[56] Agents that target the p40 subunit, including the human mAb ustekinumab and ABT874, might be expected to attenuate inflammatory processes driven by T_H1 and T_H17 T cells. These therapies are under investigation in a variety of autoimmune diseases, and ustekinumab has received regulatory approval in several countries for the treatment of psoriasis. In patients with psoriasis, ustekinumab therapy induced a substantial improvement, as measured by the psoriasis area and severity index.[57] The extent of improvement appeared to perhaps even have been larger than that achieved with TNF inhibitors, which are themselves highly effective in patients with psoriasis. The same agent has also been studied in patients with PsA and been found to have some efficacy in that condition.[58] Interestingly, the duration of clinical benefit after a few injections is prolonged and appears to far exceed the pharmacokinetic profile of the drug.

AGENTS THAT INHIBIT B CELLS

Recent data suggest that B cells might contribute significantly to the initiation and perpetuation of the immune response in various autoimmune diseases, including RA and systemic lupus erythematosus (SLE). Not only can B cells produce potentially pathologic autoantibodies (eg, rheumatoid factor and antinuclear antibody) and proinflammatory cytokines, but they can also present

antigens to T cells and provide costimulatory signals essential for T-cell activation, clonal expansion, and effector function.

CD20 inhibitor: Rituximab

Rituximab is a chimeric IgG1 mAb directed against the B-lymphocyte surface antigen CD20. It was initially approved in 1997 for the treatment of $CD20^+$ B-cell non-Hodgkin lymphoma and later for the treatment of RA in 2006. CD20 is a cell-surface molecule restricted to the surface of pre-B through activated mature B cells. Rituximab is thought to induce lysis of $CD20^+$ B cells through several mechanisms, including complement activation, antibody-dependent cell-mediated cytotoxicity, and induction of apoptosis. Depletion of B cells can last up to 9 months or longer after a single course of therapy. Rituximab has been shown to improve the signs and symptoms of disease, functional status, and quality of life and slow radiographic progression of disease in patients with RA.[59,60] Although rituximab can be used alone or in combination with DMARDs, the combination therapy yielded better clinical outcomes. Also, patients who are seropositive for rheumatoid factor had greater clinical response compared with rheumatoid factor–seronegative patients. In smaller studies rituximab has shown promising results in the treatment of other autoimmune diseases, such as SLE, primary Sjögren syndrome, idiopathic thrombocytopenic purpura, chronic inflammatory demyelinating polyneuropathy, and vasculitis. Additional trials are underway that should answer questions regarding dosing, treatment intervals, safety, and tolerability in these conditions.

Despite the potential for immunodeficiency related to depletion of mature B cells, no significant increases in infections, either serious or opportunistic, were reported in patients with RA and non-Hodgkin lymphoma treated with rituximab. The overall levels of serum immunoglobulin generally remain stable during treatment. This could be related to preserved function of plasma cells, which lack CD20 and are therefore not depleted by rituximab. However, if rituximab is used as a recurrent or maintenance therapy for autoimmune conditions, this might become more of a safety concern because plasma cells are not replenished by memory B cells. Thus far, some patients have undergone more than 4 cycles of rituximab without increased risk of adverse events.[61] Other notable adverse effects include rare neutropenia, reactivation of hepatitis B, and progressive multifocal leukoencephalopathy (PML). Three cases of PML in patients receiving rituximab for non–FDA-approved conditions, mainly SLE, have been reported.[62] The exact role of rituximab in the development of PML remains unknown given its rare occurrence, but it highlights the importance of pharmacovigilance and potential unforeseen long-term adverse effects related to biologic agents. Although treatment has overall been well tolerated, infusions have been associated with hypersensitivity reactions, Stevens-Johnson syndrome, and type III serum sickness–like illness and cytokine release syndrome. The infusion reactions are more common during the first infusion and might occur more in patients with lymphoma than in those with RA.[33,34] Lastly, given the potential for suboptimal response, vaccinations should be administered before rituximab, if possible.

Anti-IgE antibody: Omalizumab

This antibody was developed to aid in the management of severe asthma with an allergic component. Omalizumab binds IgE with high affinity, considerably reducing levels of free IgE and inhibiting its interaction with the IgE receptor. The clinical improvement correlated well with the measurement of biologic markers.[63] Its administration to patients with severe asthma with low to moderately increased serum IgE levels results in a 26% decrease in the asthma exacerbation rate and a 50% decrease in severe exacerbations and emergency department visits, as well as a reduction in systemic corticosteroid use.[64] It has also been shown to be useful to reduce symptoms in patients with corticosteroid-resistant chronic urticaria.

AGENTS THAT INHIBIT CELL ADHESION, MIGRATION, OR BOTH

Activated T lymphocytes must migrate to sites of inflammation and lymph tissue to exert their diverse effects. The entry of lymphocytes into specific sites occurs through several specific interactions between the adhesion molecules on lymphocytes, including the integrins and their ligands on endothelial cells. Particularly important for lymphocyte migration and homing are LFA-1 and its counterreceptors, intercellular adhesion molecule (ICAM) 1 and ICAM-2, and very late antigen-4 and its counterreceptor, vascular cell adhesion molecule 1.

Integrin inhibitors: Natalizumab

Natalizumab, approved in 2004 for the treatment of MS, is a recombinant humanized IgG4 mAb directed against the α_4 subunit of $\alpha_4\beta_1$; it also binds to and inhibits the function of the $\alpha_4\beta_7$ integrins, the ligand of which is mucosal addressin cell adhesion molecule 1. $\alpha_4\beta_1$ integrin, an adhesion molecule present on leukocytes, has been implicated in the pathogenesis of MS by facilitating migration of lymphocytes into the site of disease. In addition to blocking the migration of lymphocytes into the central nervous system and intestinal parenchyma, natalizumab induces T-cell apoptosis and anergy and prevents T-cell binding to osteopontin and fibronectin, thereby attenuating T cell–mediated inflammation. In 2 large clinical trials, natalizumab, either alone or in combination with IFN-β-1a was associated with significantly lower relapse rates and disability and fewer new MS lesions on magnetic resonance imaging.[65] However, shortly after FDA approval, natalizumab was temporarily withdrawn from the market after 3 cases of PML were reported. Similar to rituximab, the exact role of natalizumab in the development of PML remains unknown.

CD11a inhibitor: Efalizumab

Efalizumab, approved in 2003 for the treatment of psoriasis, is a humanized IgG1 mAb directed against the cell adhesion molecule CD11a. CD11a is an α subunit of the LFA-1 molecule on T cells that binds to ICAM-1 on antigen-presenting cells and endothelial cells. In addition to inhibiting activation of T cells, efalizumab also blocks trafficking of lymphocytes into the skin by blocking LFA-1/ICAM-1 interaction. Efalizumab has been shown to provide greater improvement in symptoms of skin psoriasis after 3 months of therapy, with a continued increase in response if therapy was continued for another 3-month cycle.[66] However, the development of PML among several patients treated with efalizumab led to its withdrawal in 2009.

TABLE II. Clinical use of human immunoglobulin preparations

Primary immunodeficiency diseases (that result in defect in antibody responses)
Secondary immunodeficiency conditions (with impaired antibody responses)
HIV infection
B-cell leukemia
Use of chemotherapy or radiotherapy
Autoimmune syndromes
Hematologic: ITP, autoimmune hemolytic anemia
Rheumatologic: RA, vasculitis, Kawasaki disease, uveitis, SLE
Endocrinologic: Autoimmune diabetes mellitus, Graves ophthalmopathy
Neurologic: Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, myasthenia gravis, dermatomyositis
Dermatologic: TEN, Steven-Johnson syndrome
Infectious diseases
CMV
Rotavirus
Parvovirus B19

ITP, Immune thrombocytopenic purpura; *TEN*, toxic epidermal necrolysis; *CMV*, cytomegalovirus.

IMMUNOGLOBULINS

Therapeutic use

Immunoglobulin concentrates derived from human plasma have been used since the 1940s and were used initially in the management of viral diseases, such as hepatitis. Bruton published the first report of the use of immunoglobulins to treat a patient with an immune defect who presented with agammaglobulinemia and frequent infections. This resulted in the increase of the gammaglobulin fraction in the patient's serum and a reduction in the number of infections. Currently, human immunoglobulin preparations are derived from pooled plasma of up to 10,000 individual donors per batch of immunoglobulin products, introducing safety concerns regarding the transmission of blood-borne infectious diseases. This is addressed by means of donor screening for infectious diseases and by introducing in the manufacturing process several steps to remove viral particles. There are 2 forms of administration: subcutaneous immunoglobulin (SCIG) and intravenous immunoglobulin (IVIG).[67]

In addition to its use as antibody replacement, IVIG preparations are indicated as an immunomodulator in many inflammatory conditions, such as idiopathic thrombocytopenic purpura (Table II). Only 6 of these indications are approved by the FDA: the treatment of primary immunodeficiencies, HIV infection, Kawasaki disease, and immune thrombocytopenic purpura and the prevention of infections in B-cell leukemias and in patients undergoing bone marrow transplantation.[68] The anti-inflammatory properties of immunoglobulins have been attributed to different mechanisms, including those mediated by neutralization of autoantibodies and anti-idiotypic antibodies and neutralization of toxins and T-cell superantigens and those mediated by the modulation of the Fc receptors in the cells of the immune system. More recently, Anthony et al[69] showed that the anti-inflammatory activity of immune globulins can be explained by the action of Fc fragments containing sialic acid on macrophages inducing the expression of the inhibitory FcγIII receptor. Both human and murine recombinant sialylated Fc proteins were able to suppress inflammation in a murine model of arthritis. This finding might lead to the development of a therapeutic anti-inflammatory agent that is not derived from human plasma and that reduces safety concerns and availability shortages.

Dosage and adverse reactions

In the United States several IVIG preparations and 1 SCIG preparation are commercially available.[68] They differ in their method of purification, osmolality and IgG concentration, IgA and sodium contents, stabilizer (to prevent IgG aggregation; eg, glycine, sucrose, or maltose), and pH; however, they are administered similarly, except when adverse reactions occur, such as idiosyncratic reactions in a particular patient or if suspected hypersensitivity to IgA leads to the use of those products with undetectable IgA. Patients with diabetes mellitus should avoid products containing sugar molecules as stabilizers. IVIG is used as an anti-inflammatory agent at 1 to 2 g/kg in 1 dose or divided in 2 daily doses. The IVIG dose used for replacement in patients with antibody deficiencies is 400 to 600 mg/kg administered every 3 to 4 weeks to maintain a trough IgG level of at least 500 mg/mL and reduce the frequency of infections. Because of increased immunoglobulin catabolism or protein loss, some patients might require even higher doses, which need to be optimized to each patient, also taking into consideration the clinical assessment. Because of the volume limitations for subcutaneous administration, SCIG is not used for inflammatory disorders and is recommended to be administered weekly for immune deficiencies, with doses that correspond to the IVIG dose mentioned above (approximately 100 mg/kg/wk). No differences of efficacy to prevent infections have been found in clinical trials comparing IVIG and SCIG. The weekly subcutaneous administration of immunoglobulins provides a tighter range of serum IgG levels, which is of advantage for patients who experience side effects associated with peak IgG concentrations. Although side effects associated with SCIG infusions are at the infusion site, IVIG side effects are not common, although they can be severe, including back pain, fever, hypotension, thrombosis, headaches, and skin rashes. Premedication with antihistaminic agents, nonsteroidal anti-inflammatory drugs, and corticosteroids and hydration with normal saline are common measures used to prevent these symptoms. Serious adverse effects, such as aseptic meningitis, seizures, anaphylaxis, pulmonary edema, and thrombosis, have been rarely reported. Therefore it is recommended that IVIG be administered with medical monitoring for early detection and management of these possible events.

FUTURE DIRECTIONS

The factors that drove the initial introduction of the biologic agents—a clinical need for better outcomes, greater delineation of pathophysiology allowing definition of various targets, and progress in biotechnology allowing development of agents—will

no doubt continue to fuel progress in this area. It can be expected that additional mAbs and fusion receptors, both directed at existing targets and against novel targets, will continue to be developed and brought to the clinic. Along with the number of agents, it is anticipated that the conditions for which these agents are used will also expand. For existing biologic agents, a number of questions remain as to the optimum treatment paradigms (eg, sequence of biologic agents) and most appropriate patient populations for their use; this will be germane for newer agents as well. As always, the balance between achieving higher levels of efficacy, with disease remission being the ultimate goal, need to be balanced against safety considerations. For macromolecules, such as mAbs and soluble receptors, there is the potential for optimizing their characteristics, including ease of use, immunogenicity, and cost. For certain targets, it is possible that small-molecule inhibitors might be developed that can address some of these issues. However, because these molecules can be anticipated to have pharmacokinetic, mechanistic, and other important differences from their macromolecular counterparts, this might translate into variable safety and efficacy. Therefore newer agents of a different class, even those whose putative target is the same as existing therapies, need to be assessed with the same rigor as the currently available agents.

REFERENCES

1. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Furst D, Weisman MH, et al. Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. Arthritis Rheum 2004;50:1051-65.
2. Lipsky PE, van der Heijde DM, St Claire EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. N Engl J Med 2000;343:1594-602.
3. Moreland LW, Weinblatt ME, Keystone EC, Kremer JM, Martin RW, Schiff MH, et al. Etanercept treatment in adults with established rheumatoid arthritis: 7 years of clinical experience. J Rheumatol 2005;33:854-61.
4. van der Heijde D, Klareskog L, Rodriguez-Valverde V, Codreanu C, Bolosiu H, Melo-Gomes J, et al. Comparison of etanercept and methotrexate, alone and combined, in the treatment of rheumatoid arthritis: two-year clinical and radiographic results from the TEMPO study, a double-blind, randomized trial. Arthritis Rheum 2006;54:76-81.
5. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, et al. Adalimumab, a fully human anti-tumor necrosis factor-α monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial [published erratum appears in Arthritis Rheum 2003; 48:855]. Arthritis Rheum 2003;48:35-45.
6. Furst DE, Schiff MH, Fleischmann RM, Strand V, Birbara CA, Compagnone D, et al. Adalimumab, a fully human anti-tumor necrosis factor-α monoclonal antibody, and concomitant standard antirheumatic therapy for the treatment of rheumatoid arthritis: results of STAR (Safety Trial of Adalimumab in Rheumatoid arthritis). J Rheumatol 2003;30:2563-71.
7. Kay J, Matteson EL, Dasgupta B, Nash P, Durez P, Hall S, et al. Golimumab in patients with active rheumatoid arthritis despite treatment with methotrexate. Arthritis Rheum 2008;58:964-75.
8. Keystone E, van der Heijde D, Mason D Jr, Landewe R, van Vollenhoven R, Combe B, et al. Certolizumab pegol plus methotrexate is significantly more effective than placebo plus methotrexate in active rheumatoid arthritis. Arthritis Rheum 2008;58:3319-29.
9. St Clair EW, van der Heijde DMFM, Smolen JS, Maini RN, Bathon JM, Emery P, et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis. Arthritis Rheum 2004;50:3432-43.
10. Genovese MC, Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, et al. Etanercept versus methotrexate in patients with early rheumatoid arthritis: two year radiographic and clinical outcomes. Arthritis Rheum 2002;46:1443-50.
11. Breedveld FC, Weisman MH, Kavanaugh AF, Cohen SB, Pavelka K, van Vollenhoven R, et al. The PREMIER study: a multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheum 2006;54:26-37.
12. Landewe R, van der Heijde D, Klareskog L, van Vollenhoven R, Fatenejad S. Disconnect between inflammation and joint destruction after treatment with etanercept plus methotrexate: results from the trial of etanercept and methotrexate with radiographic and patient outcomes. Arthritis Rheum 2006;54:3119-25.
13. Lovell DJ, Ruperto N, Goodman S, Reiff A, Jung L, Jarosova K, et al. Adalimumab with or without methotrexate in juvenile rheumatoid arthritis. N Engl J Med 2008; 359:810-20.
14. Ruperto N, Lovell DJ, Cuttica R, Wilkinson N, Woo P, Espada G, et al. A randomized, placebo-controlled trial of infliximab plus methotrexate for the treatment of polyarticular-course juvenile rheumatoid arthritis. Arthritis Rheum 2007;59: 3096-106.
15. Antoni CE, Kavanaugh A, van der Heijde D, Beutler A, Keenan G, Zhou B, et al. Two-year efficacy and safety of infliximab treatment in patients with active psoriatic arthritis: findings of the Infliximab Multinational Psoriatic Arthritis Controlled Trial (IMPACT). J Rheumatol 2008;35:869-76.
16. Mease PJ, Kivitz AJ, Burch FX, Siegel EL, Cohen SB, Ory P, et al. Etanercept treatment of psoriatic arthritis: safety, efficacy, and effect on disease progression. Arthritis Rheum 2004;50:2264-72.
17. Mease PJ, Gladman DD, Ritchlin CT, Ruderman EM, Steinfeld SD, Choy EH, et al. Adalimumab for the treatment of patients with moderately to severely active psoriatic arthritis: result of a double-blind, randomized, placebo-controlled trial. Arthritis Rheum 2005;52:3279-89.
18. Rozenblit M, Lebwohl M. New biologics for psoriasis and psoriatic arthritis. Dermatol Ther 2009;22:56-60.
19. van der Heijde D, Dijkmans B, Geusens P, Sieper J, DeWoody K, Williamson P, et al. Efficacy and safety of infliximab in patients with ankylosing spondylitis. Arthritis Rheum 2005;52:582-91.
20. Baraliakos X, Brandt J, Listing J, Haibel H, Sörensen H, Rudwaleit M, et al. Outcome of patients with active ankylosing spondylitis after two years of therapy with etanercept: clinical and magnetic resonance imaging data. Arthritis Rheum 2005; 53:856-63.
21. Sieper J, Baraliakos X, Listing J, Brandt J, Haibel H, Rudwaleit M, et al. Persistent reduction of spinal inflammation as assessed by magnetic resonance imaging in patients with ankylosing spondylitis after 2 years of treatment with the anti-tumor necrosis factor agent infliximab. Rheumatology 2005;44:1525-30.
22. Van der Heijde D, Landewe R, Baraliakos X, Houben H, van Tubergen A, Williamson P, et al. Radiographic findings following two years of infliximab therapy in patients with ankylosing spondylitis. Arthritis Rheum 2008;58:3063-70.
23. van der Heijde D, Pangan AL, Schiff MH, Braun J, Borofsky M, Torre J, et al. Adalimumab effectively reduces the signs and symptoms of active ankylosing spondylitis in patients with total spinal ankylosis. Ann Rheum Dis 2008;67:1218-21.
24. Inman RD, Davis JC Jr, Heijde D, Diekman L, Sieper J, Kim SI, et al. Efficacy and safety of golimumab in patients with ankylosing spondylitis: results of a randomized, double-blind, placebo-controlled, phase III trial. Arthritis Rheum 2008;58: 3402-12.
25. Present DH, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med 1999;340:1398-405.
26. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353:2462-76.
27. Hanauer SB, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh D, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology 2006;130:323-33.
28. Sandborn WJ, Hanauer SB, Katz S, Safdi M, Wolf DG, Baerg RD, et al. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. Gastroenterology 2001;121:1088-94.
29. Sandborn WJ, Feagan BG, Stoinov S, Honiball PJ, Rutgeerts P, Mason D, et al. Certolizumab pegol for the treatment of Crohn's disease. N Eng J Med 2007; 357:228-38.
30. Schreiber S, Khaliq-Kareemi M, Lawrence IC, Thomsen OO, Hanauer SB, McColm J, et al. Maintenance therapy with certolizumab pegol for Crohn's disease. N Eng J Med 2007;357:239-50.
31. Baert F, Noman M, Verneire S, Van Assche G, D'Haens G, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003;348:601-8.
32. Hyrich KL, Lunt M, Watson KD, Symmons DPM, Silman AJ. for the British Society for Rheumatology Biologics Register. Outcomes after switching from one anti-tumor necrosis factor a agent to a second anti-tumor necrosis factor a agent in patients with rheumatoid arthritis. Arthritis Rheum 2007;56:13-20.
33. Kapoor D, Ruderman EM. Safety of newer therapies in rheumatoid arthritis. Rheum Dis Clin Updates 2007;2:1-9.
34. Lee SJ, Kavanaugh A. Adverse events related to biologic agents. J Allergy Clin Immunol 2005;116:900-5.

35. Ross JB, Finlayson LA, Klotz PJ, Langley RG, Gaudet R, Thompson K, et al. Use of anakinra (Kineret) in the treatment of familial cold autoinflammatory syndrome with a 16-month follow-up. J Cutan Med Surg 2008;12:8-16.
36. Hoffman HM, Throne ML, Amar NJ, Sebai M, Kivitz AJ, Kavanaugh A, et al. Efficacy and safety of rilonacept (interleukin-1 trap) in patients with cryopyrin-associated periodic syndrome. Arthritis Rheum 2008;58:2443-52.
37. Smolen JF, Beaulieu A, Rubbert-Roth A, Ramos-Remus C, Rovensky J, Alecock E, et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomized trial. Lancet 2008;371:987-97.
38. Genovese MC, McKay JD, Nasonov EL, Mysler EF, da Silva NA, Alecock E, et al. Interleukin-6 receptor inhibition with tocilizumab reduces diseae activity in rheumatoid arthritis with inadequate response to disease-modifying antirheumatic drugs. Arthritis Rheum 2008;58:2968-80.
39. Emery P, Keystone E, Tony HP, Cantagrel A, van Vollenhoven R, Sanchez A, et al. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomized placebo-controlled trial. Ann Rheum Dis 20008;67:1516-1523.
40. Nishimoto N, Hashimoto J, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, et al. Study of active controlled monotherapy used for rheumatoid arthritis, an IL-6 inhibitor (SAMURAI): evidence of clinical and radiographic benefit from an x ray reader-blinded randomized controlled trial of tocilizumab. Ann Rheum Dis 2007; 66:1162-7.
41. Welzel TM, Morgan TR, Bonkovsky HL, Naishadham D, Pfeifer RM, Wright EC, et al. Variants in interferon-alpha pathway genes and response to pegylated interferon-alpha 2a plus reibavirin in the treatment of hepatitis C virus infection in the hepatitic C antiviral long-term treatment against cirrhosis trial. Hepatology 2009; 49:1847-58.
42. Coppin C, Le L, Porzsolt F, Wilt T. Targeted therapy for advanced renal cell carcinoma. Cochrane Database Syst Rev 2008 (2):CD006017.
43. Durelli L, Conti L, Clerico M, Boselli D, Contessa G, Ripellino P, et al. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta. Ann Neurol 2009;65:499-509.
44. Holland SM. Chronic Granulomatous Disease. Clin Rev Allergy Immunol 2009 [Epub ahead of print].
45. Tarhini AA, Kirkwood JM, Gooding WE, Moschos S, Agarwala SS. A phase 2 trial of sequential temozolomide chemotherapy followed by high-dose interleukin 2 immunotherapy for metastatic melanoma. Cancer 2008;113:1632-40.
46. Mitsuyasu R, Gelman R, Cherng DW, Landay A, Fahey J, Reichman R, et al. The virologic, immunologic, and clinical effects of interleukin 2 with potent antiretroviral therapy in patients with moderately advanced human immunodeficiency virus infection: a randomized controlled clinical trial—AIDS Clinical Trials Group 328. Arch Intern Med 2007;167:597-605.
47. Levy Y, Lacabaratz C, Weiss L, Viard JP, Goujard C, Lelièvre JD, et al. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. J Clin Invest 2009; 119:997-1007.
48. Waldmann TA. Anti-Tac (daclizumab, Zenapax) in the treatment of leukemia, autoimmune diseases, and in the prevention of allograft rejection: a 25-year personal odyssey. J Clin Immunol 2007;27:1-18.
49. Genovese MC, Schiff M, Luggen M, Becker JC, Aranda R, Teng J, et al. Efficacy and safety of the selective co-stimulation modulator abatacept following 2 years of treatment in patients with rheumatoid arthritis and an inadequate response to anti-tumour necrosis factor therapy. Ann Rheum Dis 2008;67:547-54.
50. Kremer JM, Geneant HK, Moreland LW, Russell AS, Emery P, Abud-Mendoza C, et al. Results of a two-year followup study of patients with rheumatoid arthritis who received a combination of abatacept and methotrexate. Arthritis Rheum 2008;58:953-63.
51. Schiff M, Keiserman M, Codding C, Songcharoen S, Berman A, Nayiager S, et al. Efficacy and safety of abatacept or infliximab vs placebo in ATTEST: a phase III, multi-centre, randomised, double-blind, placebo-controlled study in patients with rheumatoid arthritis and an inadequate response to methotrexate. Ann Rheum Dis 2008;67:1096-103.
52. Weinblatt M, Combe B, Covucci A, Aranda R, Becker JC, Keystone E. Safety of the selective costimulation modulator abatacept in rheumatoid arthritis patients receiving background biologic and nonbiologic disease-modifying antirheumatic drugs: a one-year randomized, placebo-controlled study. Arthritis Rheum 2006; 54:2807-16.
53. Sugiyama H, McCormick TS, Cooper KD, Korman N. Alefacept in the treatment of psoriasis. Clin Dermatol 2008;26:503-8.
54. Krueger GG, Gottlieb AB, Sterry W, Korman N, van de Kerkhof P. A multiceter, open-label study of repeat courses of intramuscular alefacept in combination with other psoriasis therapies in patients with chronic plaque psoriasis. J Dermatol Treat 2008;19:146-55.
55. Mease PJ, Gladman DD, Keystone EC. Alefacept in Psoriatic Arthritis Study Group. Alefacept in combination with methotrexate for the treatment of psoriatic arthritis. Arthritis Rheum 2006;54:1638-45.
56. Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, et al. Divergent pro-and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med 2003;98:1951-7.
57. Krueger GG, Langley RG, Leonardi C, Yeilding N, Guzzo C, Wang Y, et al. A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. N Engl J Med 2007;356:580-92.
58. Gottlieb A, Menter A, Mendelsohn A, Shen YK, Li S, Guzzo C, et al. Ustekinumab, a human interleukin 12/23 monoclonal antibody, for psoriatic arthritis: randomized, double-blind, placebo-controlled, crossover trial. Lancet 2009;373:633-40.
59. Emery P, Fleischmann R, Filipowicz-Sosnowska A, Schechtman J, Szczepanski L, Kavanaugh A, et al. The efficacy and safety of Rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: results of a phase IIB randomized, double-blind, placebo-controlled, dose-ranging trial. Arthritis Rheum 2006; 54:1390-400.
60. Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, Genovese MC, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum 2006;54:2793-806.
61. Keystone E, Fleischmann R, Emery P, Furst DE, van Vollenhoven R, Bathon J, et al. Safety and efficacy of additional courses of rituximab in patients with active rheumatoid arthritis: an open-label extension analysis. Arthritis Rheum 2007;56: 3896-908.
62. Calabrese LH, Molloy ES. Progressive multifocal leucoencephalopathy in the rheumatic disease: assessing the risks of biological immunosuppressive therapies. Ann Rheum Dis 2008;67(suppl III):iii64-5.
63. Slavin RG, Ferioli C, Tannenbaum SJ, Martin C, Blogg M, Lowe PJ. Asthma symptom re-emergence after omalizumab withdrawal correlates well with increasing IgE and decreasing pharmacokinetic concentrations. J Allergy Clin Immunol 2009;123:107-13.
64. Humbert M, Beasley R, Ayres J, Slavin R, Hébert J, Bousquet J, et al. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. Allergy 2005;60:309-16.
65. Hutchinson M, Kappos L, Calabresi PA, Confavreux C, Giovannoi G, Galetta SL, et al. The efficacy of natalizumab in patients with relapsing multiple sclerosis: subgroup analyses of AFFIRM and SENTINEL. J Neurol 2009;256:405-45.
66. Toth DP, Papp K, Gratton D. Long-term efficacy of up to 15 months' efalizumab therapy in patients with moderate-to-severe chronic plaque psoriasis. Dermatol Ther 2008;21(suppl 3):S6-14.
67. Ballow M. Immunoglobulin therapy: methods of delivery. J Allergy Clin Immunol 2008;122:1038-9.
68. Orange JS, Hossny EM, Weiler CR, Ballow M, Berger M, Bonilla FA, et al. Use of intravenous immunoglobulin in human disease: a review of evidence by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology. J Allergy Clin Immunol 2006;117(suppl):S525-53.
69. Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV. Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science 2008;320:373-6.

Transplantation immunology: Solid organ and bone marrow

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Development of the field of organ and tissue transplantation has accelerated remarkably since the human MHC was discovered in 1967. Matching of donor and recipient for MHC antigens has been shown to have a significant positive effect on graft acceptance. The roles of the different components of the immune system involved in the tolerance or rejection of grafts and in graft-versus-host disease have been clarified. These components include antibodies, antigen-presenting cells, helper and cytotoxic T-cell subsets, immune cell-surface molecules, signaling mechanisms, and cytokines. The development of pharmacologic and biological agents that interfere with the alloimmune response has had a crucial role in the success of organ transplantation. Combinations of these agents work synergistically, leading to lower doses of immunosuppressive drugs and reduced toxicity. Reports of significant numbers of successful solid-organ transplantations include those of the kidneys, liver, heart, and lung. The use of bone marrow transplantation for hematologic diseases, particularly hematologic malignancies and primary immunodeficiencies, has become the treatment of choice in many of these conditions. Other sources of hematopoietic stem cells are also being used, and diverse immunosuppressive drug regimens of reduced intensity are being proposed to circumvent the mortality associated with the toxicity of these drugs. Gene therapy to correct inherited diseases by means of infusion of gene-modified autologous hematopoietic stem cells has shown efficacy in 2 forms of severe combined immunodeficiency, providing an alternative to allogeneic tissue transplantation. (J Allergy Clin Immunol 2010;125:S324-35.)

Key words: *Bone marrow transplantation, solid-organ transplantation, graft rejection, graft-versus-host disease*

Abbreviations used
ADA: Adenosine deaminase
ALG: Antilymphocyte globulin
APC: Antigen-presenting cell
ATG: Antithymocyte globulin
CGD: Chronic granulomatous disease
GVHD: Graft-versus-host disease
IL-2R: IL-2 receptor
SCID: Severe combined immunodeficiency

Efforts to transplant organs or tissues from one human subject to another had been unsuccessful for many decades until the discovery of the human MHC in 1967.[1] Identification of this genetic region launched the field of clinical organ and tissue transplantation. In 1968, the World Health Organization Nomenclature Committee designated that the leukocyte antigens controlled by the closely linked genes of the human MHC be named HLA (for human leukocyte antigen). This chapter reviews general immunologic concepts that have supported the success of human organ and tissue transplantation and summarizes current medical progress in the field of transplantation medicine.

TRANSPLANTATION ANTIGENS

MHC

Histocompatibility antigens are tissue cell-surface antigens capable of inducing an immune response in a genetically dissimilar (allogeneic) recipient, resulting in the rejection of the tissues or cells bearing those antigens. The genes that encode these antigens reside in the MHC region on the short arm of human chromosome 6 (Fig 1). The HLA complex contains more than 200 genes, more than 40 of which encode leukocyte antigens.[2,3] These genes and their encoded cell-surface and soluble protein products are divided into 3 classes (I, II, and III) on the basis of their tissue distribution, structure, and function.[3-5] MHC class I and II genes encode codominantly expressed HLA cell-surface antigens, and class III genes encode several components of the complement system, all of which share important roles in immune function.

Class I MHC antigens are present on all nucleated cells and are each composed of a 45-kd α heavy chain encoded by genes of the HLA-A, HLA-B, or HLA-C loci on chromosome 6 and associated noncovalently with a 12-kd protein, β_2-microglobulin, encoded by a gene on chromosome 15 (Fig 2).[3] MHC class II antigens have a more limited tissue distribution and are expressed only on B lymphocytes, activated T lymphocytes, monocytes, macrophages, Langerhans cells, dendritic cells, endothelium, and epithelial cells.[5] Each is a heterodimer composed of noncovalently associated α and β chains of approximately 230 amino acids encoded by genes of the HLA-D region (Fig 2). On cells expressing both class I and class II HLA antigens, there are 3 class I antigens and 3 or more (usually 4) class II heterodimers.

Class III genes are located between the HLA-B and HLA-D loci and determine the structure of 3 components of the complement system: C2, C4, and factor B.[3,4] HLA antigens are inherited in a Mendelian dominant manner. Because of the closeness of the different loci of the MHC and the resultant low crossover frequency, however, HLA genes are almost always inherited

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together. To date, 3756 different class I and II HLA gene alleles have been identified.[2] The fixed combination of these genetic determinants present in 1 chromosome of a subject is referred to as a haplotype. Chromosome 6 is an autosome, and therefore all subjects have 2 HLA haplotypes (1 for each chromosome), and there are only 4 possible combinations of haplotypes among the offspring of any 2 parents. Thus there is a 25% probability that biological siblings will have identical HLA alleles.

The ABO system

ABO incompatibility does not cause stimulation in mixed leukocyte cultures, indicating that ABO compatibility is of much less importance than HLA compatibility in graft survival. However, ABO incompatibility can result in hyperacute rejection of primarily vascularized grafts, such as those of the kidney and heart.[6] This is thought to occur because (1) ABO blood group antigens are highly expressed on kidney and cardiac grafts, particularly those from patients who are blood group A or B antigen secretors, and (2) preformed naturally occurring antibodies to blood group substances are present in mismatched recipients. Advances in immunosuppressive therapies to prevent immune rejection of the graft have more recently allowed performance of organ transplantations across the ABO barrier.[7]

Donor-recipient HLA matching

Two laboratory methods are used to pair donors and recipients for transplantation. The first matching method involves the determination of HLA antigens on donor and recipient leukocytes by using either serologic or DNA-typing methods. The second method is functional and involves the measurement of the response of immunocompetent cells from the recipient to antigens present on donor cells (and *vice versa* for bone marrow transplantation). Results of both methods are generally consistent with each other. Disparities that are serologically detected are referred to as antigen mismatches, whereas differences that can be identified only by DNA-based typing are called allele mismatches. Because these methods take considerable time to perform, results are not known in time for some solid-organ transplantations, such as lung transplantations, which are performed based on immediate organ availability. Since 2000, the National Donor Matching Program performs HLA typing of donor volunteers exclusively using a DNA-based method, the PCR single-strand oligonucleotide probe. Currently, approximately 60% of volunteer donors on the National Donor Matching Program Registry had their HLA types determined by using this method. Efforts continue to improve the efficiency of HLA typing and to reduce the costs of the assays.[8]

Donor-recipient serologic cross-matching

Serologic cross-matching is of particular importance to the success of primarily vascularized grafts, such as those of the kidney and heart. Serum from the prospective recipient is tested against cells from the potential donor for the presence of antibodies to red blood cell or HLA antigens. The presence of such antibodies correlates with hyperacute renal graft rejection.[6] For this reason, a positive serologic cross-match result has been considered a contraindication to renal transplantation, although therapeutic strategies, such as the use of plasmapheresis, are proposed when the mismatch cannot be avoided.[7]

Usefulness of HLA typing in clinical organ and tissue transplantation

Although typing for intrafamilial transplants of all types is clearly of great value, the usefulness of HLA typing in cadaveric kidney grafting has been a point of controversy since cyclosporine became available.[9] Although short-term survival rates did not appear to be that different for closely or poorly matched cadaveric kidneys, the degree of HLA matching does correlate with long-term survival.[10] Until 1980, only HLA-identical siblings could be used as bone marrow donors because both graft rejection and lethal graft-versus-host disease (GVHD) were common complications if this was not the case.[11] Fortunately, the development during the past 3 decades of techniques to rigorously deplete postthymic T cells from donor marrow has permitted numerous successful half-HLA-matched marrow transplantations with no or minimal GVHD.[12,13]

MECHANISMS OF GRAFT REJECTION

Role of alloimmune antibodies

The strongest evidence for a role for antibodies in graft rejection is the hyperacute rejection of primarily vascularized organs, such as the kidney and heart. High titers of antidonor antibodies can be demonstrated in recipients presenting with these reactions.[6] These antibodies combine with HLA antigens on endothelial cells, with subsequent complement fixation and accumulation of polymorphonuclear cells. Endothelial damage then occurs, probably as a result of enzymes released from polymorphonuclear leukocytes; platelets then accumulate, thrombi develop, and the result is renal cortical necrosis or myocardial infarction.[14]

Leukocytes and cytokines in graft rejection

Allograft rejection results from the coordinated activation of alloreactive T cells and antigen-presenting cells (APCs). Although acute rejection is a T cell–dependent process, the destruction of the allograft results from a broad array of effector immune mechanisms. Cell-cell interactions and the release by primed T_H cells of multiple types of cytokines (IL-2, IL-4, IL-5, IL-7, IL-10, IL-15, TNF-α, and IFN-γ) recruit not only immunocompetent donor-specific $CD4^+$ T cells, $CD8^+$ cytotoxic T cells, and antibody-forming B cells but also nonspecific inflammatory cells, which constitute the majority of cells infiltrating an allograft.[15] Other cells specific to the transplanted organ might play a role in the balance of tolerance and rejection, such as the Kupffer cells and the sinusoidal epithelial cells in the liver.[16]

Stimulation of $CD4^+$ T cells through their antigen receptors is not sufficient to initiate T-cell activation unless costimulation is provided by interaction of other ligand-receptor pairs present on the surfaces of T cells and APCs during the encounter. Some of these interactive pairs include the T-cell surface molecule CD2 and its ligand CD58 on APCs, CD11a/CD18-CD54, CD5-CD72, CD40 ligand–CD40, and CD28–CD80 or CD86. $CD4^+$ T-cell anergy or tolerance induction occurs when the T-cell receptor interacts with the APC unless signals are provided through 1 or more of these receptor-ligand interactions (particularly through CD40 ligand–CD40 and CD28–CD80 or CD86) or by cytokines (eg, IL-1 and IL-6 from the APC). Thus T-cell accessory proteins and their ligands on APCs are target molecules for antirejection therapy.[17,18] If costimulation does occur, the $CD4^+$ T cell becomes activated, which leads to stable

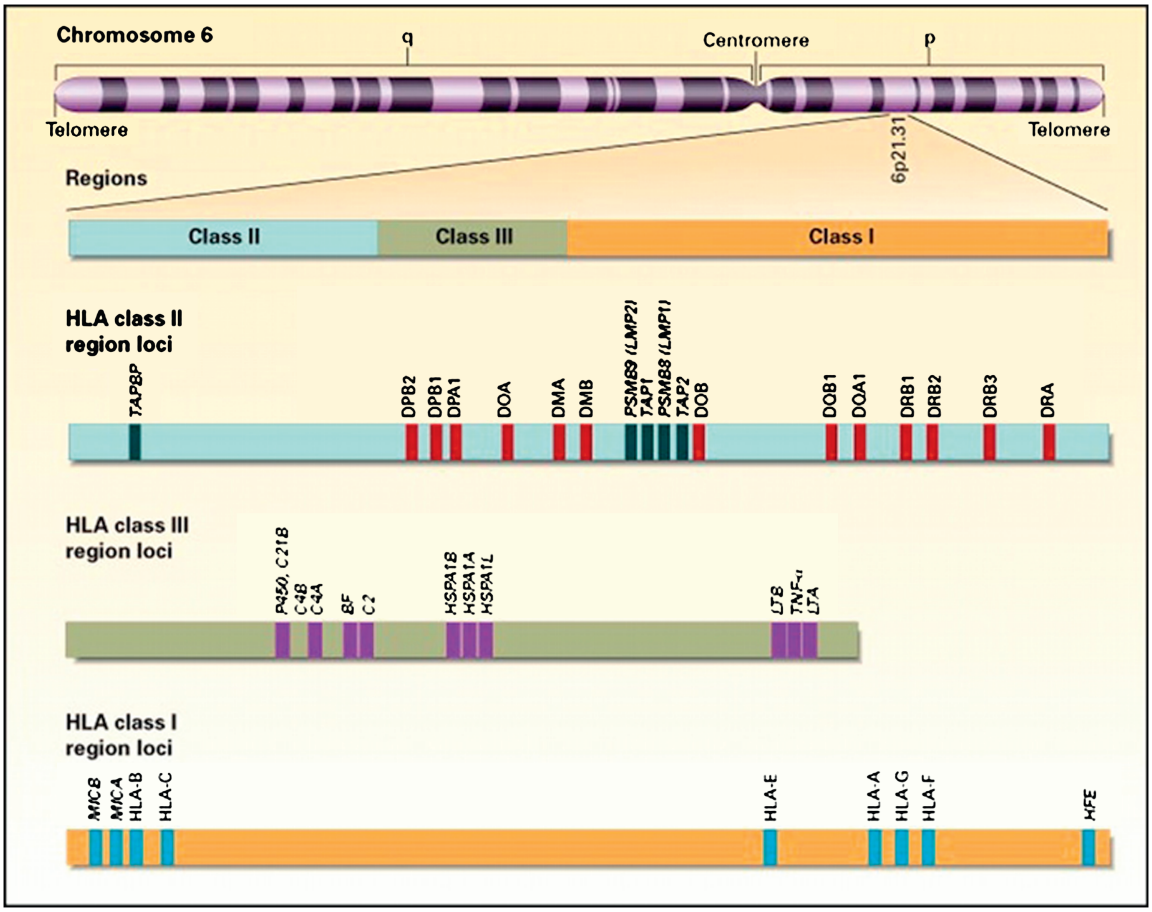

FIG 1. Location and organization of the HLA complex on chromosome 6. *BF*, Complement factor B; *C2*, complement component 2; *C4A*, complement component 4A; *C4B*, complement component 4B; *LTA*, lymphotoxin A; *LTB*, lymphotoxin B; *TAP1*, transporter of antigenic peptides 1; *TAP2*, transporter of antigenic peptides 2. Reprinted with permission from Klein and Sato.[3]

transcription of genes important in T-cell activation. $CD8^+$ T cells recognize antigenic peptides displayed on MHC class I molecules and represent a major cytotoxic effector lymphocyte population in graft rejection. Donor class I molecules on donor APCs in the graft directly activate cytotoxic effector lymphocytes. However, CD8 activation also requires a costimulatory second signal, as well as an IL-2 signal. Activated $CD8^+$ T cells proliferate and mature into specific alloreactive clones capable of releasing granzyme (serine esterase), perforin, and toxic cytokines, such as TNF-α. More recently, the identification of T_H17 effector cells (proinflammatory) and regulatory T cells (downregulators of immune activation) has improved our understanding of the development of graft tolerance or rejection.[19] Stimulation of the B cell by antigen occurs through its antigen receptor (surface immunoglobulin), but costimulation is also required for B-cell activation. This costimulation can be provided by cytokines released by T cells or through many of the same T-cell protein–ligand pairs important in T-cell–APC costimulation because these ligands are also present on B cells. B-cell contribution to the immune rejection of organ transplants is not limited to the production of alloimmune antibodies but also involves antigen presentation and the secretion of proinflammatory cytokines.[20]

Once T-cell activation has occurred, autocrine T-cell proliferation continues as a consequence of the expression of the IL-2 receptor (IL-2R). Interaction of IL-2 with its receptor triggers the activation of protein tyrosine kinases and phosphatidylinositol 3–kinase, resulting in translocation into the cytosol of an IL-2R–bound serine-threonine kinase, Raf-1. This in turn leads to the expression of several DNA-binding proteins, such as c-Jun, c-Fos, and c-Myc, and to progression of the cell cycle. The consequence of all of these events is the development of graft-specific, infiltrating cytotoxic T cells. Cytokines from the T cells also activate macrophages and other inflammatory leukocytes and cause upregulation of HLA molecules on graft cells. The activated T cells also stimulate B cells to produce anti-graft antibodies. Ultimately, if not recognized and managed, all these cellular and humoral factors constitute the rejection process that destroys the graft.

IMMUNOSUPPRESSION

More information on immunosuppresion regimens can be found in Table I.

Currently, there is no method that will suppress the host's immune response to antigens of the graft and at the same time maintain other immune responses. Nonspecific immunosuppressive agents are needed to prevent rejection of the transplanted organ, which can occur even though HLA-matched donors are

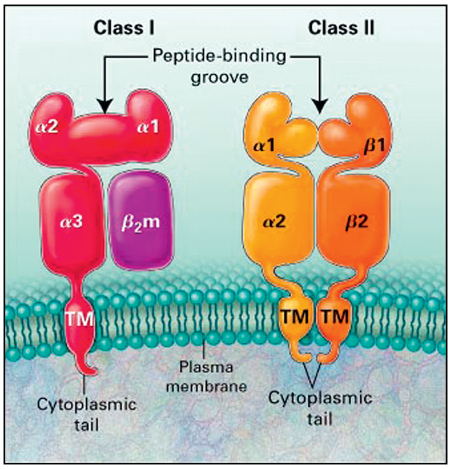

FIG 2. Structures of HLA class I and II molecules. β_2-Microglobulin *(β_2m)* is the light chain of the class I molecule. *TM,* Transmembrane component. Reprinted with permission from Klein and Sato.[3]

used. The development of immunosuppressive strategies during the past 4 decades reflects enormous progress in understanding the cellular and molecular mechanisms that mediate allograft rejection.[21] The success of transplantation between unrelated donors and recipients can be attributed to implementation of these strategies. These agents depress both specific and nonspecific immunity, and they render the recipient more susceptible to both infection and malignancy. Indeed, infection is the most important cause of transplant-recipient death. Thus all patients must have the immunosuppressive regimen fine tuned to prevent rejection yet minimize the risk of infection: too high a dose, and infection supervenes; too small a dose, and the graft is rejected.

The immunosuppressive agents initially used in most transplant centers for nearly 2 decades were corticosteroids, azathioprine, and cyclosporine. Several new agents have been introduced during the past few years: mycophenolate mofetil, which has a similar but more effective mode of action to that of azathioprine; tacrolimus, which has a mode of action and side effects similar to those of cyclosporine; and sirolimus, which blocks IL-2–induced T-cell cycle progression.

Immunosuppressive agents can be categorized by whether they (1) interrupt lymphocyte cell division, (2) deplete lymphocytes, (3) interfere with lymphocyte maturational events, (4) interfere with immune cell costimulation, (5) modulate ischemia–reperfusion injury, or (6) facilitate induction of tolerance.[22] They can also be grouped into those used for induction therapy, for prophylaxis against rejection, for reversal of acute rejection episodes, and for maintenance of immunosuppression.

mAbs to lymphocytes and to cytokine receptors

Antibodies from animals immunized with human lymphoid cells are useful agents for induction therapy, as well as for reversal of acute rejection episodes.[23] They consist of the IgG fraction of serum from horses or rabbits immunized with either human lymphocytes (antilymphocyte globulin [ALG]) or thymocytes (antithymocyte globulin [ATG; thymoglobulin]) or of mAbs (murine or humanized) to T-cell surface antigens (eg, CD3 [OKT3]). In general, ALG, ATG, and OKT3 decrease the onset, severity, and number of rejection episodes. Prevention of graft rejection has also been approached by inhibiting cytokines from interacting with their receptors. Chimeric or humanized murine anti–IL-2R α chain antibodies (daclizumab and basiliximab) have been developed for clinical use. The advantage of these mAbs to the IL-2R α chain is that such molecules are present only on activated T cells; therefore the main effect is on T cells possibly activated by graft antigens.

Calcineurin inhibitors

The main action of calcineurin inhibitors (cyclosporine and tacrolimus) is that they prevent the synthesis of IL-2 and other cytokines that might be produced by T cells activated by allografts.[21] Through its hydrophobicity, cyclosporine enters cell membranes to gain access to and bind to the cytoplasmic isomerase protein cyclophilin. The complex then inhibits calcineurin, an intracellular phosphatase critical for the translocation of signals from the T-cell receptor to the nucleus. In this manner it blocks transcription of the *IL2* gene. In addition, it also blocks the synthesis of other cytokines and thereby interferes with activated $CD4^+$ helper T-cell function. As a consequence, T-cell proliferation and differentiation of precursor cytotoxic lymphocytes are blocked. Tacrolimus binds to a cytoplasmic isomerase protein in the same way that cyclosporine does, but it binds to a different one, the FK-binding protein.[24] The complex formed inhibits calcineurin to prevent T-cell receptor signal transduction to the cell nucleus, blocking cell activation. Tacrolimus thus inhibits synthesis of IL-2, IL-3, IFN-γ, and other cytokines; it was found to be 100 times more potent than cyclosporine as an immunosuppressive agent.[24]

Cytokine receptor signal transduction inhibitors

Sirolimus (Rapamune; Wyeth, Madison, NJ) has a structure similar to tacrolimus, and its activity is also dependent on its binding to the FK-binding protein. However, the complex formed does not inhibit calcineurin but instead prevents the phosphorylation of the p70S6 kinase. This action blocks signal transduction from many cell-surface cytokine receptors, including the IL-2, IL-4, IL-15, and IL-10 receptors. Both *in vitro* and *in vivo* studies have shown a synergistic effect of sirolimus with cyclosporine, as would be expected because sirolimus prevents cytokine receptor signaling and cyclosporine inhibits cytokine production. In addition, sirolimus selectively preserves the development of regulatory T cells.[25] No agent is the perfect nonspecific immunosuppressive drug. Anti-lymphocyte antibodies (including anti-CD3, anti-CD6, and anti-CD52 antibodies), nucleoside synthesis inhibitors, steroids, cyclosporine (or tacrolimus), anti–IL-2R α chain (anti-CD25), and sirolimus all affect allorecognition and antigen-driven T-cell proliferation at different points in the T-cell activation process. Thus the combined use of several of these types of agents provides a synergistic effect rather than a merely additive effect.

SOLID-ORGAN TRANSPLANTATION

The explosive growth of transplantation since the discovery of HLA in 1967 is attested to by the fact that, according to the Global Database on Donation and Transplantation gathering data from 97 countries, in 2007 around 100,000 solid-organ transplantations were performed per year worldwide: 68,250 are kidney transplantations (45% from living donors), 19,850 are liver transplantations (14% from living donors), 5,179 are heart transplantations, 3,245 are lung transplantations, and 2,797 are pancreas transplantations.[26]

TABLE I. Immunosuppresion regimens

Immunosuppression regimen	Immunologic target	Specific use	Major adverse effects
Radiation, anti-metabolite agents	Hematopoietic stem cells, leukocytes	BMT	Cytopenias, opportunistic infections, diarrhea, alopecia, veno-occlusive disease, long-term organ damage: endocrine abnormalities, growth delay, hypodontia, cognitive delay, sterility
Calcineurin inhibitors, anti-lymphocyte antibodies, anti-cytokine antibodies, anti-metabolite agents, and corticosteroids	Lymphocytes	In solid-organ transplantation and BMT: prevention and treatment of graft rejection and GVHD	Opportunistic infections, lymphopenia, renal dysfunction, seizures, hypertrichosis, hypertension, gastritis, osteoporosis, cataracts, growth delay

BMT, Bone marrow transplantation.

Kidney transplantation

Despite major improvements in dialysis techniques, renal transplantation remains the treatment of choice for end-stage renal disease in patients of nearly all ages.[27] Estimates of new cases of end-stage renal disease are at 300 cases per million persons annually, with an increasing trend.[27] For adults and most children, the renal transplantation operation has become standardized. The earlier practice of removing the patient's diseased kidneys 2 to 3 weeks before transplantation has not been carried out routinely in recent years, except for patients with hypertension or infection, and nephrectomy is now performed at the time of transplantation.

Immunosuppressive regimens. Until cyclosporine became available in the early 1980s, most centers used a combination of azathioprine (Imuran; Prometheus Laboratories, Inc, San Diego, Calif) and prednisone to prevent graft rejection. Beginning in 1983, many centers began to use cyclosporine (in lieu of azathioprine) with lower doses of prednisone for immunosuppression.[27,28] Cyclosporine has been given in varying doses at different centers but has generally been given intravenously during or just after transplantation and on the day after. It is then subsequently administered orally and gradually tapered, depending on signs of toxicity or rejection and blood levels. Trough blood levels are periodically monitored, and doses are adjusted to maintain levels of greater than 200 ng/mL. Prednisone is given on the day of transplantation and gradually reduced during the course of 12 weeks. In many centers the induction agents consist of one of the anti–IL-2R α chain antibodies, daclizumab or basiliximab, along with steroids, mycophenolate mofetil (instead of azathioprine), and tacrolimus (instead of cyclosporine). Some transplantation surgeons are combining plasmapheresis, intravenous immunoglobulin, and immunosuppressive drugs for patients who are highly sensitized and have high titers of alloantibodies.[29,30] Acute rejection episodes are treated with intravenous pulses of high-dose methylprednisolone. Among the most useful agents have been ALG for 5 days, ATG for 5 days, and OKT3 for 1 to 14 days. Another anti-lymphocyte mAb, anti-CD52 or alemtuzumab, has also been used successfully, although with differences in the incidence of opportunistic infections.[31,32]

Rejection. Rejection is the most common problem during the 3 months immediately after kidney grafting.[27] Except for hyperacute rejection, most such episodes can be partially or completely reversed by one of the previously described immunosuppressive agents. Rejection episodes are classified as follows (Table II).

Hyperacute rejection occurs within the first 48 hours after the anastomosis takes place in recipients with preformed anti-leukocyte antibodies. It is characterized by fever and anuria. The binding of cytotoxic antibodies to the vascular endothelium activates complement, with subsequent aggregation of neutrophils and platelets, resulting in thrombosis. This is an irreversible event, and the only treatment option is immediate graft removal.

Accelerated rejection occurs on the third to fifth day after transplantation. It is accompanied by fever, graft swelling, oliguria, and tenderness. It is thought to be mediated by non–complement-fixing antibodies to antigens present in the donor kidney. Histopathologically, it is characterized by vascular disruption with hemorrhage. The most effective treatments are anti-lymphocyte reagents, with or without plasmapheresis; these have a success rate of about 60% in reversing this process.

Acute rejection, the most common form, is due to a primary allogeneic response occurring within the first 6 to 90 days after transplantation. It is mediated by both T cells and antibodies, which cause tubulitis and vasculitis, respectively. High-dose pulses of steroids and anti-lymphocyte reagents are effective in reversing the T-cell response about 80% to 90% of the time, but anti-lymphocyte antibodies only reverse the vasculitis about 60% of the time.

Chronic rejection occurs when the tenuous graft tolerance is disturbed 2 or more months after transplantation. It is characterized by marked proteinuria, occasional hematuria, hypertension, and the nephritic syndrome. The primary mediator of this type of rejection is antibody. A kidney biopsy is usually necessary to distinguish rejection from cyclosporine or tacrolimus nephrotoxicity. This process is usually treatment resistant, although progression might be slowed by immunosuppressive regimens.

Efficacy. Renal grafts from HLA-identical sibling donors have a 10-year survival of about 74%. Those transplants from "6 HLA antigen–matched" cadavers have currently a 1-year survival of 95%. The estimated graft survival has slowly improved over time, and the most recent data, from the 1998-1999 cohort, is estimated at 11.6 years, according to national statistics. Grafts from living donors have a higher estimated lifespan of 15 years.[27,33]

Liver and intestinal transplantation

Liver transplantation had its inception in 1963, when the diseased liver of a 3-year-old child with extrahepatic biliary

TABLE II. Solid-organ rejection patterns: Renal rejection as an example

Type	Time after transplantation	Signs and symptoms	Rapidity of onset	Immune component	Pathologic findings	Treatment	Success rate (%)
Hyperacute	<24 h	Fever, anuria	Hours	Antibody and complement	Polymorphonuclear neutrophil deposition and thrombosis	None	0
Accelerated	3-5 d	Fever, graft swelling, oliguria, tenderness	1 d	Non–complement-fixing antibody	Vascular disruption hemorrhage	ALG, ATG, anti-CD3	60
Acute	6-90 d	Oliguria, salt retention, graft swelling, tenderness, sometimes fever	Days to weeks	T cells and antibody	Tubulitis, endovasculitis	Steroids, ALG, ATG, anti-CD3	60-90
Chronic	>60 d	Edema, hypertension, proteinuria, occasional hematuria	Months to years	Antibody	Vascular onion skinning	None	0

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atresia was replaced.[34] Although that patient died, subsequent successes have established liver transplantation as the standard therapy for advanced chronic liver disease.[35] Since 1983, the 1-year survival rates have increased from 25% to 78%, depending on the age and health of the recipient, the underlying condition, and various clinical considerations.

Liver transplantation is indicated for chronic end-stage liver disease, fulminant acute liver failure, and cancer limited to the liver.[36] As with renal transplantation, combined therapy targeting several facets of the potential rejection process is used for liver transplantation.

Anti–IL-2R α chain antibodies are given intravenously on the day of transplantation, followed by tacrolimus, which is given intravenously initially and orally thereafter and then by mycophenolate mofetil and steroids tapered slowly over a year. Survival has increased by 20% in the last 2 decades with tacrolimus-based immunosuppression.[37]

Although this improvement might be the result of several factors, retransplantation as a result of acute or chronic rejection has not occurred in patients treated with tacrolimus. Similar to all solid-organ transplantation, lack of suitable donors is a major problem for liver transplantation. Since 1988, this organ shortage has been approached by partial hepatectomies of living related donors. Donor safety is much greater with use of the left lateral segment.[38]

Intestinal transplantation is offered to patients who have intestinal failure (caused by short-bowel syndrome, mucosal disease, motility disorders, and tumors) and who present with severe complications of parenteral nutrition, such as cholestatic liver disease and recurrent loss of central venous access.[39] Advances in surgical techniques, control of immune rejection, and treatment of infections have improved the outcomes over time. In 2008, 185 intestinal transplantations were performed in the United States. The 1-year rate of patient survival has increased from 57% in 1997 to 80% in 2005 and to 90% if the data are limited to centers that perform the largest number of transplantations.

Heart, heart-lung, and lung transplantation

The various forms of cardiomyopathy are the most common indications for heart transplantation, followed by congenital heart disease. Approximately 25% of heart transplant recipients are infants.[22] Immunosuppressive regimens for heart transplantation are similar in many respects to those already described for renal and hepatic grafts. Usually an anti–IL-2R α chain mAb is given for induction therapy on the day of transplantation, along with high-dose intravenous methylprednisolone. Prednisone is given after the operation and maintained orally until it is discontinued after the first normal findings from an endomyocardial biopsy. Tacrolimus is then begun as the primary immunosuppressive agent with or without mycophenolate.[22]

Since the introduction of cyclosporine 26 years ago, the results of cardiac transplantation have improved greatly. The International Heart Transplantation Registry has shown a 4-year survival of 71% for patients receiving cyclosporine- or tacrolimus-based triple immunosuppression therapy. Survival is influenced by the age of the recipient; patients younger than 40 years have a better survival.[40] Lung transplantation has been performed for the following major diagnostic categories: cystic fibrosis, pulmonary vascular disease, bronchiolitis obliterans, pulmonary alveolar proteinosis, and pulmonary fibrosis, with 4-year survival at approximately 50%.[22,41]

BONE MARROW TRANSPLANTATION

Since 1955, more than 240,000 bone marrow transplantations have been performed worldwide at 450 centers in 47 countries for the treatment of more than 50 different fatal diseases (Table III).[42] Most of these transplantations have been done by reinfusing stored autologous marrow cells collected before the patient receives intensive chemotherapy or irradiation. Annually, 25,000 to 35,000 autologous transplantations are performed compared with approximately 15,000 allogeneic transplantations. Certain unique problems distinguish bone marrow transplantation from transplantation of solid organs, such as the kidney, liver, and heart. The first problem is that immunocompetent cells, both in the recipient and in the donor marrow or blood, have the potential to reject each other, resulting in graft rejection on the one hand and GVHD on the other.[43] The second concern is that successful unfractionated marrow grafting usually requires strict donor and recipient MHC class II antigen compatibility to minimize such reactions. Finally, except for patients with severe combined immunodeficiency (SCID), complete DiGeorge anomaly, or identical twin donors, even HLA-identical recipients must be pretreated with cytotoxic and myeloablative agents to prevent graft rejection.[43] Diseases treated successfully by allogeneic bone marrow transplantation include radiation injury, primary

TABLE III. Conditions treated with hematopoietic stem cell transplantation

Leukemias	Acute lymphoblastic leukemia Acute myelogenous leukemia Chronic lymphocytic leukemia Chronic myelogenous leukemia
Lymphomas	Non-Hodgkin lymphoma Hodgkin disease
Plasma cell disorders	Multiple myeloma and related disorders
Solid-organ neoplasias	Breast cancer, ovarian cancer, melanoma neuroblastoma, lung cancer, sarcoma
Myelodysplastic syndromes	
Severe aplastic anemia	
Autoimmune diseases	Multiple sclerosis, systemic sclerosis, systemic lupus erythematosus
Inherited erythrocyte abnormalities	Sickle cell disease, thalassemia
Inherited metabolic diseases	Mucopolysaccharidosis type I, adrenoleukodystrophy, osteopetrosis
Primary immunodeficiencies	SCID Wiskott-Aldrich syndrome CGD Leukocyte adhesion deficiency CD40 ligand deficiency X-linked lymphoproliferative disease Hemophagocytic lymphohistiocytosis

immunodeficiencies, hemoglobinopathies, aplastic anemia, multiple myeloma, leukemia, neuroblastoma, non-Hodgkin lymphoma, inborn errors of metabolism, and certain autoimmune diseases.[44] In addition, autologous marrow transplantation has been used after lethal irradiation or chemotherapy in the treatment of patients with some hematologic malignancies, solid tumors, or breast cancer, as well as for the treatment of several autoimmune diseases.[45]

Other sources of hematopoietic stem cells for transplantation

Bone marrow is not the only source of hematopoietic stem cells. These cells are capable of reconstituting all blood cell lineages and can also be obtained from peripheral blood or cord blood. Peripheral blood–derived hematopoietic stem cells are retrieved after the donor receives granulocyte colony-stimulating factor, usually at 5 to 10 μg/kg/d for 5 days, to allow mobilization of the hematopoietic stem cells. These are then collected by means of leukapheresis, and the stem cells are positively selected by using affinity columns containing antibodies to the cell-surface markers CD34 or CD133, both of which are suggested to have the highest specificity for pluripotential hematopoiesis.[46] Cord blood is increasingly being used because of its availability and simplicity of procurement and the potential of a lower severity of GVHD without full HLA matching.[47] The number of cells in cord blood units is a limiting factor that is currently being addressed by using more than 1 donor's cord blood.

Clinical features of GVHD

Acute GVHD begins 6 or more days after transplantation (or after transfusion in the case of nonirradiated blood products).[48] Signs of GVHD include fever, a morbilliform erythematous rash, and severe diarrhea.[49] The rash becomes progressively confluent and might involve the entire body surface; it is both pruritic and painful and eventually leads to marked exfoliation. Eosinophilia and lymphocytosis develop, followed shortly by hepatosplenomegaly, exfoliative dermatitis, protein-losing enteropathy, bone marrow aplasia, generalized edema, increased susceptibility to infection, and death.[50] Skin biopsy specimens reveal basal vacuolar degeneration or necrosis, spongiosis, single-cell dyskeratosis, eosinophilic necrosis of epidermal cells, and a dermal perivascular round cell infiltration. Similar necrotic changes can occur in the liver, intestinal tract, and eventually most other tissues.

Treatment of GVHD

Many regimens have been used to mitigate GVHD in both HLA-incompatible and HLA-compatible bone marrow transplants. In MHC-compatible bone marrow transplants into patients with SCID or complete DiGeorge anomaly, it is not usually necessary to give immunosuppressive agents to prevent or mitigate the mild GVHD that might occur, although occasionally steroids are used to treat more severe forms of this condition. For unfractionated, HLA-identical marrow transplants into all patients for whom pretransplantation chemotherapy is given to prevent rejection, however, it is necessary to use prophylaxis against GVHD. Patients are usually given a combination of methotrexate, corticosteroids, and a calcineurin inhibitor daily for 6 months.[51-53] When GVHD becomes established, it is extremely difficult to treat. Antithymocyte serum, steroids, cyclosporine, tacrolimus, anti–IL-2R α chain antibodies, anti–TNF-α inhibitors, mycophenolate mofetil, and murine mAbs to human T-cell surface antigens have ameliorated some cases, but the course has been inexorably fatal in many patients similarly treated.[54-56] The best approach to GVHD reactions is prevention, and by far the best preventive approach is the removal of all postthymic T cells from the donor marrow or blood.

HLA-identical bone marrow transplantation for patients with SCID

The only adequate therapy for patients with severe forms of cellular immunodeficiency is immunologic reconstitution by means of transplantation of immunocompetent hematopoietic stem cells. Until 1980, only HLA-identical unfractionated bone marrow could be used for this purpose because of the lethal GVHD that ensued if mismatched donors were used.[57] In most cases, both T-cell and B-cell immunity have been reconstituted by such fully matched transplants, with evidence of function detected very soon after unfractionated marrow transplantation.[58] Analysis of the genetic origins of the immune cells in the engrafted patients has revealed that although the T cells are all of donor origin, the B cells are often those of the recipient.[12] Initially, it was considered that bone marrow was effective in conferring immunity in patients with SCID because it provided normal stem cells, but it is apparent from later experience with T cell–depleted marrow[59] that the early restoration of immune function after unfractionated HLA-identical marrow transplantation is caused by adoptive transfer of mature T and B cells in the donor marrow. Unfortunately, because of the lack of HLA-identical related donors, unfractionated bone marrow transplantation has not been possible for more than 85% of the immunodeficient patients who could have benefited. As a consequence, before the year 1982, most such patients died with severe infections.

HLA-haploidentical bone marrow transplantation for patients with SCID

The fact that totally HLA-disparate fetal liver cells could correct the immune defect in a few such patients without causing GVHD gave hope that HLA-disparate marrow stem cells could do the same if all donor postthymic T cells could be removed. Early success in T-cell depletion was achieved in experimental animals by treating donor marrow or spleen cells with anti–T-cell antiserum or agglutinating the unwanted cells with plant lectins.[60] The remaining immature marrow or splenic non-T cells restored lymphohematopoietic function to lethally irradiated MHC-disparate recipients without lethal GVHD. This approach was applied to human subjects in the early 1980s and has been highly successful in infants with SCID.[12,59-63]

The time to development of immune function after haploidentical stem cell grafting is quite different from that after unfractionated HLA-identical marrow grafting. Lymphocytes with mature T-cell phenotypes and functions fail to increase significantly until 3 to 4 months after transplantation; normal T-cell function is reached between 4 and 7 months.[59] B-cell function develops much more slowly, averaging 2 to 2.5 years for normalization; many do not have B-cell function developed, despite normal T-cell function.[12,13] Genetic analyses of the lymphocytes from such chimeric patients have revealed all T cells to be genetically from donor origin, whereas the B cells and APCs almost always remain those of the recipient.[61,62] These observations indicate that the thymic microenvironment of most infants with SCID is capable of differentiating half-matched normal stem cells to mature and functioning T lymphocytes that can cooperate effectively with host B cells for antibody production. Thus the genetic defect in SCID does not compromise the function of the thymus.

Efficacy of bone marrow transplantation in patients with immunodeficiency diseases

Although precise figures are not available, during the past 40 years, more than 1,200 patients worldwide with different forms of genetically determined immunodeficiency have been given bone marrow transplants in attempts to correct their underlying immune defects. Possibly because of earlier diagnosis before untreatable opportunistic infections develop, the results have improved considerably during the last 2 decades.[62-67] As would be expected, survival outcomes of HLA-matched related transplants have been superior to those of HLA-haploidentical or HLA-identical unrelated transplants in several series of patients treated in specialized centers worldwide.

SCID. Bone marrow transplantation has been more widely applied and more successful in infants with SCID than any other primary immunodeficiency. The use of pretransplantation myelosuppressive or myeloablative conditioning is advocated by some investigators to prevent graft rejection, but because infants with SCID lack T cells, there should be no need to use pretransplantation chemotherapy. The largest multicenter report of patients with SCID who received bone marrow transplantation was a European collaborative study from 1968 to 1999, including 153 patients receiving an HLA-matched related (from parent or sibling) transplant, with a survival rate of 77%, and 294 patients receiving a haploidentical HLA-matched transplant, with a survival of 54%.[63] Twenty-eight patients received an HLA-matched unrelated donor transplant, with a survival rate of 63%. These outcomes have improved in the last decade, likely because of progress in early diagnosis and medical care, specifically in the availability of newer antibacterial and antiviral agents, as well as immunosuppressive drugs for the control and prophylaxis of GVHD. In addition, difference in the use of myeloablative and rejection prophylaxis regimens with their inherent toxicity is a variable that affects the survival rate. The largest series of patients with SCID receiving bone marrow transplantation in the United States reported 161 patients who did not receive pretransplantation conditioning.[62,68] Sixteen of them received an HLA-matched related donor transplant, with 100% survival. The others received a haploidentical HLA-matched related donor transplant, with a long-term (up to 26 years) survival rate of 77%. Nevertheless, this is a major accomplishment because SCID is 100% fatal without marrow transplantation or, in the case of adenosine deaminase (ADA)–deficient SCIDs, enzyme replacement therapy. Of note, those who underwent transplantation earlier than 3.5 months of age had a survival of 94%, possibly reflecting the influence of opportunistic infections as determinants of transplantation success. These studies and others have shown that such transplants can provide normal numbers of T cells and normalize T-cell function in all known molecular types of SCID. Thus there appears to be no survival advantage in performing such transplantations *in utero*[69,70] as opposed to performing them soon after birth. *In utero* transplantations carry the risks associated with the invasive procedure that involves accessing the fetus and the difficulty of monitoring the possible development of GVHD during gestation.

Other primary immunodeficiencies. The second largest group of patients with immunodeficiency given bone marrow transplants since 1968 are those with the Wiskott-Aldrich syndrome.[71,72] In a report from the International Bone Marrow Transplant Registry, 170 patients with Wiskott-Aldrich syndrome had undergone transplantation, and the 5-year probability of survival for all subjects was 70% (95% CI, 63% to 77%). Probabilities differed according to donor type: 87% (95% CI, 74% to 93%) with HLA-identical sibling donors, 52% (95% CI, 37% to 65%) with other related donors, and 71% (95% CI, 58% to 80%) with matched unrelated donors ($P = .0006$). Boys who had received a matched unrelated donor transplant before 5 years of age had survivals similar to those receiving HLA-identical sibling transplants. Of note, the incidence of autoimmunity in these patients after bone marrow transplantation is up to 20%.[72]

Patients with combined immunodeficiencies characterized by less severe T-cell defects than those seen in patients with SCID, such as ZAP70 deficiency, constitute the third largest group of patients given bone marrow transplants. Forty-five patients with Omenn syndrome were reported as having received marrow transplants, and 23 (51%) were alive at the time of the report.[61] Fourteen (54%) of 26 patients with the bare lymphocyte syndrome were alive after having been given marrow transplants.[73,74] Other disorders treated successfully with bone marrow transplantation include X-linked hyper-IgM,[75] reticular dysgenesis,[76] purine nucleoside phosphorylase deficiency,[77] cartilage hair hypoplasia, and X-linked lymphoproliferative syndrome.

Patients with complete DiGeorge syndrome have undergone both marrow and thymic transplantations. Six of 9 such patients were reported to have survived 2 to 24 years after having received unfractionated HLA-identical sibling marrow[78]; however, possible publication bias was suggested, proposing that a number of patients who might not have survived had not been taken into account.[79] Because the underlying defect in this condition is absence of the thymus, a more direct approach is to perform

thymus transplantation. To this end, 54 infants with complete DiGeorge syndrome have undergone thymic transplantation with cultured HLA-unmatched unrelated thymic tissue, with a survival rate of 69%.[80] An important immunologic difference is that the transplanted thymus allows the development of naive T cells even with a disparate HLA haplotype between donor and recipient. In contrast, patients with complete DiGeorge syndrome who receive bone marrow transplants survive with a reduced T-cell number and absent naive T-cell population.

Patients with primarily phagocytic disorders also have been shown to benefit from bone marrow transplantation. Recently, a report from Europe included data from 24 patients with chronic granulomatous disease (CGD) who had received bone marrow transplants, with 19 patients surviving.[81] At Texas Children's Hospital (Houston, Texas), 11 patients with CGD (9 with X-linked CGD and 2 with autosomal recessive CGD) have undergone transplantation, with 10 patients surviving and immunoreconstituted and a median follow-up of 25 months (unpublished data). Four of these received HLA-matched related transplants, and 6 received HLA-matched unrelated grafts. One patient who received a mismatched related (HLA 5/6 matched) transplant did not survive. Other leukocyte disorders that have been successfully treated with bone marrow transplantation include pigmentary dilution (Griscelli) syndrome, Chediak-Higashi syndrome, familial hemophagocytic histiocytosis, severe congenital neutropenia, and leukocyte adhesion deficiency.[61,82]

Efficacy of bone marrow transplantation in malignancy

Bone marrow transplantation is the therapy of choice for leukemia, lymphoma, and myelodysplastic proliferative disorders.[83] The success of marrow transplantation in curing malignancy depends on a number of factors, the most important of which are the type of malignant disease, the stage of the disease, and the age of the recipient. Most patients with acute myelogenous leukemia achieve remission after chemotherapy; however, approximately 65% of patients will relapse within 2 years.[84] During the first complete remission, consolidation chemotherapy or bone marrow transplantation are possible alternatives. In patients with intermediate-risk disease, the projected disease-free survivals at 5 years are 52% for allogeneic transplantation and 45% for autologous transplantation.[85] For patients with chronic myelogenous leukemia, allogenic bone marrow transplantation is considered primarily for pediatric patients, with a success rate of more than 80%, and for those adults who have had unsuccessful medical treatment with tyrosine kinase inhibitors.[83,86] Three-year overall survival is variable among different series, reaching up to 80%. The best survival rates with the lowest probability of relapse occurred in patients younger than 20 years who had acute nonlymphocytic leukemia and underwent transplantation in first remission and in patients with chronic myelogenous leukemia who underwent transplantation in the chronic phase.[87]

The rationale for allogeneic bone marrow transplantation in patients with leukemia is the hope that the leukemic cells can be reduced or eliminated by means of irradiation or chemotherapy and that the grafted allogeneic normal T cells can then reject any remaining leukemic cells.[88] Supporting a need for T cells in the graft is the fact that T cell–depleted bone marrow transplants have been associated with a higher degree of leukemia recurrence.[89]

Efficacy of bone marrow transplantation in hemoglobinopathies, osteopetrosis, metabolic storage diseases, and severe autoimmunity

Bone marrow transplantation has been highly effective for the treatment of homozygous β-thalassemia, with survivals reaching 70% to 80% for marrow transplants from HLA-identical siblings.[90] Likewise, HLA-identical bone marrow transplantation has also been successful for patients with sickle cell disease, with 59 patients known to have been treated, 55 of whom were surviving, with 50 free of sickle cell disease.[91] The European Bone Marrow Transplantation Group reported on 69 patients with autosomal recessive osteopetrosis who received HLA-identical or haploidentical bone marrow transplants between 1976 and 1994.[92,93] Recipients of genotypically HLA-identical marrow had an actuarial probability for 5-year survival of up to 60%, with osteoclast function of 79% of the survivors. Mucopolysaccharidosis type I (Hurler disease) and adrenoleukodystrophy, but not other lysosomal storage diseases, have been successfully treated with bone marrow transplantation when performed before significant organ damage occurs, as an alternative to enzyme replacement.[94] Autologous and allogeneic bone marrow transplantation protocols have been used with relative success in patients with severe autoimmunity. In a large collaborative study of more than 500 patients with autoimmune conditions, survival was 80%, with sustained improvement in 70% of the survivors.[95]

Nonmyeloablative bone marrow transplantation

For patients with pre-existing organ damage, there is significant morbidity and mortality from traditional conditioning regimens with busulfan and cyclophosphamide or irradiation. Because of this, there has been increasing interest in developing conditioning regimens that are less toxic.[96]

This has been accomplished by using either total lymphoid irradiation or a combination of nucleoside analogs and anti–lymphocyte antibody preparations. Although these regimens are significantly less cytotoxic than high-dose alkylating agents and total-body irradiation, they are profoundly immunosuppressive. Opportunistic infections, such as the reactivation of cytomegalovirus, remain clinical obstacles when nonmyeloablative stem cell transplantations are performed with these agents, especially in elderly and previously immunosuppressed patients. GVHD prophylaxis with cyclosporine and methotrexate, with added mycophenolate mofetil in some cases, has been necessary because GVHD is common after nonmyeloablative transplantation.

Gene therapy for primary immunodeficiencies

Gene therapy trials in the last decade have shown "proof of concept" that genetic disorders can be modified and even cured. Significant progress was made in patients with X-linked SCID, ADA-deficient SCID, and X-linked CGD. The reports by Cavazzana-Calvo et al[97] and Hacein-Bey-Abina et al[98,99] of successful gene therapy in infants with X-linked SCID represented a major step forward because repeated efforts to achieve gene correction of ADA-deficient SCID had failed during the decade before 2000. Subsequently, Gaspar et al[100] reported a similar gene therapy protocol for X-linked SCID conducted in London, confirming the efficacy of this novel approach. The group at the Hôpital Necker in Paris treated 11 patients with X-linked SCID with

gene-corrected autologous bone marrow cells. Nine infants had normal T- and B-cell functions after the treatments. Two did not improve and were given allogeneic bone marrow transplants. The 9 patients who did acquire normal immune function did not require intravenous immunoglobulin infusions and were at home without any medication. Four of the 10 patients treated in London have poor B-cell reconstitution and are dependent on immunoglobulin supplementation. Natural killer cell reconstitution in this molecular type of SCID is also poor, which is similar to that seen in patients who receive bone marrow transplantation.

However, serious adverse events with this therapy occurred in 4 patients treated at the Hôpital Necker and 1 patient treated in London.[99] Shortly before varicella developed, the first patient was discovered to have a high white blood cell count as a result of an expanded clonal population of circulating γδ-positive T cells. The white blood cell count became much higher and became a leukemic-like process that was treated with chemotherapy. The T-cell clone was shown to carry the inserted retroviral gene vector within an intron in a gene on chromosome 11 called *LMO2*. *LMO2* is an oncogene that is aberrantly expressed in acute lymphoblastic leukemia of childhood.[101] Similarly, the other 3 patients in that protocol and 1 of the 10 patients treated in London had T-cell proliferation with upregulation of the expression of not only *LMO2* but also of other oncogenes. Fortunately, 4 of these patients responded to conventional chemotherapy regimens and are presently in remission, with a relatively normal quality of life. Insertional oncogenesis has long been known to be a potential complication of retroviral vector gene transfer because retrovirus integration might occur within oncogenes in the genome. This complication has been thought to be unlikely with such vectors because the vectors cannot reproduce themselves and cannot repeatedly insert into the cell's chromosomes to increase the likelihood of malignant change. Before these cases, malignant changes had not been seen in any human subjects given retroviral vectors for gene transfer. Considering the success of bone marrow transplantation for recipients of HLA-matched related donor grafts and for those who are treated in early infancy, new gene therapy trials for X-linked SCID are now being developed with the objective of reducing their oncogenesis potential, such as with the use of lentivirus-based gene vectors.[102]

Gene therapy trials for ADA deficiency were initiated in the early 1990s, with targeting of peripheral lymphocytes and later CD34-enriched bone marrow cells. The success of these trials was modest, resulting in detection of a small proportion of gene-modified cells in peripheral blood but no evidence of immunologic benefits.[103] The required concomitant use of polyethylene glycol–modified bovine ADA is considered to have been a contributing cause to the failures in the US trials. Recently, 2 European research groups reported gene therapy trials for ADA deficiency using low-dose busulfan pretherapy without polyethylene glycol–modified bovine ADA or (in those patients who were receiving it) withdrawing the enzyme for a few weeks before infusion of the gene-modified cells.[104,105] Eleven of the 15 patients treated with this approach (10 in Italy and 5 in London) showed good immunoreconstitution. Of note, there have not been cases of leukemia or lymphoma in the cases of ADA-deficient SCID that have been corrected by gene therapy, although insertions of gene vectors near oncogenes similar to the X-linked SCID trials have been observed.

A small number of patients with X-linked CGD have been treated with gene therapy approaches.[106] In the United States initial efforts in 1997 by Malech and collaborators resulted in the detection of genetically corrected cells, although in minimal proportion (<1% of granulocytes). A more recent European trial adding a myeloablative regimen before infusion of the gene-corrected cells showed a larger proportion of gene-modified cells, although with only transient expression of the gene. The treatment provided initial clinical benefit, including resolution of severe and chronic fungal and bacterial infections. Patients in one of the trials demonstrated cell expansion as a result of insertional mutagenesis and required bone marrow transplantation, which was curative in one of 2 patients.[107] Efforts aimed to improve the expression of the gene and to reduce oncogenesis are underway.

CONCLUSIONS

Advances in transplantation immunology have allowed the exponential growth of organ and tissue transplantation in medicine over the last 3 decades. Newer immunosuppressive agents have allowed the control of solid-organ and tissue rejection and GVHD, even when HLA incompatibility is present. For the treatment of hematologic disorders, including primary immunodeficiencies, hematopoietic stem cell transplantation is not only feasible but is also the treatment of choice in many cases. Future developments in the field of transplantation immunology will hopefully include novel immunosuppressors with less toxicity and more specificity to control graft rejection while sparing overall immunity and thereby enabling better infection control. Gene therapy has shown promise in curing severe primary immunodeficiencies; however, problems with this approach urgently need to be addressed, the most important of which is insertional mutagenesis seen with the gene vectors used to date.

REFERENCES

1. Bach FH, Amos DB. Hu-1: major histocompatibility locus in man. Science 1967; 156:1506-8.
2. Holdsworth R, Hurley CK, Marsh SG, Lau M, Noreen HJ, Kempenich JH, et al. The HLA dictionary 2008: a summary of HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens. Tissue Antigens 2009;73:95-170.
3. Klein J, Sato A. The HLA system: first of two parts. N Engl J Med 2000;343: 702-9.
4. Marsh SG. WHO Nomenclature Committee for Factors of the HLA System. Nomenclature for factors of the HLA system, update. Tissue Antigens June 2009; 2009(74):364-6.
5. Klein J, Sato A. The HLA system: second of two parts. N Engl J Med 2000;343: 782-6.
6. Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O. Hyperacute rejection of kidney allografts associated with pre-existing humoral antibodies against donor cells. Lancet 1966;2:662-5.
7. Thielke J, Kaplan B, Benedetti E. The role of ABO-incompatible living donors in kidney transplantation: state of the art. Semin Nephrol 2007;27:408-13.
8. Spellman S, Setterholm M, Maiers M, Noreen H, Oudshoorn M, Fernandez-Viña M, et al. Advances in the selection of HLA-compatible donors: refinements in HLA typing and matching over the first 20 years of the National Marrow Donor Program Registry. Biol Blood Marrow Transplant 2008;14(suppl):37-44.
9. Kahan BD. Cyclosporine: a revolution in transplantation. Transplant Proc 1999; 31(suppl):14S-5S.
10. Zou Y, Stastny P. The role of major histocompatibility complex class I chain-related gene A antibodies in organ transplantation. Curr Opin Organ Transplant 2009;14:414-8.
11. Mickelson EM, Fefer A, Storb R, Thomas ED. Correlation of the relative response index with marrow graft rejection in patients with aplastic anemia. Transplantation 1976;22:294-330.
12. Buckley RH, Schiff SE, Schiff RI, Markert L, Williams LW, Roberts JL, et al. Hematopoietic stem cell transplantation for the treatment of severe combined immunodeficiency. N Engl J Med 1999;340:508-16.
13. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. Annu Rev Immunol 2004;22:625-55.

14. Al-Lamki RS, Bradley JR, Pober JS. Endothelial cells in allograft rejection. Transplantation 2008;86:1340-8.
15. Karczewski J, Karczewski M, Glyda M, Wiktorowicz K. Role of TH1/TH2 cytokines in kidney allograft rejection. Transplant Proc 2008;40:3390-2.
16. Knechtle SJ, Kwun J. Unique aspects of rejection and tolerance in liver transplantation. Semin Liver Dis 2009;29:91-101.
17. Snanoudj R, de Préneuf H, Créput C, Arzouk N, Deroure B, Beaudreuil S, et al. Costimulation blockade and its possible future use in clinical transplantation. Transpl Int 2006;19:693-704.
18. Li XC, Rothstein DM, Sayegh MH. Costimulatory pathways in transplantation: challenges and new developments. Immunol Rev 2009;229:271-93.
19. Atalar K, Afzali B, Lord G, Lombardi G. Relative roles of Th1 and Th17 effector cells in allograft rejection. Curr Opin Organ Transplant 2009;14:23-9.
20. Balin SJ, Platt JL, Cascalho M. Noncognate function of B cells in transplantation. Transpl Int 2009;22:593-8.
21. Kahan BD. Forty years of publication of transplantation proceedings—the second decade: the cyclosporine revolution. Transplant Proc 2009;41:1423-37.
22. Webber SA, McCurry K, Zeevi A. Heart and lung transplantation in children. Lancet 2006;368:53-69.
23. Brennan DC, Daller JA, Lake KD, Cibrik D, Del Castillo D. Thymoglobulin Induction Study Group. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. N Engl J Med 2006;355:1967-77.
24. Webster AC, Woodroffe RC, Taylor RS, Chapman JR, Craig JC. Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: meta-analysis and meta-regression of randomised trial data. BMJ 2005;331:810.
25. Coenen JJ, Koenen HJ, van Rijssen E, Hilbrands LB, Joosten I. Rapamycin, and not cyclosporin A, preserves the highly suppressive CD27+ subset of human CD4 + CD25+ regulatory T cells. Blood 2006;107:1018-23.
26. Matesanz R, Mahillo B, Alvarez M, Carmona M. Global observatory and database on donation and transplantation: world overview on transplantation activities. Transplant Proc 2009;41:2297-301.
27. Magee CC, Pascual M. Update in renal transplantation. Arch Intern Med 2004; 164:1373-88.
28. Knoll G. Trends in kidney transplantation over the past decade. Drugs 2008; 68(suppl 1):3-10.
29. Lefaucheur C, Nochy D, Andrade J, Verine J, Gautreau C, Charron D, et al. Comparison of combination Plasmapheresis/IVIg/anti-CD20 versus high-dose IVIg in the treatment of antibody-mediated rejection. Am J Transplant 2009; 9:1099-107.
30. Uchida J, Iwai T, Kato M, Machida Y, Naganuma T, Kumada N, et al. A novel approach to successful ABO-incompatible high-titer renal transplantation. Transplant Proc 2008;40:2285-8.
31. Kauffman HP, Stormstad AS, Sampson D, Stawicki AT. Randomized steroid therapy of human kidney transplant rejection. Transplant Proc 1979;11:36-8.
32. Clatworthy MR, Friend PJ, Calne RY, Rebello PR, Hale G, Waldmann H, et al. Alemtuzumab (CAMPATH-1H) for the treatment of acute rejection in kidney transplant recipients: long-term follow-up. Transplantation 2009;87:1092-5.
33. Paramesh A, Zhang R, Yau CL, Balamuthusamy S, Shenava R, Killackey M, et al. Long-term outcome of single pediatric donor kidney transplants between African-American and non-African-American adults. Clin Nephrol 2009;72:55-61.
34. Starzl TE, Esquivel C, Gordon R, Todo S. Pediatric liver transplantation. Transplant Proc 1987;19:3230-5.
35. Jain A, Mazariegos G, Kashyap R, Kosmach-Park B, Starzl TE, Fung J, et al. Pediatric liver transplantation. A single center experience spanning 20 years. Transplantation 2002;73:941-7.
36. Mowat AP. Liver disorders in children: the indications for liver replacement in parenchymal and metabolic diseases. Transplant Proc 1987;19:3236-41.
37. Pillai AA, Levitsky J. Overview of immunosuppression in liver transplantation. World J Gastroenterol 2009;15:4225-33.
38. Hong JC, Yersiz H, Farmer DG, Duffy JP, Ghobrial RM, Nonthasoot B, et al. Long term outcomes for whole and segmental liver grafts in adult and pediatric liver transplant recipients: a 10- year comparative analysis of 2,988 cases. J Am Coll Surg 2009;208:682-9.
39. Fishbein TM. Intestinal transplantation. N Engl J Med 2009;361:998-1008.
40. Aurora P, Edwards LB, Christie J, Dobbels F, Kirk R, Kucheryavaya AY, et al. Registry of the International Society for Heart and Lung Transplantation: eleventh official pediatric lung and heart/lung transplantation report—2008. J Heart Lung Transplant 2008;27:978-83.
41. Hertz MI, Aurora P, Christie JD, Dobbels F, Edwards LB, Kirk R, et al. Registry of the International Society for Heart and Lung Transplantation: a quarter century of thoracic transplantation. J Heart Lung Transplant 2008;27:937-42.
42. Horowitz M. The role of registries in facilitating clinical research in BMT: examples from the Center for International Blood and Marrow Transplant Research. Bone Marrow Transplant 2008;42(suppl 1):S1-2.
43. Martin PJ, Hansen JA, Storb R, Thomas ED. Human marrow transplantation: an immunological perspective. Adv Immunol 1987;40:379-438.
44. Appelbaum FR. Hematopoietic-cell transplantation at 50. N Engl J Med 2007; 357:1472-5.
45. Ljungman P, Urbano-Ispizua A, Cavazzana-Calvo M, Demirer T, Dini G, Einsele H, et al. European Group for Blood and Marrow. Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: definitions and current practice in Europe. Bone Marrow Transplant 2006;37:439-49.
46. Padmanabhan A, Reich-Slotky R, Jhang JS, Dael S, Crowder T, Colovai AI, et al. Use of the haematopoietic progenitor cell parameter in optimizing timing of peripheral blood stem cell harvest. Vox Sang 2009;97:153-9.
47. Kurtzberg J. Update on umbilical cord blood transplantation. Curr Opin Pediatr 2009;21:22-9.
48. Dwyre DM, Holland PV. Transfusion-associated graft-versus-host disease. Vox Sang 2008;95:85-93.
49. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. Transplantation 1974;18:295-304.
50. Deeg HJ, Antin JH. The clinical spectrum of acute graft-versus-host disease. Semin Hematol 2006;43:24-31.
51. Carnevale-Schianca F, Leisenring W, Martin PJ, Furlong T, Schoch G, Anasetti C, et al. Longitudinal assessment of morbidity and acute graft-versus-host disease after allogeneic hematopoietic cell transplantation: retrospective analysis of a multicenter phase III study. Biol Blood Marrow Transplant 2009;15:749-56.
52. Finke J, Bethge WA, Schmoor C, Ottinger HD, Stelljes M, Zander AR, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. Lancet Oncol 2009;10:855-64.
53. Ogawa H, Soma T, Hosen N, Tatekawa T, Tsuboi A, Oji Y, et al. Combination of tacrolimus, methotrexate, and methylprednisolone prevents acute but not chronic graft-versus-host disease in unrelated bone marrow transplantation. Transplantation 2002;74:236-43.
54. Adkins D, Ratanatharathorn V, Yang H, White B. Safety profile and clinical outcomes in a phase I, placebo-controlled study of siplizumab in acute graft-versus-host disease. Transplantation 2009;88:198-202.
55. Pidala J, Kim J, Anasetti C. Sirolimus as primary treatment of acute graft-versus-host disease following allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2009;15:881-5.
56. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-vs-host disease. Lancet 2009;373: 1550-61.
57. Bortin MM, Rimm AA. Severe combined immunodeficiency disease. Characterization of the disease and results of transplantation. JAMA 1977;238:591-600.
58. Sindel LJ, Buckley RH, Schiff SE, Ward FE, Mickey GH, Huang AT, et al. Severe combined immunodeficiency with natural killer cell predominance: abrogation of graft-versus-host disease and immunologic reconstitution with HLA-identical bone marrow cells. J Allergy Clin Immunol 1984;73:829-36.
59. Buckley RH, Schiff SE, Sampson HA, Schiff RI, Markert ML, Knutsen AP, et al. Development of immunity in human severe primary T cell deficiency following haploidentical bone marrow stem cell transplantation. J Immunol 1986;136: 2398-407.
60. Reisner Y, Itzicovitch L, Meshorer A, Sharon N. Hematopoietic stem cell transplantation using mouse bone marrow and spleen cells fractionated by lectins. Proc Natl Acad Sci U S A 1978;75:2933-6.
61. Buckley RH, Fischer A. Bone marrow transplantation for primary immunodeficiency diseases. In: Ochs HD, Smith CIE, Puck JM, editors. Primary immunodeficiency diseases: a molecular and genetic approach. 2nd ed New York: Oxford University Press; 2006. p. 669-83.
62. Sarzotti-Kelsoe M, Win CM, Parrott RE, Cooney M, Moser BK, Roberts JL, et al. Thymic output, T-cell diversity, and T-cell function in long-term human SCID chimeras. Blood 2009;114:1445-53.
63. Antoine C, Muller S, Cant A, Cavazzana-Calvo M, Veys P, Vossen J, et al. Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. Lancet 2003;361: 553-60.
64. Grunebaum E, Mazzolari E, Porta F, Dallera D, Atkinson A, Reid B, et al. Bone marrow transplantation for severe combined immune deficiency. JAMA 2006; 295:508-18.
65. Mazzolari E, Forino C, Guerci S, Imberti L, Lanfranchi A, Porta F, et al. Long-term immune reconstitution and clinical outcome after stem cell transplantation for severe T-cell immunodeficiency. J Allergy Clin Immunol 2007;120:892-9.
66. Neven B, Leroy S, Decaluwe H, Le Deist F, Picard C, Moshous D, et al. Long-term outcome after haematopoietic stem cell transplantation of a single-centre cohort of 90 patients with severe combined immunodeficiency: long-term outcome of HSCT in SCID. Blood 2009;113:4114-24.

67. Patel NC, Chinen J, Rosenblatt HM, et al. Outcomes of patients with severe combined immunodeficiency treated with hematopoietic stem cell transplantation with and without preconditioning. J Allergy Clin Immunol 2009;124: 1062-9.
68. Railey MD, Lokhnygina Y, Buckley RH. Long-term clinical outcome of patients with severe combined immunodeficiency who received related donor bone marrow transplants without pre-transplant chemotherapy or post-transplant GVHD prophylaxis. J Pediatr 2009;155:834-40.
69. Wengler GS, Lanfranchi A, Frusca T, Verardi R, Neva A, Brugnoni D, et al. In-utero transplantation of parental CD34 haematopoietic progenitor cells in a patient with X-linked severe combined immunodeficiency (SCIDX1). Lancet 1996;348:1484-7.
70. Flake AW, Roncarolo MG, Puck JM, Almeida-Porada G, Evans MI, Johnson MP, et al. Treatment of X-linked severe combined immunodeficiency by in utero transplantation of paternal bone marrow. N Engl J Med 1996;335:1806-10.
71. Ozsahin H, Cavazzana-Calvo M, Notarangelo LD, Schulz A, Thrasher AJ, Mazzolari E, et al. Long-term outcome following hematopoietic stem-cell transplantation in Wiskott-Aldrich syndrome: collaborative study of the European Society for Immunodeficiencies and European Group for Blood and Marrow Transplantation. Blood 2008;111:439-45.
72. Filipovich AH, Stone JV, Tomany SC, Ireland M, Kollman C, Pelz CJ, et al. Impact of donor type on outcome of bone marrow transplantation for Wiskott-Aldrich syndrome: collaborative study of the International Bone Marrow Transplant Registry and the National Marrow Donor Program. Blood 2001;97: 1598-603.
73. Klein C, Lisowska-Grospierre B, LeDeist F, Fischer A, Griscelli C. Major histocompatibility complex class II deficiency: clinical manifestations, immunologic features, and outcome. J Pediatr 1993;123:921-8.
74. Klein C, Cavazzana-Calvo M, Le Deist F, Jabado N, Benkerrou M, Blanche S, et al. Bone marrow transplantation in major histocompatibility complex class II deficiency: a single center study of 19 patients. Blood 1995;85:580-7.
75. Duplantier JE, Seyama K, Day NK, Hitchcock R, Nelson RP Jr, Ochs HD, et al. Immunologic reconstitution following bone marrow transplantation for X-linked hyper IgM syndrome. Clin Immunol 2001;98:313-8.
76. Bertrand Y, Müller SM, Casanova JL, Morgan G, Fischer A, Friedrich W. Reticular dysgenesis: HLA non-identical bone marrow transplants in a series of 10 patients. Bone Marrow Transplant 2002;29:759-62.
77. Broome CB, Graham ML, Saulsbury FT, Hershfield MS, Buckley RH. Correction of purine nucleoside phosphorylase deficiency by transplantation of allogeneic bone marrow from a sibling. J Pediatr 1996;128:373-6.
78. McGhee SA, Lloret MG, Stiehm ER. Immunologic reconstitution in 22q deletion (DiGeorge) syndrome. Immunol Res 2009;45:37-45.
79. Markert ML. Treatment of infants with complete DiGeorge anomaly. J Allergy Clin Immunol 2008;121:1063.
80. Markert ML, Devlin BH, Alexieff MJ, Li J, McCarthy EA, Gupton SE, et al. Review of 54 patients with complete DiGeorge anomaly enrolled in protocols for thymus transplantation: outcome of 44 consecutive transplants. Blood 2007; 109:4539-47.
81. van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic granulomatous disease: the European experience. PLoS One 2009;4:e5234.
82. Qasim W, Cavazzana-Calvo M, Davies EG, Davis J, Duval M, Eames G, et al. Allogeneic hematopoietic stem-cell transplantation for leukocyte adhesion deficiency. Pediatrics 2009;123:836-40.
83. Rowley S, Friedman TM, Korngold R. Hematopoietic stem cells transplantation for malignant diseases. In: Rich RR, editor. Clinical immunology. Principles and practice. Philadelphia: Elsevier; 2008. p. 1223-36.
84. Shipley JL, Butera JN. Acute myelogenous leukemia. Exp Hematol 2009;37: 649-58.
85. Koreth J, Schlenk R, Kopecky KJ, Honda S, Sierra J, Djulbegovic BJ, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. JAMA 2009;301:2349-61.
86. Fernandez HF, Kharfan-Dabaja MA. Tyrosine kinase inhibitors and allogeneic hematopoietic cell transplantation for chronic myeloid leukemia: targeting both therapeutic modalities. Cancer Control 2009;16:153-7.
87. Sharathkumar A, Thornley I, Saunders EF, Calderwood S, Freedman MH, Doyle J. Allogeneic bone marrow transplantation in children with chronic myelogenous leukemia. J Pediatr Hematol Oncol 2002;24:215-9.
88. Barrett AJ. Understanding and harnessing the graft-versus-leukaemia effect. Br J Haematol 2008;142:877-88.
89. Goerner M, Weber-Nordt R, Hoepfner S, Benner A, Luft T, Ho AD. Addition of a low fixed number of CD3+ cells to CD34-enriched allografts: effects on engraftment, graft-versus-host disease, and survival after related and unrelated peripheral stem cell transplantation. J Hematother Stem Cell Res 2003;12:309-20.
90. Lucarelli G, Andreani M, Angelucci E. The cure of thalassemia by bone marrow transplantation. Blood Rev 2002;16:81-5.
91. Walters MC, Patience M, Leisenring W, Rogers ZR, Aquino VM, Buchanan GR, et al. Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. Biol Blood Marrow Transplant 2001;7:665-73.
92. Gerritsen EJ, Vossen JM, Fasth A, Friedrich W, Morgan G, Padmos A, et al. Bone marrow transplantation for autosomal recessive osteopetrosis. J Pediatr 1994;125: 896-902.
93. Mazzolari E, Forino C, Razza A, Porta F, Villa A, Notarangelo LD. A single-center experience in 20 patients with infantile malignant osteopetrosis. Am J Hematol 2009;84:473-9.
94. Orchard PJ, Blazar BR, Wagner J, Charnas L, Krivit W, Tolar J. Hematopoietic cell therapy for metabolic disease. J Pediatr 2007;151:340-6.
95. Gratwohl A, Passweg J, Bocelli-Tyndall C, Fassas A, van Laar JM, Farge D, et al. Autologous hematopoietic stem cell transplantation for autoimmune diseases. Bone Marrow Transplant 2005;35:869-79.
96. Sandmaier BM, Mackinnon S, Childs RW. Reduced intensity conditioning for allogeneic hematopoietic cell transplantation: current perspectives. Biol Blood Marrow Transplant 2007;13(suppl 1):87-97.
97. Cavazzana-Calvo M, Hacein-Bey S, deSaint Basile G, Gross F, Yvon E, Nusbaum P, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 2000;288:669-72.
98. Hacein-Bey-Abina S, Le Deist F, Carlier F, Bouneaud C, Hue C, De Villartay JP, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N Engl J Med 2002;346:1185-93.
99. Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy for SCID-X1. J Clin Invest 2008;118:3132-42.
100. Gaspar HB, Parsley KL, Howe S, King D, Gilmour KC, Sinclair J, et al. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. Lancet 2004;364:2181-7.
101. Rabbitts TH. LMO T-cell translocation oncogenes typify genes activated by chromosomal translocations that alter transcription and developmental processes. Genes Dev 1998;12:2651-7.
102. Throm RE, Ouma AA, Zhou S, Chandrasekaran A, Lockey T, Greene M, et al. Efficient construction of producer cell lines for a SIN lentiviral vector for SCID-X1 gene therapy by concatemeric array transfection. Blood 2009;113:5104-10.
103. Onodera M, Nelson DM, Sakiyama Y, Candotti F, Blaese RM. Gene therapy for severe combined immunodeficiency caused by adenosine deaminase deficiency: improved retroviral vectors for clinical trials. Acta Haematol 1999; 101:89-96.
104. Aiuti A, Cattaneo F, Galimberti S, Benninghoff U, Cassani B, Callegaro L, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med 2009;360:447-58.
105. Santilli G, Thornhill SI, Kinnon C, Thrasher AJ. Gene therapy of inherited immunodeficiencies. Expert Opin Biol Ther 2008;8:397-407.
106. Kang EM, Malech HL. Advances in treatment for chronic granulomatous disease. Immunol Res 2009;43:77-84.
107. Ott MG, Schmidt M, Schwarzwaelder K, Stein S, Siler U, Koehl U, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. Nat Med 2006;12:401-9.

Embryonic and adult stem cell therapy

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There are many types of stem cells. All share the characteristics of being able to self-renew and to give rise to differentiated progeny. Over the last decades, great excitement has been generated by the prospect of being able to exploit these properties for the repair, improvement, and/or replacement of damaged organs. However, many hurdles, both scientific and ethical, remain in the path of using human embryonic stem cells for tissue-engineering purposes. In this report we review current strategies for isolating, enriching, and, most recently, inducing the development of human pluripotent stem cells. In so doing, we discuss the scientific and ethical issues associated with this endeavor. Finally, progress in the use of stem cells as therapies for type 1 diabetes mellitus, congestive heart failure, and various neurologic and immunohematologic disorders, and as vehicles for the delivery of gene therapy, is briefly discussed. (J Allergy Clin Immunol 2010;125:S336-44.)

Key words: *Stem cells, human embryonic stem cells, induced pluripotent stem cells, regenerative medicine, gene therapy, cell therapy*

Abbreviations used

AHSCT:	Autologous hematopoietic stem cell transplantation
G-CSF:	Granulocyte-colony stimulating factor
GVHD:	Graft-versus-host disease
GVL:	Graft-versus-leukemia
hESC:	Human embryonic stem cell
hESC-CM:	Human embryonic stem cell–derived cardiomyocyte
HSC:	Hematopoietic stem cell
HSCT:	Hematopoietic stem cell transplantation
iPSC:	Induced pluripotent stem cell
LVEF:	Left ventricular ejection fraction
MSC:	Mesenchymal stem cell
NK:	Natural killer
NSC:	Neural stem cell
SCID:	Severe combined immunodeficiency
T1DM:	Type 1 diabetes mellitus
UCB:	Umbilical cord blood

Stem cells are not homogeneous but exist instead as part of a developmental continuum. The most primitive of the cells is the totipotent stem cell. This cell has the potential to develop into a complete embryo (ie, to form any type of cell, including extraembryonic tissues [embryonic membranes, umbilical cord, and placenta]). This unique property is evanescent. It appears with fertilization of the egg and disappears by the time the embryo reaches the 4- to 8-cell stage. With subsequent divisions, embryonic stem cells lose the ability to generate an entire organism. However, they are capable of differentiating into cells present in all 3 embryonic germ layers, namely ectoderm, mesoderm, and endoderm, and on this basis are called pluripotent. With subsequent divisions, cells become more and more restricted in their ability to differentiate into multiple lineages. They are then called multipotent; that is, they are capable of forming a limited number of cell types. This is the property of adult stem cells, also referred to as somatic stem cells or nonembryonic stem cells, which are able to self-renew during the lifetime of the organism and to generate differentiated daughter cells. In the adult, tissues are in a perpetual state of flux under homeostatic conditions. Even in the absence of injury, they are continuously producing new cells to replace those that have worn out. For this reason, adult stem cells can be found in a metabolically quiescent state in most specialized tissues of the body, including the brain, bone marrow, liver, skin, and gastrointestinal tract. These cells are scarce, however, and with the relative exception of hematopoietic stem cells (HSCs), they are difficult to isolate. Typically, preparations of these cells are often contaminated with more differentiated progenitor cells, which decreases the long-term efficiency of the product because progenitor cells are fixed with respect to cell fate and do not self-renew.

One could argue that 3 major technologic achievements have driven the field of stem cell therapeutics. The first occurred in 1961, when the pioneering studies of Till and McCulloch,[1] using a revolutionary *in vivo* bioassay, unequivocally demonstrated the existence of HSCs. The second major enabling technologic leap occurred in 1998, when Thomson et al[2] reported the isolation of human embryonic stem cells (hESCs) from blastocysts and the creation of hESC lines for study. The most recent was reported by Yamanaka's group[3] in 2006, which induced the formation of pluripotent stem cells from murine fibroblasts.

Each of these advances has furthered the ability of researchers to use stem cells for basic research on cell-lineage fate and development, as well as for drug testing, modeling, and treating disease. It is in the latter area, in particular, that exciting progress has been made over the last few years.

SOURCES OF STEM CELLS

Having defined the different types of stem cells, we will now describe currently available sources of stem cells.

hESCs

hESCs are characterized by self-renewal, immortality, and pluripotency. Ongoing attempts to use hESCs in the laboratory finally came to fruition in 1998 with the creation of several human

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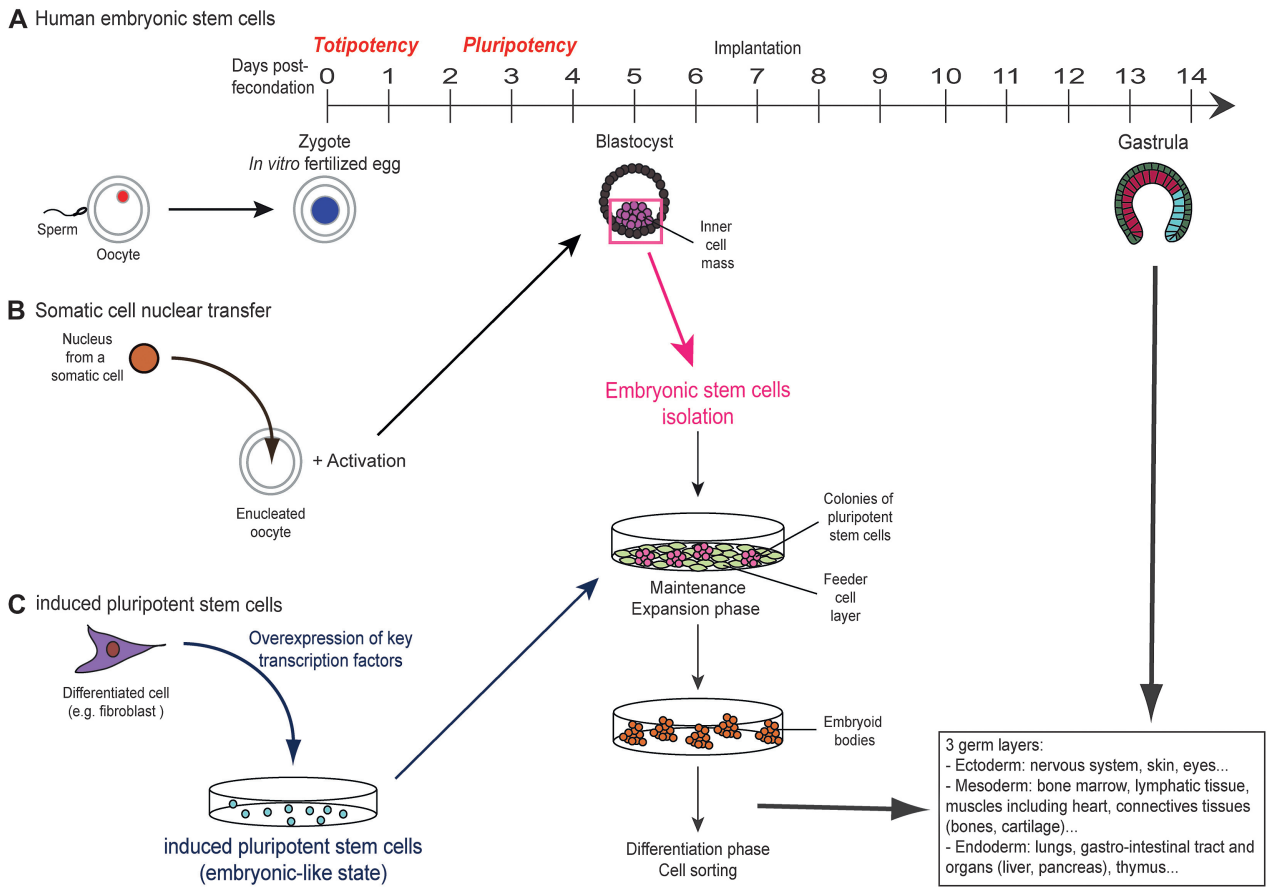

FIG 1. Isolation, generation, and culture of pluripotent stem cells. **A**, After isolation, typically from the inner cell mass of the blastocyst made by means of *in vitro* fertilization, hESCs are expanded in culture. They are classically grown on feeder cell layers, the purpose of which is to expand the cells while maintaining their undifferentiated state (maintenance/expansion phase). Initially those feeder layers were of xenogeneic origin (irradiated murine embryonic fibroblasts), but human feeder layers are being developed and will likely be used with increasing frequency in the future. When removed from feeder layers and transferred to suspension cultures, hESCs begin to form 3-dimensional multicellular aggregates of differentiated and undifferentiated cells termed embryoid bodies. Plated cultures of embryoid bodies spontaneously display a variety of cellular types from the 3 germ lineages at various differentiation stages. Theoretically, cells can be sorted according to differentiation markers, can be differentiated into any desired cells by adding specific growth factors (differentiation phase), or both. On a more practical level, it is difficult to induce hESC differentiation into a specific lineage, and highly definite culture protocols have to be developed for each desired cell type. **B**, Somatic cell nuclear transfer consists of injecting the nucleus from a somatic cell into an enucleated oocyte, followed by activation stimuli. The resulting embryo can be used to generate an hESC line (therapeutic cloning). **C**, iPSCs are generated from differentiated cells that have been reprogrammed to acquire a pluripotent state through overexpression of the key transcription factors *Oct4*, *Sox2*, and either *c-Myc* and *Klf4* or *Nanog* and *Lin28*. Overexpression can be achieved with viral vectors or proteins with or without histone-modifying chemicals. Once they are undifferentiated, they can be grown in culture like hESCs.

hESC lines,[2,4] although the cloning efficiency of such lines is still very low. Typically, hESCs are derived from the inner cell mass of 5-day-old blastocysts. The blastocysts in turn are made from unused embryos generated by means of *in vitro* fertilization for infertility problems. (It is important to note that the unused embryos can only be used for research purposes with the written informed consent of the parents.) Cells derived from earlier developmental stages can also be used.[5] At the other extreme, pluripotent cells have been isolated from the primordial germ cells of the gonadal ridge of the 5- to 9-week-old embryo. These are cells that normally become either oocytes or spermatozoa. Ironically, spontaneous differentiation in long-term *in vitro* culture of these so-called embryonic germ cells impeded their availability for research.[6] Accordingly, other strategies were and are still being developed with different methods and cell sources. For example, single-cell biopsy of the embryo[5] using a procedure not dissimilar to that used in preimplantation genetic diagnosis and that critically avoids the destruction of the embryo has been used with success, as have parthenogenesis of an unfertilized oocyte[7] and spermatogonial cells from adult human testis.[8] The latter developments are particularly exciting because they would allow the production of histocompatible cells that could be used in the donor. Technical methods for culture of hESCs are depicted in Fig 1, *A*.

Hundreds of hESC lines have been generated thus far. The first human stem cell line bank opened in 2004 in the United Kingdom (http://www.ukstemcellbank.org.uk/). The National Institutes of

Health registry (http://stemcells.nih.gov/research/registry/) has also archived a number of hESC lines and established criteria for demonstration of the pluripotency of these lines. Specifically, cells should be able to give rise to any cell lineage of the body and thus to form a teratoma (a tumor containing tissues from the 3 primary germ layers) *in vivo* after injection in an immune-compromised animal and should be capable of unlimited self-renewal.

Nuclear reprogramming and induced pluripotency

Nuclear reprogramming is a procedure that causes changes in gene expression that allow a cell of one type to develop into a cell of another type.[9] Recent strategies for generating stem cells are focused on nuclear reprogramming of differentiated cells to force them to become pluripotent. An example is somatic cell nuclear transfer (Fig 1, *B*). This consists of injection of the nucleus of a somatic cell into an enucleated oocyte.[10] The resulting pluripotent cells are genetically matched with the cell donor (this technique is thereby often called "therapeutic cloning"), except for the mitochondrial DNA, which comes from the egg. Another method is accomplished by means of cell fusion with an hESC, which can produce cells with some stem cell characteristics.[11]

A very recent and very exciting advance in reprogramming has been the generation of induced pluripotent stem cells (iPSCs). First reported in 2006 using murine fibroblasts,[3] iPSCs can be made from multiple murine and human somatic cell types,[12,13] and it is now possible to create patient-specific iPSCs.[14] iPSCs can be generated from differentiated cells by using retroviral-mediated expression of core transcription factors known to be required for maintenance of pluripotency and proliferation of embryonic stem cells.[3] These genes are *Oct4*, *Sox2*, and either *c-Myc* and *Klf4* or *Nanog* and *Lin28* (Fig 1, *C*). iPSCs exhibit similar features to embryonic stem cells, including cell morphology, cell-surface markers, growth properties, telomerase activity, expression, and epigenetic marks (ie, methylation or acetylation of histones, which result in changes in gene expression) of pluripotent cell–specific genes[12,13] but not global gene expression signatures.[15] They can give rise to cells derived from all 3 germ layers *in vitro* and *in vivo*, and murine iPSCs injected into murine blastocysts have been shown to contribute to embryonic development.[3]

Using pluripotent stem cells in the clinic: Scientific/medical issues

A number of scientific/medical issues need to be addressed before stem cells can be considered safe for clinical applications. The first hurdle is the tumorigenic potential of pluripotent cells (hESCs and iPSCs). Because pluripotency is evidenced by the ability to form teratomas when transplanted in immunodeficient mice, the concern exists that these cells could form malignant tumors in their new host. One strategy for dealing with this problem is to select pure populations of more committed cells for transfer. Demonstrating genetic and epigenetic stability will therefore be important before these cells are used clinically. In fact, karyotypic abnormalities have been described in several hESC lines, although changes might be at least partially dependent on culture techniques.[16]

In additional to biologic issues directly affecting the stem cell product, it is imperative that controlled, standardized practices and procedures be followed to maintain the integrity, uniformity, and reliability of the human stem cell preparations. Because stem cells are both maintained and expanded *in vitro* before transplantation, culture conditions compatible with human administration must be used. Feeder cells and sera of animal origin have to be reduced and ideally avoided to reduce the potential risk of contamination by xenogeneic protein and pathogens. Finally, transplantation of hESCs into patients is also limited by potential HLA incompatibility. Consequently, life-long immunosuppressive therapy, which can lead to infections and organ-based toxic side effects, such as nephropathy, might be required to prevent graft rejection. In this regard iPSCs hold great promise because they are histocompatible with the patient and because their use avoids one of the major ethical concerns (see below) associated with hESCs.

Although iPSCs solve the tissue-barrier problem, they too have technical drawbacks that are presently limiting their use. First is the issue of the risk of insertional mutagenesis caused by viral integration into the genome. This is of particular concern because patients who have received gene-modified lymphoid cells have had aggressive leukemias as a result of this phenomenon (see below).[17] The possibility that iPSCs might be generated with non-integrating expression plasmids or adenoviral vectors is being explored in the murine system and appears possible.[18,19]

Another risk is reactivation of a viral oncogene, such as *c-Myc*, used to engineer the cells. Here there are data to suggest that the use of histone-modifying chemicals, such as the histone deacetylase inhibitor valproic acid, improves reprogramming efficiency and avoids the need to use *c-Myc* and that *Oct4* and *Sox2* alone are then sufficient in the generation of human iPSCs.[20] Recently, iPSCs were successfully obtained from murine fibroblasts cultured without any genetic material at all by using only valproic acid and recombinant proteins for the necessary transcription factors.[21] However, even as technical hurdles are overcome, generation of iPSCs still suffers from low efficiency and high cost, although no doubt these problems will be solved in time as well. In particular, the reprogramming efficiency is typically less than 1% but could depend on the differentiation stage of the cells. Indeed, the efficiency of iPSC generation has been recently increased to 28% with the use of hematopoietic stem and progenitor cells.[22]

Adult stem cells

The best-known example of the adult stem cell is the HSC, which is located in the bone marrow niche. HSCs and progenitors can be readily harvested from bone marrow and umbilical cord blood (UCB). They can even be collected from peripheral blood after mobilization from the marrow with granulocyte colony-stimulating factor (G-CSF) with or without CXCR4 antagonist.[23] HSCs are characterized by the expression of cell-surface markers, which allows for their isolation. In human subjects the HSC surface phenotype is typically lineage-specific antigen negative (lin^-), $CD34^+CD38^-CD133^+$c-Kit/$CD117^+CD59^+$Thy1/$CD90^+CXCR4^+$. Apart from differentiating into all myeloid and lymphoid lineages, HSCs have been shown to be able to differentiate *in vitro* into cells of nonhematopoietic lineages. However, such plasticity was probably an experimental artifact that is currently explained by the presence of heterogeneous populations of non-HSCs in hematopoietic organs or by the phenomenon of cell fusion.

Mesenchymal stem cells (MSCs) are another type of adult multipotent cells that are capable of differentiating into various mesodermal cell lineages, including myocytes, osteoblasts,

chondroblasts, fibroblasts, adipocytes, and other stromal elements. MSCs are present in almost all organs, but for therapeutic purposes, they are most conveniently isolated from bone marrow and UCB. MSCs can be organ specific. Consequently, populations isolated from various sources, although morphologically similar, might be functionally different. For example, MSCs isolated from the umbilical cord do not have the same abilities to give rise to osteoblasts, chondrocytes, and cardiomyocytes as bone marrow–derived MSCs.[24] MSCs can be readily expanded *ex vivo* and manipulated, if needed, to acquire specific properties. The International Society for Cellular Therapy recommended changing their name to "multipotent mesenchymal stromal cells" because the majority of MSCs lack complete "stemness" property and proposed minimal criteria for standardization of preparations.[25] Human MSCs must be plastic adherent; express CD105, CD73 and CD90; lack hematopoietic markers (CD45, CD34, CD14 or CD11b, CD79α or CD19 m and HLA-DR); and be able to differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro*. MSCs display trophic, anti-inflammatory, and immunomodulatory capacities, both through secretion of soluble factors (indoleamine 2,3-dioxygenase, IL-6, TGF-β1, hepatocyte growth factor, inducible nitric oxide synthase, and prostaglandin) and direct cell-to-cell interaction with immune cells. *In vitro* MSCs suppress effector and cytotoxic T-cell, B-cell, natural killer (NK) cell, and dendritic cell activity and can induce regulatory T cells.[26] However, how MSCs assist in repairing a damaged organ is still unclear. Mounting evidence suggests that direct substitution of injured cells by *in situ* differentiated MSCs is unlikely (although still argued). Establishment of a favorable environment or niche for reconstruction of the tissue by intrinsic stem cells *per se* seems more likely. Regardless, because of their low immunogenicity and claimed beneficial effects on organ regeneration (whatever the mechanism), MSCs are being examined in an increasing number of regenerative medicine applications, as well as in inflammatory and immunologic diseases.

Finally, amniotic fluid, UCB, and the placenta are other sources of nonembryonic stem cells. However, it is not clear yet whether they are pluripotent or multipotent and how clinically useful they will be.

POTENTIAL CLINICAL USES OF STEM CELLS

Stem cells are postulated to have a tremendous number of applications, but tissue engineering seems to generate the greatest excitement. Stem cells can be used in regenerative medicine, immunotherapy, and gene therapy. Animal models and clinical studies have shown that transplantation of stem cells from diverse origins can successfully treat many acute and chronic diseases, such as immunohematologic disorders, type 1 diabetes mellitus (T1DM), Parkinson disease, neuronal destruction, and congestive heart failure.

Hematology-immunology

During the last 50 years, allogeneic hemopoietic stem cell transplantation (HSCT) has progressively become a common procedure for the treatment of a variety of inherited or acquired immunohematologic diseases, including thalassemias, sickle cell disease, Fanconi anemia, inborn errors of metabolism, severe aplastic anemia, severe combined immunodeficiency (SCID), and other primary immune deficiencies. HSCT is also widely used for the treatment of hematologic malignancies, such as acute myeloid and lymphoid leukemias, chronic myeloid leukemia and other myeloproliferative syndromes, myelodysplastic disorders, lymphoma, myeloma, and even solid tumors, such as renal cell cancer, breast cancer, ovarian carcinoma, and neuroblastoma.[27]

The aim of allogeneic HSCT in malignancies is not only a substitution of the malignant bone marrow but also a form of adoptive immunotherapy. In the context of HLA compatibility, donor allogeneic T lymphocytes detect differences in minor histocompatibility antigens in both the host and the tumor and can destroy the residual malignant cells, thereby contributing to the cure of the patient. This is the graft-versus-tumor or graft-versus-leukemia (GVL) effect. Particularly in the case of disease relapse after transplantation, donor lymphocyte infusions might induce or enhance a GVL effect and reinduce the patient into remission.

Major complications of HSCT include organ toxicity from the conditioning regimen used and graft-versus-host disease (GVHD), in which the donor's immune system destroys the recipient's normal tissues, particularly the skin, gastrointestinal tract, and liver. Other important complications of HSCT are graft failure, infertility, growth retardation in children, and secondary cancers thought to arise as a result of chronic immunosuppression and DNA-damaging preparative regimens.

In an effort to decrease these complications, several strategies have been developed. First among these are the reduced-intensity conditioning regimens, or so-called minitransplantations, which have made HSCT available to older and less fit patients.[28] Significant immunosuppression in these patients and GVHD as a result of the conditioning regimen remain serious problems and have suggested to many that the use of the term "mini" is misleading with respect to potential complications.

Second, the cell source has also been examined with respect to complications. Several studies convincingly show that $CD34^+$ cells harvested from peripheral blood engraft faster but are associated with a higher incidence of GVHD.[29]

Finding a histocompatible donor remains a problem for many patients in need of HSCT. Ideally, one would like to use an HLA-matched sibling as a donor. If such a donor is not available, then a matched unrelated donor is an acceptable alternative. This can be a major problem for minority groups underrepresented in registries of volunteer donors, information on which is centralized in the Bone Marrow Donors Worldwide database. In the absence of a compatible donor, HLA-haploidentical mismatched HSCT from a relative can be performed,[30] generally T depleted to avoid GVHD. In that context NK cells could facilitate engraftment and display an antileukemic activity without GVHD.[31]

Finally, UCB transplantation has the potential to significantly enlarge the number of potential HSCT recipients. UCBs are rapidly and easily available from cord blood banks and can be used when only partially HLA matched because they are much less likely to induce acute and chronic GVHD.[32] Remarkably, the GVL effect seems to be preserved, likely as a result of NK cells present in the cord blood preparation.[33] Issues that remain to be solved are delayed engraftment, prolonged T lymphopenia, and defective thymopoiesis.[34] In addition, it is not currently possible to perform donor lymphocyte infusions in the case of relapse, but an ongoing clinical trial is testing *ex vivo* expansion of UCB T cells. Because the number of HSCs per unit of UCB is small, thereby limiting their ability to be used for adult patients, strategies using combined units or *ex vivo* expanded cells are being developed.[35,36]

Autologous hematopoietic stem cell transplantation (AHSCT) is commonly performed in certain settings as well. Its main role is

to lessen the period of aplasia (rescue therapy) after high-dose chemotherapy and thereby lessen the risk of infection and bleeding. When used for the treatment of hematologic malignancies and solid tumors, HSCs to be used for AHSCT are commonly collected after few cycles of chemotherapy to lessen contamination of the graft by tumor cells. Moreover, autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus, might benefit from AHSCT. It is thought that immunoablative therapy resets the immune system by eliminating autoreactive T and B lymphocytes, lessening memory T cells, increasing thymus-derived naive T cells, generating a diverse but distinct T-cell receptor repertoire,[37] and promoting regulatory T cells.[38] Several recent studies have shown, as might be expected, that nonmyeloablative and low-intensity myeloablative regimens have fewer treatment-related complications and mortality than high-intensity myeloablative regimens[39] and that best results might be obtained during the inflammatory phase of autoimmune disease. Allogeneic HSCT has also been used for the treatment of autoimmune disease on the theory of both immune reset and correction of the genetic predisposition, but the risks of GVHD and infection are substantial, and therefore this form of therapy should be reserved only for treatment of very serious and refractory disease.[39]

Finally, the immunomodulatory capability of MSCs is being tested in the treatment of patients with systemic lupus erythematosus, multiple sclerosis, Crohn disease, amyotrophic lateral sclerosis, and T1DM. In solid organ transplantation and HSCT, results from preclinical animal studies, although controversial, suggest that donor-derived MSCs could have a tolerogenic effect and might therefore be used for prevention and/or treatment of graft rejection and GVHD.[40-42]

T1DM

T1DM is an autoimmune disease resulting from the destruction of pancreatic insulin-producing β cells in the islets of Langerhans. Insulin replacement therapy, even when rigorously controlled, is often not efficient enough to prevent long-term complications of the disease. Transplantation of a whole pancreas or isolated mature islets can restore proper glucose regulation, but the former is a morbid high-complication procedure, and the latter appears to be only a transient solution.[43] For these reasons, other sources of β cells suitable for transplantation are being sought. Pancreatic stem cells have been identified in ductal epithelium of injured pancreas on the basis of expression of the transcription factor neurogenin 3.[44] Efforts are now being directed toward methods to expand these cells *ex vivo* or to stimulate their proliferation *in vivo*.

Generation of insulin-secreting cells from hESCs and iPSCs holds great promise for the cure of T1DM. hESC-derived pancreatic endoderm can be differentiated *in vivo* into glucose-responsive insulin-secreting cells in immunodeficient mice with streptozotocin-induced diabetes (a β cell–selective destruction).[45] Unfortunately, teratoma formation was found in approximately 15% of the recipient mice and is a safety concern. The immunologic incompatibility of hESCs could be resolved by using bioengineered porous capsules, which are designed to protect the graft from immune cells but remain permissive to the passage of small molecules. In regard to iPSCs, a particularly exciting study from Zhou et al[46] reported the reprogramming of differentiated murine pancreatic exocrine cells into β-like cells *in vivo*. These investigators found that transient expression of 3 key developmental transcription factors, neurogenin 3, MafA, and Pancreatic duodenal homeobox-1 (Pdx1), by adenoviral vectors injected directly into the pancreas was sufficient to reprogram exocrine cells into insulin-secreting cells responding to hyperglycemia.

The immunomodulatory effects of MSCs are also being explored in the setting of T1DM. At the time of diagnosis, β-cell destruction is often not yet complete. In theory, amelioration of the immune attack might allow the survival of the residual islet cells. In diabetic immunodeficient mice human MSCs decreased hyperglycemia and increased endogen insulin levels and β-cell numbers.[47] Clinical studies testing allogeneic MSCs in patients with recently diagnosed diabetes are ongoing.

Finally, nonmyeloablative AHSCT was performed in 23 patients with early-onset T1DM.[48] Twenty enjoyed a variable insulin-free period, and 12 of these patients remained insulin free after a mean follow-up of 31 months. Interestingly, benefit was demonstrated in those patients with transient responses as well. In this small group (8 patients) daily insulin doses were significantly diminished, and C-peptide levels (reflective of endogenous insulin synthesis) were increased.

Diseases of the nervous system

It was thought for a long time that nerve cells do not divide in the adult mammalian brain, but this has now been shown to be incorrect. Neurogenesis not only occurs during prenatal and postnatal development but also in adults. Neurogenic niches have been identified in the subventricular zone of the lateral ventricles and in the subgranular zone of the dentate gyrus of the hippocampus. In brain-injury models neural stem cells (NSCs) proliferate in those neurogenic regions and are even able to migrate toward the site of damage.[49] NSCs are multipotent and capable of self-renewing. *In vitro* they cluster in "neurospheres," which are able to differentiate into the 3 major neuroectodermal lineages (neurons, astrocytes, and oligodendrocytes).

Neural stem/progenitor cells can be isolated from embryonic, fetal, or adult brain tissue by sorting cells on the basis of nestin expression primarily, as well as other markers, and can then be placed into culture for expansion. Clearly, for autologous stem cell therapy, more accessible NSCs are required. Curiously, dental pulp and peridontium have been shown to be sources of NSC, as well as olfactory mucosa, which is readily harvested by means of nasal biopsy.[50] Investigations for NSC-based therapy are ongoing for various neurologic diseases. The neural repair probably results from a replacement of defective cells but also from neuroprotective, trophic, and immunomodulatory effects. To date, the ideal NSC source, schedule, and route of transplantation have not been established and are likely to be disease specific. The use of NSCs to treat Parkinson disease might be instructive in this regard.

Parkinson disease is an incurable, progressive, neurodegenerative disease that affects dopaminergic neurons. Levodopa, which is converted to dopamine in the brain, is the mainstay of treatment, but most patients acquire tachyphylaxis to its effects over time. In contrast to patients with diabetes, in whom transplantation of islet cells is a therapeutic option, implantation of fully differentiated dopamine-releasing neurons into the brain is not presently feasible because such cells do not survive. Transplantation of embryonic/fetal nigral dopaminergic neurons was tested in 2 double-blind, placebo-controlled trials, but results

were not as encouraging as those from previous open-trial reports.[51,52] However, modest clinical improvement was noted in some patients, and striatal fluorodopa uptake was significantly enhanced. Unfortunately, several patients subsequently had dyskinesias. Postmortem examination of the brains of some patients provided evidence that transplanted dopamine neurons can differentiate and survive for many years without immunosuppression. However, it appeared that at least some of the grafted tissue was involved by disease over a period lasting from 9 to 16 years.[53] Widespread application of this therapy will likely be limited as long as access to fetal donor tissue is required. Moreover, the safety of those cells is not completely assessed because they have not been tested in a large number of patients and because development of cerebral mass lesions of donor origin was reported in a patient less than 6 years after fetal transplantation for Huntington disease.[54] Accordingly, finding alternative sources of NSCs for therapeutic purposes is the object of intense investigation.

In one case autologous NSCs were harvested by means of cerebral biopsy, expanded, and differentiated into dopaminergic neurons *ex vivo* and then injected back to the patient's putamen 9 months later.[55] Clinical evaluation and fluorodopa uptake were improved after transplantation, but all benefits had disappeared by 5 years. In animal studies other stem cell types partially alleviated Parkinson symptoms, including MSCs,[56] olfactory NSCs,[57] hESC-derived neurons,[58] and iPSCs.[59] Human clinical trials with cells of these types can therefore be anticipated.

Unlike Parkinson disease, which theoretically requires replacement of only 1 cell type, therapies for other neurologic diseases, such as stroke and spinal cord injury, in which large numbers of cells of many types (neurons, glia, and endothelial cells) are destroyed, face much larger hurdles. In recent years, human NSCs from diverse origins, including hESCs, HSCs, and MSCs, have been tested in preclinical models of ischemic stroke. These experiments have enabled the development of treatment strategies and have demonstrated the critical importance of transplantation timing for clinical success. Only a few clinical studies have been performed for the treatment of stroke. Stereotactic injection of neuronal cells derived from embryonal carcinoma cell line (NT2/D1) did not display significant benefits compared with control results, but some patients experienced improvement.[60] In other studies an investigation into the utility of fetal porcine cells was stopped because of adverse events (temporary worsening of deficits and seizures) in 2 of 5 patients,[61] whereas intravenous infusion of MSCs was shown to be safe without significant benefit. HSCs are currently being evaluated in phase I/II protocols. The injection of specific growth factors to stimulate proliferation of intrinsic neuroprogenitors in the brain is a novel approach to this problem.[62]

Spinal cord injury often results in permanent motor deficiency, sensory deficiency, or both, thereby rendering treatment particularly challenging. In a small number of paraplegic and quadriplegic patients, olfactory NSCs have been injected into intralesional and perilesional areas.[50] Feasibility and safety were acceptable, but unfortunately, clinical improvement remained slight after 3 years of follow-up. Logically, early-phase treatment might yield the best results. In fact, a phase I/II clinical trial tested infusion of HSCs into the spinal cord associated with G-CSF injections: the Association Impairment Scale grade improved in 30.4% of patients treated quite early (<8 weeks) after the initial lesion; however, no enhancement was noted when the treatment was performed later.[63]

Cardiac repair

Congestive heart failure afflicts millions of persons around the world, with 400,000 new cases being reported each year in the United States alone. The most common cause is coronary artery disease. After myocardial necrosis has occurred, the cell loss is irreversible, and although many medical and surgical treatments are available for the subsequent congestive heart failure, the long-term prognosis of these patients remains guarded, with a 5-year mortality of 50%. Transplanting stem cells would have clear advantages to transplanting a heart because it would obviate the constraint of a donor and, in case of autologous cells, for the requisite immunosuppression.

hESC-derived cardiomyocytes (hESC-CMs) have been successfully generated. Intramyocardial injection of hESC-CMs a few days after infarction in immunodeficient rodents seemed to enhance left ventricular ejection fraction (LVEF) compared with that seen in a control group when evaluated at 4 weeks.[64] Unfortunately, this enhancement was not sustained after 12 weeks of follow-up. Another study suggested that a coinfusion of hESC-CMs and MSCs in mice was of benefit because, according to the authors, a "synergistic trophic effect that enhanced repair of injured host tissue" was brought about.[65] Importantly, no teratoma was found in animals receiving hESC-CMs.[64,65]

Despite a controversial plasticity *in vitro*, a considerable amount of data from actual preclinical studies suggest that it is unlikely that transdifferentiation of HSCs and MSCs into functional cardiomyocytes happens to any significant degree *in vivo*. In very specific culture conditions, MSCs might be driven toward differentiating into cardiomyocyte-like cells at a very low frequency (approximately 0.07%) that would not be enough for cardiac repair.[24] It is now generally agreed that the transplanted cells exert their beneficial role through paracrine effects and by creating a favorable trophic environment for intrinsic cell recovery, enhancing angiogenesis, and limiting ventricular remodeling.[66] A study comparing the efficacy of transplanted bone marrow mononuclear cells, MSCs, skeletal myoblasts, and fibroblasts in mice with experimental myocardial infarcts was carried out and showed that HSCs had the most beneficial effect on left ventricular function in that model.[67] Recently, infusion of endogenous cardiac stem cells isolated from endomyocardial biopsy specimens and expanded *ex vivo* appeared to enhance myocardial viability and LVEF in a murine infarction model.[68]

Intracoronary infusions of HSCs[69] or MSCs[70] have been performed a few days after percutaneous coronary intervention for acute myocardial infarction. Despite contradictory results of clinical trials, meta-analyses reported moderate but significant benefits of such therapy compared with the condition of control patients, with improvement in LVEF, infarct size, and end-systolic volume.[71] In patients with chronic ischemic disease, intracoronary and intramyocardial injections of HSCs are associated with modest enhancements as well.[71] Other cell types are also being tested, such as skeletal myoblasts (although lack of electrical synchronization with cardiomyocytes could potentially be arrhythmogenic) or endothelial progenitor cells.

Stem cells and gene therapy

The goal of gene therapy is to cure diseases caused by malfunctioning genes. It does so by substituting the function of a normal gene for the one that causes disease. Until now, the most commonly used procedure in human gene therapy clinical trials is

the insertion of a normal copy of the target gene in a nonspecific location into the host genomic DNA. The therapeutic transgene is packaged into a delivery vehicle, which is typically a replication-deficient virus. Nonintegrating virus (adenovirus or adeno-associated virus) can be used in nondividing cells, such as neurons and cardiomyocytes. In dividing cells, such as stem cells, vectors that integrate into host DNA, such as γ-retrovirus or lentivirus, are required to have a transmission to daughter cells.

Stem cells are of great benefit to cell-based gene therapy because they are self-renewing and thus might reduce or eliminate the necessity for repeated administrations of the therapeutic cells. Single-gene inherited diseases are particularly good candidates for gene therapy. In theory the host's own stem cells can be repaired through genetic engineering and then used in an autologous transplantation. This avoids all the risks of transplanted allogeneic cells, including the risks associated with long-term immunosuppression, as well as GVHD, in patients receiving HSCT. The first clinical trials with engineered HSCs involved patients with genetic immunodeficiency diseases, such as adenosine deaminase–deficient SCID.[72] Trials have also been carried out in patients with X-linked SCID (γ-common [γ-c] cytokine receptor deficient or SCID-X1)[17] and chronic granulomatous disease.[73] The clinical results have been quite promising but have been marred by the development of leukemia, which has been shown to be caused by insertional mutagenesis in a number of these patients (see below). Gene therapy for hemoglobinopathies, such as β-thalassemia and sickle cell disease, are ongoing. Easily accessible mucosal and skin stem cells are also being used, for example in treatment of diseases such as junctional epidermolysis bullosa.[74]

These early studies revealed problems that need to be addressed, such as difficulties controlling protein levels without endogenous gene regulatory regions, maintenance of gene expression through long periods, low protein production, and insertional mutagenesis of the retroviral transgene vector. Indeed, the major side effect was thus far the occurrence of T cell–acute lymphoblastic leukemia in 5 of 19 patients successfully treated for SCID-X1 in 2 distinct French and British trials.[17,75] In all cases the retroviral vector was found in the leukemic clone, integrated near a proto-oncogene, and particularly before the LIM domain–only 2 in 4 cases and was associated with acquired somatic mutations. γ-Retroviral vectors were subsequently shown to integrate preferentially in the 5′ ends of genes[76] near transcription start sites in a nonrandom manner near genes that provide selective advantage to the clone. Interestingly, when the same retroviral vector, the murine leukemia virus, was used to deliver other transgenes, no case of leukemia was observed, suggesting that γ-c receptor overexpression might be involved in the oncogenesis.

New techniques are being developed to enhance efficiency and to avoid the risk of insertional oncogenesis. First, safer delivery systems are being developed. For example, HIV-derived lentivirus is able to transduce nondividing cells, is easier to use, and can induce less mutagenesis than γ-retrovirus, as assessed in murine models.[77] Other modifications under development include the use of inducible and tissue-specific promoters, a weaker viral promoter/enhancer, and self-inactivating retroviral vectors and introduction of suicide genes. At last, the improvement of direct gene correction with homologous recombination (a normal copy of the gene is switched with the defective allele) is promising in murine models.[78] That last technology could be particularly significant in the treatment of dominant genetic diseases.

The potential utility of hESCs and iPSCs was discussed earlier, but the use of such cells is under active investigation for human gene therapy.

Finally, new attempts are focusing on cell-based delivery vehicles for tumor-specific therapy. MSCs appear to be good agents for this purpose because, in addition to their properties described above, they have been shown to migrate toward tumors. For example, MSCs engineered to express the TNF-related apoptosis-inducing ligand induced apoptosis in tumor cells, reduced tumor growth *in vivo*, and prolonged survival in murine models of human glioma.[79]

ETHICAL ISSUES

The use of hESCs in medical research has drawn much attention from many sectors of the public. Religious, historical, cultural, medical, and other points of view have contributed to a very vigorous and wide-ranging discourse over the use of these materials.[80] Some consider research with hESCs to be inherently immoral because these individual's believe that life begins with fertilization of the ovum, and the destruction of an embryo with the potential to develop into a viable human being is thought tantamount to infanticide. For this reason, the American federal government severely restricted access and use of hESCs in 2001. These restrictions have now been largely overturned by the Obama administration. In contrast, proponents of this line of research insist that the potential benefits to humankind from this research mitigate such concerns. They also argue that hESCs are made from unwanted fertilized ovum that would likely be destroyed in any event.

Stem cells created by means of nuclear transfer share the same ethical concerns. Furthermore, because these cells have the potential to generate a complete embryo, they also raise the even more highly charged possibility of cloning human beings, so-called reproductive cloning. Many organizations and countries have already banned reproductive cloning of human beings. Because this procedure can be used to generate stem cells for therapeutic purposes, in countries where this type of cloning is legal, such as Australia and the United Kingdom, the created embryos must be destroyed within 14 days. Federal laws in the United States are not clear on the legality of therapeutic cloning, but the Obama administration has pledged establishment of strict guidelines to ensure that cloning research will not be used for human reproduction.

Because of the shortage of human oocytes, generation of human-animal chimeras was legalized in 2008 in the United Kingdom for research purposes only. A human nucleus is transferred into an animal's oocyte, creating a hybrid embryo that must be destroyed within 2 weeks and cannot be implanted. Clearly, creation of such tissues raises even more complex issues.

Finally, the issue of financial compensation for embryo and gamete donors is also controversial, with guidelines for this problem being proposed by the International Society of Stem Cell Research (http://www.isscr.org/guidelines/index.htm). All parties involved in the debate want very much to avoid the development of an underground black market in spare embryos.

CONCLUSIONS AND PERSPECTIVES

The promise of stem cell therapeutics powers the field of regenerative medicine and has generated a huge amount of

excitement, anticipation, and hope. Accordingly, research with hESCs is increasing exponentially worldwide, particularly in the United States, where important limitations on research with such cells were overturned in 2009. Furthermore, the US Food and Drug Administration recently approved the world's first phase I clinical trial using hESC-based therapy in patients with spinal cord injury.

Nonetheless, a number of substantive scientific and ethical issues remain to be resolved before hESCs can enter the therapeutic mainstream. In the meantime, recent breakthroughs in generating iPSCs would obviate the need to solve the most vexing of these problems. In fact, it seems reasonable to hope that in the next few years many of the enabling issues relevant to iPSCs will be solved, allowing the field of regenerative medicine to deliver on its vast potential promise.

Although it is difficult to predict the ultimate utility of stem cell–based therapy at this time, it is not difficult to conclude that this is an extremely important area of scientific research. Surrounded by controversy and a good many ethical concerns, thoughtful legislative action could both foster the field and ensure continued progress. This would clearly be more desirable than having the whole endeavor driven underground and potentially into the hands of less ethical and less regulated scientists. Open discussions between political bodies and the various interest groups in the scientific, medical, and religious communities need to take place to address the concerns of each and to provide an ultimate solution that is clearly in the interest of humanity.

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REFERENCES

1. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 1961;14:213-22.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145-7.
3. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663-76.
4. Battey JF Jr, Cole LK, Goldthwaite CA Jr. Regenerative Medicine. Department of Health and Human Services. August 2006. http://stemcells.nih.gov/info/scireport/2006report.htm. Accessed December 15, 2009.
5. Klimanskaya I, Chung Y, Becker S, Lu SJ, Lanza R. Human embryonic stem cell lines derived from single blastomeres. Nature 2006;444:481-5.
6. Turnpenny L, Brickwood S, Spalluto CM, Piper K, Cameron IT, Wilson DI, et al. Derivation of human embryonic germ cells: an alternative source of pluripotent stem cells. Stem Cells 2003;21:598-609.
7. Revazova ES, Turovets NA, Kochetkova OD, Kindarova LB, Kuzmichev LN, Janus JD, et al. Patient-specific stem cell lines derived from human parthenogenetic blastocysts. Cloning Stem Cells 2007;9:432-49.
8. Conrad S, Renninger M, Hennenlotter J, Wiesner T, Just L, Bonin M, et al. Generation of pluripotent stem cells from adult human testis. Nature 2008;456:344-9.
9. Gurdon JB, Melton DA. Nuclear reprogramming in cells. Science 2008;322:1811-5.
10. French AJ, Adams CA, Anderson LS, Kitchen JR, Hughes MR, Wood SH. Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts. Stem Cells 2008;26:485-93.
11. Cowan CA, Atienza J, Melton DA, Eggan K. Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. Science 2005;309:1369-73.
12. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861-72.
13. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318:1917-20.
14. Raya A, Rodriguez-Piza I, Guenechea G, Vassena R, Navarro S, Barrero MJ, et al. Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. Nature 2009;460:53-9.
15. Chin MH, Mason MJ, Xie W, Volinia S, Singer M, Peterson C, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 2009;5:111-23.
16. Mitalipova MM, Rao RR, Hoyer DM, Johnson JA, Meisner LF, Jones KL, et al. Preserving the genetic integrity of human embryonic stem cells. Nat Biotechnol 2005;23:19-20.
17. Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 2008;118:3132-42.
18. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. Science 2008;322:949-53.
19. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. Science 2008;322:945-9.
20. Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol 2008;26:1269-75.
21. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 2009;4:381-4.
22. Eminli S, Foudi A, Stadtfeld M, Maherali N, Ahfeldt T, Mostoslavsky G, et al. Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. Nat Genet 2009;41:968-76.
23. Flomenberg N, Devine SM, Dipersio JF, Liesveld JL, McCarty JM, Rowley SD, et al. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. Blood 2005;106:1867-74.
24. Martin-Rendon E, Sweeney D, Lu F, Girdlestone J, Navarrete C, Watt SM. 5-Azacytidine-treated human mesenchymal stem/progenitor cells derived from umbilical cord, cord blood and bone marrow do not generate cardiomyocytes in vitro at high frequencies. Vox Sang 2008;95:137-48.
25. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-7.
26. Selmani Z, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, et al. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4 + CD25highFOXP3+ regulatory T cells. Stem Cells 2008;26:212-22.
27. Demirer T, Barkholt L, Blaise D, Pedrazzoli P, Aglietta M, Carella AM, et al. Transplantation of allogeneic hematopoietic stem cells: an emerging treatment modality for solid tumors. Nat Clin Pract Oncol 2008;5:256-67.
28. Storb R. Can reduced-intensity allogeneic transplantation cure older adults with AML? Best Pract Res Clin Haematol 2007;20:85-90.
29. Cutler C, Giri S, Jeyapalan S, Paniagua D, Viswanathan A, Antin JH. Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. J Clin Oncol 2001;19:3685-91.
30. Aversa F, Terenzi A, Tabilio A, Falzetti F, Carotti A, Ballanti S, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. J Clin Oncol 2005;23:3447-54.
31. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 2002;295:2097-100.
32. Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Oiwa-Monna M, et al. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. Blood 2007;109:1322-30.
33. Lu X, Kondo Y, Takamatsu H, Ohata K, Yamazaki H, Takami A, et al. CD16 + CD56- NK cells in the peripheral blood of cord blood transplant recipients: a unique subset of NK cells possibly associated with graft-versus-leukemia effect. Eur J Haematol 2008;81:18-25.
34. Komanduri KV, St John LS, de Lima M, McMannis J, Rosinski S, McNiece I, et al. Delayed immune reconstitution after cord blood transplantation is characterized by impaired thymopoiesis and late memory T-cell skewing. Blood 2007;110:4543-51.
35. Ruggeri A, de Latour RP, Rocha V, Larghero J, Robin M, Rodrigues CA, et al. Double cord blood transplantation in patients with high risk bone marrow failure syndromes. Br J Haematol 2008;143:404-8.
36. Robinson SN, Ng J, Niu T, Yang H, McMannis JD, Karandish S, et al. Superior ex vivo cord blood expansion following co-culture with bone marrow-derived mesenchymal stem cells. Bone Marrow Transplant 2006;37:359-66.
37. Muraro PA, Douek DC, Packer A, Chung K, Guenaga FJ, Cassiani-Ingoni R, et al. Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. J Exp Med 2005;201:805-16.
38. Bayer AL, Jones M, Chirinos J, de Armas L, Schreiber TH, Malek TR, et al. Host CD4 + CD25 + T cells can expand and comprise a major component of the Treg compartment after experimental HCT. Blood 2009;113:733-43.

39. Burt RK, Loh Y, Pearce W, Beohar N, Barr WG, Craig R, et al. Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases. JAMA 2008;299:925-36.
40. Wan CD, Cheng R, Wang HB, Liu T. Immunomodulatory effects of mesenchymal stem cells derived from adipose tissues in a rat orthotopic liver transplantation model. Hepatobiliary Pancreat Dis Int 2008;7:29-33.
41. Ball LM, Bernardo ME, Roelofs H, Lankester A, Cometa A, Egeler RM, et al. Cotransplantation of ex vivo expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem-cell transplantation. Blood 2007;110:2764-7.
42. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 2008;371:1579-86.
43. Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med 2006;355:1318-30.
44. Xu X, D'Hoker J, Stange G, Bonne S, De Leu N, Xiao X, et al. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 2008;132:197-207.
45. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 2008;26:443-52.
46. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 2008;455:627-32.
47. Lee RH, Seo MJ, Reger RL, Spees JL, Pulin AA, Olson SD, et al. Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. Proc Natl Acad Sci U S A 2006;103:17438-43.
48. Couri CE, Oliveira MC, Stracieri AB, Moraes DA, Pieroni F, Barros GM, et al. C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA 2009;301:1573-9.
49. Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med 2002;8:963-70.
50. Mackay-Sim A, Feron F, Cochrane J, Bassingthwaighte L, Bayliss C, Davies W, et al. Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial. Brain 2008;131:2376-86.
51. Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N Engl J Med 2001;344:710-9.
52. Olanow CW, Goetz CG, Kordower JH, Stoessl AJ, Sossi V, Brin MF, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. Ann Neurol 2003;54:403-14.
53. Braak H, Del Tredici K. Assessing fetal nerve cell grafts in Parkinson's disease. Nat Med 2008;14:483-5.
54. Keene CD, Chang RC, Leverenz JB, Kopyov O, Perlman S, Hevner RF, et al. A patient with Huntington's disease and long-surviving fetal neural transplants that developed mass lesions. Acta Neuropathol 2009;117:329-38.
55. Lévesque MF, Neuman T, Rezak M. Therapeutic microinjection of autologous adult human neural stem cells and differentiated neurons for Parkinson's disease: five-year post-operative outcome. Open Stem Cell J 2009;1:20-9.
56. Levy YS, Bahat-Stroomza M, Barzilay R, Burshtein A, Bulvik S, Barhum Y, et al. Regenerative effect of neural-induced human mesenchymal stromal cells in rat models of Parkinson's disease. Cytotherapy 2008;10:340-52.
57. Murrell W, Wetzig A, Donnellan M, Feron F, Burne T, Meedeniya A, et al. Olfactory mucosa is a potential source for autologous stem cell therapy for Parkinson's disease. Stem Cells 2008;26:2183-92.
58. Cho MS, Lee YE, Kim JY, Chung S, Cho YH, Kim DS, et al. Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells. Proc Natl Acad Sci U S A 2008;105:3392-7.
59. Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, Soldner F, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci U S A 2008;105:5856-61.
60. Kondziolka D, Steinberg GK, Wechsler L, Meltzer CC, Elder E, Gebel J, et al. Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. J Neurosurg 2005;103:38-45.
61. Savitz SI, Dinsmore J, Wu J, Henderson GV, Stieg P, Caplan LR. Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. Cerebrovasc Dis 2005;20:101-7.
62. Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O, Kawahara N, et al. Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 2002;110:429-41.
63. Yoon SH, Shim YS, Park YH, Chung JK, Nam JH, Kim MO, et al. Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor: phase I/II clinical trial. Stem Cells 2007;25:2066-73.
64. van Laake LW, Passier R, Monshouwer-Kloots J, Verkleij AJ, Lips DJ, Freund C, et al. Human embryonic stem cell-derived cardiomyocytes survive and mature in the mouse heart and transiently improve function after myocardial infarction. Stem Cell Res 2007;1:9-24.
65. Puymirat E, Geha R, Tomescot A, Bellamy V, Larghero J, Trinquart L, et al. Can mesenchymal stem cells induce tolerance to cotransplanted human embryonic stem cells? Mol Ther 2009;17:176-82.
66. Hamamoto H, Gorman JH 3rd, Ryan LP, Hinmon R, Martens TP, Schuster MD, et al. Allogeneic mesenchymal precursor cell therapy to limit remodeling after myocardial infarction: the effect of cell dosage. Ann Thorac Surg 2009;87:794-801.
67. van der Bogt KE, Sheikh AY, Schrepfer S, Hoyt G, Cao F, Ransohoff KJ, et al. Comparison of different adult stem cell types for treatment of myocardial ischemia. Circulation 2008;118(suppl):S121-9.
68. Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. Circulation 2007;115:896-908.
69. Dill T, Schachinger V, Rolf A, Mollmann S, Thiele H, Tillmanns H, et al. Intracoronary administration of bone marrow-derived progenitor cells improves left ventricular function in patients at risk for adverse remodeling after acute ST-segment elevation myocardial infarction: results of the Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction study (REPAIR-AMI) cardiac magnetic resonance imaging substudy. Am Heart J 2009;157:541-7.
70. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. Am J Cardiol 2004;94:92-5.
71. Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. Arch Intern Med 2007;167:989-97.
72. Aiuti A, Cattaneo F, Galimberti S, Benninghoff U, Cassani B, Callegaro L, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med 2009;360:447-58.
73. Ott MG, Schmidt M, Schwarzwaelder K, Stein S, Siler U, Koehl U, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. Nat Med 2006;12:401-9.
74. Mavilio F, Pellegrini G, Ferrari S, Di Nunzio F, Di Iorio E, Recchia A, et al. Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. Nat Med 2006;12:1397-402.
75. Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempski H, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest 2008; 118:3143-50.
76. Wu X, Li Y, Crise B, Burgess SM. Transcription start regions in the human genome are favored targets for MLV integration. Science 2003;300:1749-51.
77. Montini E, Cesana D, Schmidt M, Sanvito F, Ponzoni M, Bartholomae C, et al. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. Nat Biotechnol 2006;24:687-96.
78. Wu LC, Sun CW, Ryan TM, Pawlik KM, Ren J, Townes TM. Correction of sickle cell disease by homologous recombination in embryonic stem cells. Blood 2006; 108:1183-8.
79. Kim SM, Lim JY, Park SI, Jeong CH, Oh JH, Jeong M, et al. Gene therapy using TRAIL-secreting human umbilical cord blood-derived mesenchymal stem cells against intracranial glioma. Cancer Res 2008;68:9614-23.
80. Leist M, Bremer S, Brundin P, Hescheler J, Kirkeby A, Krause KH, et al. The biological and ethical basis of the use of human embryonic stem cells for in vitro test systems or cell therapy. ALTEX 2008;25:163-90.

Study questions

Overview of the immune response

Learning objectives: "Overview of the immune response"

1. To understand the fundamental ways in which the innate and the adaptive arms of the immune system work together to help the host recognize, inactivate, and clear pathogenic microbes, neoplastic cells, toxins, and other exogenous threats.
2. To understand the mechanisms used by the innate and the adaptive arms of the immune response to distinguish self from non-self so that the immune effector mechanisms can be focused on appropriate targets and avoid damage to the host's normal tissues.

Question 1. For $CD8^+$ T lymphocytes to recognize virally infected cells, the infected cells must —

A. express functional HLA-DM molecules.
B. express functional transporter associated with antigen presentation (TAP) 1 and 2 proteins.
C. have trafficked through a germinal center.
D. extinguish expression of its class I HLA molecules.

Question 2. The HLA invariant chain —

A. controls loading of viral peptide fragments into class I HLA molecules.
B. is delivered to HLA molecules in endosomes.
C. prevents loading of antigenic peptides into class II HLA molecules until it is proteolytically degraded.
D. differs in primary amino acid sequence among different subjects in the population.

Question 3. For T_H cells, a functional T-cell receptor requires all of the following except

A. the CD3 complex.
B. coexpression of CD4.
C. rearranged α and β chains.
D. β_2-microglobulin.

Question 4. Which of the following statements is true?

A. The extracellular domains of Toll-like receptors (TLRs) are homologous to the corresponding domains of the IL-1 receptor.
B. All TLRs are cell-surface proteins.
C. TLRs are found on macrophages, dendritic cells, neutrophils, and endothelial cells.
D. TLR4 is activated by CpG DNA.

Study questions

Innate immunity

Learning objectives: "Innate immunity"

1. To appreciate the contribution of the innate immune system to host defense.
2. To understand the cellular and humoral elements involved in innate immune responses.
3. To be aware of the molecular strategies used by the innate immune system to sense infection or tissue damage.
4. To recognize how innate immune defects contribute to human disease and how the innate immune system can be modulated to prevent or treat illness.

Question 1. Which of the following statements regarding activation of Toll-like receptor (TLR) 9 in allergic responses is true?

A. Activation of TLR9 expressed by eosinophils inhibits generation of prostaglandin D_2.
B. Activation of TLR9 expressed by $CD4^+$ cells inhibits generation of T_H2 cytokines.
C. Activation of TLR9 expressed by dendritic cells inhibits T_H2 cell generation of cytokines.
D. Activation of TLR9 expressed by endothelial cells inhibits recruitment of T_H2 cells to sites of allergic inflammation.

Question 2. Which of the following human diseases is primarily caused by a defect in the innate immune system?

A. IL-1 receptor–associated kinase 4 (IRAK4) deficiency
B. severe combined immunodeficiency (SCID)
C. X-linked agammaglobulinemia (XLA)
D. DiGeorge syndrome

Question 3. One recognition strategy used by the innate immune system is to detect conserved microbial components. Which of the following is an example of a well-characterized innate immune system receptor-ligand pair?

A. TLR4 and peptidoglycan
B. T-cell receptor and H1N1 influenza A peptide
C. caspase 1 and the muramyl dipeptide component of peptidoglycan
D. TLR5 and flagellin

Question 4. Aluminum-containing vaccine adjuvants (alum) appear to mediate their beneficial immunostimulatory effects through which of the following molecules of the innate immune system?

A. TLR1/6 heterodimers
B. nucleotide oligomerization domain–like receptor family, pyrin domain–containing 3 (NLRP3, also called *NALP3* or *cryopyrin*)
C. myeloid differentiation primary response gene 88 (MyD88)
D. killer cell immunoglobulin-like receptor (KIR)

Study questions

Adaptive immunity

Learning objectives: "Adaptive immunity"

1. To understand the process of T-cell development, including the mechanisms of somatic genetic rearrangements that generate T-cell receptor diversity.
2. To recognize the different functional subsets of effector T cells.
3. To know the subunits that comprise the immunoglobulin pre-B cell receptor, which is critical for B-cell development in the bone marrow.
4. To know the critical processes of antigen-dependent B-cell development, which takes place in germinal centers.

Question 1. Which of the following statements concerning T cells is true?

A. On full maturation, T cells exiting the thymus express both CD4 and CD8.
B. CD8 serves as a coreceptor by binding to nonpolymorphic domains on MHC class II molecules.
C. T-cell activation leads both to release of intracellular calcium stores and to influx of extracellular calcium.
D. Newborn screening for severe combined immunodeficiency is performed by counting T cells on a blood spot.

Question 2. Which of the following statements concerning effector T cells is true?

A. $CD25^+$ regulatory T cells express the transcription factor retinoic acid receptor related orphan receptor γt (RORγt).
B. T_H17 cells arise from T_H0 precursors under the influence of IL-4 and IFN-γ.
C. Killing of virally infected target cells by cytolytic T lymphocytes is mediated by complement.
D. Natural killer T cells express αβ T-cell receptors and CD56.

Question 3. The pre-B cell receptor expressed on developing B cells in the bone marrow consists of —

A. IgM heavy chain, κ or λ light chain, Ig-α and Ig-β.
B. IgM heavy chain, surrogate light chain, CD20.
C. IgD heavy chain, surrogate light chain, Ig-α and Ig-β.
D. IgM heavy chain, surrogate light chain, Ig-α and Ig-β.

Question 4. Which of the following takes place predominantly in germinal centers?

A. immunoglobulin gene rearrangement
B. expression of IgD on the B-cell surface
C. immunoglobulin class-switching and somatic hypermutation
D. large-scale antibody secretion

Study questions

Structure and function of immunoglobulins

Learning objectives: "Structure and function of immunoglobulins"

1. To understand the molecular basis of immunoglobulin gene rearrangement.
2. To gain insight into the structural features of immunoglobulin that allow an individual antibody to distinguish between antigens.
3. To understand the contribution of immunoglobulin heavy chain structure to effector functions, such as complement activation and antibody-dependent cellular cytotoxicity.
4. To recognize that classes of immunoglobulin heavy chains differentially contribute to innate and adaptive immune responses.

Question 1. To generate their antigen receptors, developing B cells must undergo a complex process of DNA gene rearrangements that begins with precise cutting of the DNA strands and ends with the imprecise, in-frame joining of the ends of the nonhomologous sequences that encode the various portions of the future variable domain. Which of the following proteins is most critical for immunoglobulin rearrangement?

A. activation-induced cytidine deaminase
B. κ light chain
C. recombination-activating gene (RAG) 1 and 2
D. surrogate light chain (λ14.1 [λ5] and V_{preB})
E. terminal deoxynucleotidyl transferase (TdT)

Question 2. Activation of complement is one mechanism by which antibodies can kill cells. However, not all antibodies can bind complement, and some bind it better than others. Of the following isotypes, which one activates complement best?

A. IgA
B. IgD
C. IgE
D. IgG3
E. IgG4

Question 3. Which of the following functions cannot be performed by IgA?

A. binding Fcϵ receptors on mast cells
B. blocking pathogen adhesion
C. facilitation of antibody-dependent cellular cytotoxicity
D. mucosal transport
E. neutralizing toxins

Question 4. As a glycoprotein, there are potential N- and O-linked sites on the protein backbone of an immunoglobulin. Which of the following statements regarding immunoglobulin glycosylation is true?

A. All immunoglobulins are glycosylated the same.
B. Aglycosylated immunoglobulins function the same as glycosylated immunoglobulins.
C. Aberrantly glycosylated immunoglobulins play a role in some disease manifestations.
D. Fucose is the only sugar moiety on an immunoglobulin.

Study questions

Immunologic messenger molecules: Cytokines, interferons, and chemokines

Learning objectives: "Immunologic messenger molecules: Cytokines, interferons, and chemokines"

1. To recognize how different cytokines modulate cellular immune function.
2. To describe how the different T-cell populations develop and the role that cytokines play in modulating this response.
3. To understand how chemokines are grouped into separate families based on structure and function to modulate cell recruitment under inflammatory and homeostatic conditions.

Question 1. IL-6 and the IL-6 family of cytokines trigger signal transducer and activator of transcription 3 phosphorylation through which of the following receptors?

A. glycoprotein 130
B. shared γ chain
C. shared β chain (CD131)
D. nuclear factor IL-6
E. oncostatin M receptor α chain

Question 2. Which of the following is the master regulator for T_H17-like lymphocytes?

A. T-bet transcription factor
B. GATA-3
C. retinoic acid receptor–related orphan receptor γt
D. signal transducer and activator of transcription 3
E. Forkhead box protein 3

Question 3. Which of the following cytokines does not use the shared γ chain as part of its receptor?

A. IL-4
B. thymic stromal lymphopoietin
C. IL-7
D. IL-15
E. IL-21

Question 4. Which of the following chemokines is not involved in T_H1-like recruitment?

A. CCL3 (macrophage inflammatory protein 1α)
B. CCL4 (macrophage inflammatory protein 1β)
C. CCL5 (RANTES)
D. CCL11 (eotaxin)
E. CCL17 (thymus and activation-regulated chemokine).

Study questions

IgE, mast cells, basophils, and eosinophils

Learning objectives: "IgE, mast cells, basophils, and eosinophils"

1. To understand the biology of IgE, mast cells, basophils, and eosinophils.
2. To understand the role of IgE, mast cells, basophils, and eosinophils in disease.

Question 1. Which of the following regarding the high-affinity IgE receptor FcεRI is true?

A. The γ chain amplifies signaling through the receptor.
B. The β chain is absent on basophils.
C. The α chain binds to the C2 domain of the Fc region of IgE.
D. The β chain associates with Lyn kinase.

Question 2. All of the following are produced by basophils after activation except —

A. GM-CSF.
B. granzyme B.
C. IL-4.
D. prostaglandin D_2.

Question 3. Which of the following is associated with eosinopenia?

A. Addison disease
B. sepsis
C. Kimura disease
D. Omenn syndrome

Question 4. Which of the following statements is true regarding tryptase?

A. Anaphylaxis to food allergens is always associated with an increase in total serum tryptase levels.
B. Baseline serum tryptase is composed of predominantly the mature β-tryptase.
C. Tryptase is stabilized in secretory granules by heparin.
D. Protryptase is the predominant form of tryptase stored in the secretory granules of mast cells.

Study questions

Genetics of allergic disease

Learning objectives: "Genetics of allergic disease"

1. To comprehend the principles of study design for genetic and genomic approaches to studying allergic disease.
2. To identify single nucleotide polymorphisms (SNPs) that have been identified as potential markers for allergic disease in the latest genome-wide association studies.
3. To apply knowledge of genetic studies to the pharmacogenetics of allergic disease.
4. To analyze mechanisms of genetic susceptibility to allergic disease and their associated candidate genes.

Question 1. Which of the following approaches to studying the genetics of allergic disease would be most appropriate to identifying the role of variation in a candidate gene in susceptibility to allergic disease?

A. positional cloning/linkage studies examining transmission of genetic markers with clinical phenotype in families
B. examination of "tagging" SNPs that capture the common variation in a defined region of the genome in a case-control cohort
C. using a genome-wide association study approach to assess variation across the whole genome to find polymorphisms associated with allergic disease
D. examining the effect of an amino acid variant on protein function in *in vitro* studies

Question 2. SNPs in or near which of the following genes have been found to be associated with asthma or allergic phenotypes in genome-wide association studies?

A. *ORMDL*
B. *CHRNA3* (nicotinic acetylcholine receptor subunit)
C. *IL13* (IL-13)
D. *SH2B3* (SH2B adaptor protein 3)

Question 3. Which of the following genes have SNPs that have been associated with pharmacogenetic responses in asthma treatment?

A. *CYP1A1* (cytochrome P450 1A1)
B. *ADRB2* (β_2-adrenergic receptor)
C. *IL5* (IL-5)
D. *CD14*

Question 4. Which of the following pairs of mechanisms and genes correctly matches a proposed disease susceptibility mechanism for allergic disease with a relevant candidate gene?

A. modulation of the effect of environmental risk factors for allergic disease–*IL13*
B. loss of epithelial barrier function–*FLG* (filaggrin)
C. regulation of atopic inflammation–*ORMDL3*
D. tissue response genes–*PHF11*

Study questions

Asthma: Clinical expression and molecular mechanisms

Learning objectives: "Asthma: Clinical expression and molecular mechanisms"

1. To understand the importance of viral respiratory tract infections in asthma inception and exacerbations.
2. To recognize the potential contribution of various comorbidities to asthma control.
3. To understand the contribution of allergic sensitization to asthma expression and management.

Question 1. When asthma severity and control are being evaluated, which of the following factors is part of the assessment of the risk domain?

A. pulmonary function
B. symptoms
C. exacerbations
D. rescue albuterol use

Question 2. Which of the following viruses is the most frequent respiratory tract infection associated with asthma exacerbations?

A. metapneumovirus
B. rhinovirus
C. respiratory syncytial virus
D. parainfluenza

Question 3. Which of the following pain medications can be safely given to an asthmatic subject sensitive to aspirin?

A. ibuprofen
B. naproxen
C. acetaminophen
D. indomethacin

Question 4. Which of the following medications has been associated with an increased risk for severe asthma exacerbations when used as the only treatment?

A. inhaled corticosteroids
B. theophylline
C. leukotriene receptor antagonists
D. long-acting β-agonists

Study questions

Rhinitis and sinusitis

Learning objectives: "Rhinitis and sinusitis"

1. To understand the mechanism of dust mite allergen sensitization in the nasal mucosa.
2. To understand the association between nonallergic rhinitis and eosinophilia.
3. To understand the pathologic processes involved in chronic rhinosinusitis (CRS) with or without nasal polyps.
4. To learn the clinical significance of hyperdensities on sinus computed tomographic (CT) scanning in a patient with CRS.

Question 1. Which of the following processes involved during natural allergen sensitization through the nasal mucosa in patients with allergic rhinitis is most specific for dust mite antigen?

A. elaboration of thymic stromal lymphopoietin by nasal epithelial cells
B. local and systemic production of allergen-specific IgE
C. enhancement through induction of Toll-like receptor 4 (TLR4) signaling
D. interaction of dust mite antigen with interepithelial and subepithelial dendritic cells

Question 2. Which of the following subtypes of nonallergic rhinitis is most likely to be associated with eosinophilia?

A. irritant-induced rhinitis
B. cold-induced rhinitis
C. vasomotor rhinitis
D. rhinitis associated with aspirin sensitivity (aspirin-exacerbated respiratory disease)

Question 3. Which of the following pathologic processes implicated in the pathogenesis of CRS is most specific for CRS with nasal polyposis?

A. T_H2-type immune hyperresponsiveness (production of IL-5 and IL-13) directed toward colonizing fungi in sinus secretions
B. glandular hyperplasia
C. formation of bacterial biofilm on sinus mucosal tissue
D. local production of IgE directed against staphylococcal enterotoxins (ie, superantigens) from *Staphylococcus aureus*

Question 4. In patients with CRS, the sinus CT scan might reveal hyperdensities within an opacified sinus cavity. Which of the following statements best describes the significance of hyperdensities?

A. They are pathognomonic of allergic fungal rhinosinusitis.
B. They are suggestive of the presence of necrotizing infection (abscess formation).
C. They are often associated with mucocele formation.
D. They are suggestive of the presence of thick inspissated secretions containing large numbers of degranulated eosinophils (allergic mucin) and possibly colonizing fungi.

Dr. Mark Dykewicz, as a member of the Board of Directors of the American Board of Allergy and Immunology, did not participate in the development or review of these questions.

Study questions

Food allergy

Learning objectives: "Food allergy"

1. To understand the epidemiologic aspects of food hypersensitivity disorders.
2. To understand the pathogenesis of food allergy.
3. To understand the clinical manifestations of food allergy.
4. To understand current and future diagnosis and management.

Question 1. Which of the following most accurately describes an epidemiologic feature of food allergy?

A. Allergy to fish/shellfish is more prevalent among children than among adults.
B. On the basis of studies from a referral center in the United States, allergy to milk and egg might be more persistent than noted in past decades, with fewer than 20% resolving these allergies by age 4 years.
C. Food allergy has approximately doubled in children over the past decade.
D. Peanut allergy resolves by school age for 35% of children given diagnoses at less than 2 years of age.

Question 2. A 27-year-old atopic man experienced mild oral pruritus when eating raw apples but tolerates apple juice and baked apple. Which of the following is most likely to be true?

A. He has an increased IgE level that binds lipid transfer protein in apple.
B. He has positive skin test results to commercial extract of apple.
C. He has an increased IgE level to Bet v 1.
D. The Maillard reaction during heating apple results in a change in conformation that abrogates IgE binding for this subject.

Question 3. Which of the following clinical descriptions is most likely to represent a food allergy?

A. A 3-year-old experiences acute, transient, nonpruritic erythema over the left cheek minutes after she ingests, on separate occasions, lemonade, spicy potato chips, and sour candy.
B. A breast-fed 5-month-old infant experiences severe vomiting, lethargy, and an increased white blood cell count with bandemia 2 hours after she is fed rice cereal. Skin test results to rice are negative.
C. An 18-year-old experiences cramps and diarrhea after ingesting a large milk shake.
D. A 47-year-old experiences facial flushing and a tingling sensation in the mouth after ingesting tuna in a restaurant. He previously tolerated all fish.

Question 4. An infant experienced urticaria and angioedema when introduced to egg, and the egg-specific IgE concentration was 4.7 kIU/L. At age 2 years, she accidentally ingested a bite of egg and experienced wheezing and generalized urticaria and around that time had an egg IgE level of 1.7 kIU/L. At age 3 years, she accidentally ingested a small amount of egg and experienced generalized urticaria. At age 3½ years, she presents for evaluation, and the serum egg IgE level was less than 0.35 kIU/L. Which of the following would be the most reasonable next step toward diagnosis?

A. Perform an open oral food challenge to egg.
B. Perform a double-blind, placebo-controlled oral food challenge to egg.
C. Perform a skin prick test to egg.
D. Allow the child to add egg to the diet.

Study questions

Drug allergy

Learning objectives: "Drug allergy"

1. To recognize the limitations of diagnostic testing in most patients with drug allergy.
2. To gain an understanding of the negative predictive value of penicillin skin testing.
3. To gain an understanding of duration, indications, and contraindications of procedures to induce drug tolerance.
4. To be able to differentiate the various drug-induced allergic reactions to aspirin and nonsteroidal anti-inflammatory drugs.

Question 1. In evaluation of a patient with drug allergies, which of the following is generally the best tool to guide management?

A. skin testing
B. *in vitro* tests
C. detailed history
D. Gell and Coombs classification

Question 2. Which of the following is true regarding penicillin allergy?

A. History is adequate for diagnosis.
B. Skin testing has high negative predictive value.
C. Cross-reactivity with cephalosporins is high.
D. Resensitization is common.

Question 3. Procedures to induce drug tolerance —

A. involve only IgE-mediated allergy.
B. cause permanent drug tolerance.
C. can take days to weeks to complete.
D. are indicated for Stevens-Johnson syndrome reactions.

Question 4. A 30-year-old man has a history of shortness of breath, urticaria, and lightheadedness 30 minutes after ingesting ibuprofen. He most likely —

A. has asthma.
B. has nasal polyposis.
C. will react to celecoxib.
D. will tolerate aspirin.

Study questions

Allergic skin diseases

Learning objectives: "Allergic skin diseases"

1. To identify common clinical patterns and sensitizing allergens in patients with contact dermatitis.
2. To understand the newest concepts regarding the immunology and treatment of chronic urticaria.

Question 1. Which of the following statements concerning autoantibodies in patients with chronic urticaria is true?

A. The autologous serum skin test is the gold standard.
B. Commercially available tests for autoantibody activity are well established.
C. The presence, titer, or both of autoantibodies to the high-affinity receptor for IgE, FcεRIα, predict clinical outcome.
D. Approximately 40% of patients with chronic urticaria have evidence of autoantibodies with the ability to activate mast cells.

Question 2. For patients with chronic urticaria unresponsive to high doses of antihistamines, the immunomodulatory drug with the best efficacy data is —

A. hydroxychloroquine.
B. cyclosporin A.
C. sulfasalazine.
D. mycophenolate.

Question 3. Which of the following statements would be true for a patient with contact dermatitis?

A. Irritant contact dermatitis commonly presents as a generalized dermatitis with vesicles extending beyond the area of contact and involving the whole hand, including the webs of fingers and the dorsal and ventral surfaces of the hands.
B. Allergic contact dermatitis often has vesicles that favor the dorsum of the hands and, less commonly, involve the palms.
C. Atopic dermatitis is not an important factor in susceptibility to persistent postoccupational dermatitis.
D. A patient with allergic contact dermatitis can use "unscented" products and botanical extracts because these products are typically free of classic fragrance ingredients.

Question 4. Which of the following is true for patch testing?

A. Patch test results are affected by oral corticosteroids, cancer chemotherapy, immunosuppressive drugs, and antihistamines but not by topical corticosteroids.
B. Allergens not found on commercially available screening series in the United States are generally irrelevant reactions, and personal products have no use as supplements in patch testing.
C. Metals (gold, potassium dichromate, nickel, and cobalt), topical antibiotics (neomycin and bacitracin), topical corticosteroids, and paraphenylenediamine (PPD) might produce positive results after 7 days.
D. Hairdressers allergic to glycerol thioglycolate in permanent wave solution and PPD in hair dye might be able to cut hair after it has been rinsed out.
E. Lanolin in medicaments is less sensitizing than lanolin in cosmetics and is a weak sensitizer in normal skin but a stronger sensitizer in damaged skin.

Study questions

Environmental and occupational allergies

Learning objectives: "Environmental and occupational allergies"

1. To know what allergens can occur in the air both indoors and outdoors and how to identify which ones are important to an individual patient.
2. To understand the methods of reducing exposure to these allergens.
3. To understand the effects of indoor and outdoor air pollution.
4. To recognize and diagnose occupational asthma.

Question 1. Concentrations of which of the following allergens are most closely related to indoor humidity?

A. *Dermatophagoides farinae*
B. *Blatella germanica*
C. *Alternaria alternata*
D. *Felis domesticus*

Question 2. Which of the following methods of controlling exposure to cat allergen is most useful?

A. keep the cat out of the bedroom
B. dispose of the cat
C. wash the cat once a week
D. use high-efficiency particle filtration

Question 3. Which of the following air pollutants increases production of IgE antibodies?

A. sulfur dioxide
B. nitric oxide
C. diesel exhaust particles
D. ozone.

Question 4. Which of the following statements about occupational asthma is true?

A. Symptoms occur only on days when the patient is at work.
B. Bronchial provocation tests are required to confirm the diagnosis.
C. Approximately 5% of asthma beginning in adulthood is due to occupational exposure.
D. All the exposures that cause occupational asthma elicit an IgE response.

Study questions

Anaphylaxis

Learning objectives: "Anaphylaxis"

1. To describe the triggers, mechanisms, and patient-specific risk factors in anaphylaxis.
2. To state the principles of risk assessment in anaphylaxis.
3. To discuss long-term risk reduction in anaphylaxis: preventive measures and emergency preparedness.

Question 1. The lifelong prevalence of anaphylaxis from all triggers in the general population is estimated at —

A. 0.001%.
B. 0.01%.
C. 0.1%.
D. 0.05% to 2%.

Question 2. You diagnose idiopathic anaphylaxis in a 50 year-old woman who has had 2 episodes during the past year. What should you do next?

A. Refer her for a bone marrow biopsy.
B. Measure her serum total tryptase level.
C. Advise her to avoid peanut, tree nuts, shellfish, and fish.
D. Prescribe prednisone, 60 mg daily, for a week and then taper the dose.

Question 3. For which of the following is a 3- to 5-year course of subcutaneous immunotherapy recommended based on randomized, double-blind, placebo-controlled trials?

A. food-induced anaphylaxis
B. medication-induced anaphylaxis
C. stinging insect venom–induced anaphylaxis
D. natural rubber latex–induced anaphylaxis

Question 4. Epinephrine is the drug of first choice in anaphylaxis because —

A. its α_1-adrenergic vasoconstrictor effects decrease mucosal edema and increase peripheral vascular resistance.
B. its α_2-adrenergic receptor effects decrease release of insulin and norepinephrine.
C. its β_1-adrenergic effects increase the rate and force of cardiac contractions.
D. its β_2-adrenergic effects increase bronchodilation and decrease mediator release.

Study questions

Primary immunodeficiencies

Learning objectives: "Primary immunodeficiencies"

1. To recognize the key diagnostic features of congenital defects of lymphocyte development and neutrophil function.
2. To learn the mainstay of treatment for patients with antibody deficiency.

Question 1. Which of the following statements concerning severe combined immunodeficiency (SCID) is true?

A. SCID is characterized by severe deficiency of T cells.
B. SCID is characterized by severe deficiency of T and B cells.
C. SCID is characterized by severe deficiency of T, B, and natural killer cells.
D. SCID is characterized by severe deficiency of both lymphocytes and neutrophils.

Question 2. Which of the following statements concerning X-linked agammaglobulinemia (XLA) is true?

A. XLA is characterized by lack of immunoglobulins in spite of a normal number of circulating B cells.
B. XLA is characterized by a virtual lack of circulating B cells.
C. In patients with XLA, the profound deficiency of immunoglobulins reflects defects of T_H lymphocytes.
D. The mainstay of treatment of patients with XLA is antibiotic prophylaxis.

Question 3. Which of the following presentations is common in patients with chronic granulomatous disease?

A. autoimmune manifestations resembling systemic lupus erythematosus
B. interstitial pneumonia caused by *Pneumocystis jiroveci*
C. purulent lymphadenitis
D. recurrent otitis media

Question 4. Which of the following statements concerning treatment with immunoglobulins is true?

A. Initial treatment for patients with agammaglobulinemia should be with intravenous immunoglobulin, 100 mg/kg every 3 weeks.
B. Subcutaneous immunoglobulins should be used at the dose of 100 mg/kg/wk in children less than 14 years of age. Beyond that age, the dose for adults is 4 g/wk.
C. The usual dose for subcutaneous immunoglobulins is 100 mg/kg/wk.
D. Patients with IgA deficiency should receive preparations enriched in IgA.

Study questions

Secondary immunodeficiencies, including HIV infection

Learning objectives: "Secondary immunodeficiencies, including HIV infection"

1. To define the concept of secondary immunodeficiency as a clinical condition in which the immune response is adversely affected by extrinsic factors.
2. To realize that the frequency of patients with secondary immunodeficiencies far exceeds the frequency of those with primary (genetic) immunodeficiencies.
3. To appreciate the many diverse factors and conditions that produce secondary immunodeficiency.
4. To understand that HIV infection is one of the best understood yet most challenging examples of a secondary immunodeficiency.

Question 1. An 18-year-old woman presents with a history of recurrent respiratory tract infections in the past 3 months. She has been previously healthy. Which of the following is the most likely cause of immunodeficiency in this patient?

A. severe combined immunodeficiency
B. HIV infection
C. X-linked agammaglobulinemia
D. hyper-IgM syndrome

Question 2. Which of the following is a characteristic of a secondary immunodeficiency?

A. The clinical presentation is variable.
B. A defect in T-cell function can always be identified.
C. Management should prioritize immunoglobulin supplementation in all cases.
D. Phagocyte chemotaxis is normal.

Question 3. Calcineurin inhibitors primarily inhibit—

A. oxidative burst.
B. complement activation.
C. T-cell activation.
D. calcium membrane receptor.

Question 4. In patients with HIV infection, which of the following is true?

A. AIDS, the advanced stage of HIV infection, develops when B cells are depleted.
B. The presence of the chemokine receptor CCR5 in cell membrane blocks HIV infection.
C. The presence of the chemokine receptor CCR5 in cell membrane is necessary for HIV infection.
D. An adenovirus-based anti-HIV vaccine has been demonstrated to reduce HIV infection in a large placebo-controlled trial.

Study questions

Immunologic rheumatic disorders

Learning objectives: "Immunologic rheumatic disorders"

1. To recognize the diagnostic utility and the prognostic significance of the rheumatoid factor and anti–cyclic citrullinated peptide (anti-CCP) antibody tests in patients with rheumatoid arthritis (RA).
2. To understand the basic mechanisms behind the biologic disease-modifying medications currently available to treat RA.
3. To identify the antibody tests that are useful in making the diagnosis of systemic lupus erythematosus (SLE) while recognizing that the diagnosis is based on the whole clinical picture and not simply the laboratory findings.

Question 1. Which of the following statements is true regarding anti-CCP antibodies in patients with RA?

A. The appearance of anti-CCP antibodies in the bloodstream coincides with the onset of clinical RA.
B. Anti-CCP antibodies are highly specific for RA.
C. Patients with RA who have anti-CCP antibodies tend to have milder disease than those who do not have the antibodies.
D. Rheumatoid factor and anti-CCP antibodies are about equally specific for RA.

Question 2. Comparing the traditional disease-modifying antirheumatic drugs (DMARDs) used to treat RA with the new biologic DMARDS, which of the following statements is true?

A. The biologic DMARDs target specific factors in the immune system, such as proinflammatory cytokines.
B. Current American College of Rheumatology recommendations include initiation of biologic DMARDs within 3 months of diagnosis of RA.
C. Traditional DMARDs, such as methotrexate, increase cardiovascular risk in patients with RA.
D. Combining biologic DMARDs with traditional DMARDs does not increase efficacy in patients with RA.

Question 3. Which of the following diseases is more common in men than in women?

A. RA
B. seronegative spondyloarthropathies
C. SLE
D. Sjögren syndrome

Question 4. Which of the following statements is true regarding antibodies in SLE?

A. A patient with arthralgias and a low titer of antinuclear antibody (ANA) is likely to have SLE.
B. Patients with SLE frequently have negative ANA test results.
C. The presence of anti-Ro (SSA) rules out SLE in favor of Sjögren syndrome.
D. Anti-Smith antibodies are highly specific for SLE.

Study questions

Vasculitis

Learning objectives: "Vasculitis"

1. To describe the diagnostic yield from biopsy specimens of different organs in patients with Wegener granulomatosis.
2. To identify the sites of organ involvement in patients with Churg-Strauss syndrome.
3. To recognize the antigen associations of antineutrophil cytoplasmic antibodies (ANCAs).
4. To distinguish the prominent clinical features of giant cell arteritis (GCA).

Question 1. Which of the following biopsies of a clinically involved site has the highest likelihood of yielding a diagnosis of Wegener granulomatosis?

A. sinus
B. kidney
C. lung
D. gastrointestinal mucosa

Question 2. Which of the following is the most common organ system affected by vasculitis in patients with Churg-Strauss syndrome?

A. peripheral nerve
B. heart
C. kidney
D. gastrointestinal tract

Question 3. Which of the following antigens do ANCAs most commonly target in patients with Wegener granulomatosis?

A. myeloperoxidase
B. proteinase 3
C. human neutrophil elastase
D. bactericidal permeability–increasing protein

Question 4. Which of the following statements is true regarding GCA?

A. Visual loss occurs in 50% to 60% of patients.
B. Isolated polymyalgia rheumatica requires treatment with 40 to 60 mg/d prednisone.
C. An increased sedimentation rate occurs in less than 50% of patients with GCA.
D. Large-vessel involvement of the aorta or its primary branches occurs in 27% of cases.

Study questions

Immunologic endocrine disorders

Learning objectives: "Immunologic endocrine disorders"

1. To understand general HLA and autoantibody testing for type 1 diabetes and the associated celiac disease.
2. To understand the genetics of 2 major monogenic forms of autoimmune type 1 diabetes.
3. To become familiar with major autoantibodies measured in patients with polyendocrine syndromes.
4. To recognize the similarities and differences between the major autoimmune polyendocrine syndromes.

Question 1. Which of the following statements concerning type 1 diabetes is true?

A. The highest-risk genotype for type 1 diabetes is DR4/4.
B. Islet autoantibodies typically appear years before the development of diabetes.
C. Insulin autoantibodies are most common in adults rather than children with diabetes.
D. Celiac disease and anti-transglutaminase autoantibodies are not increased in patients with type 1 diabetes.

Question 2. Immune dysfunction, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome and autoimmune polyendocrine syndrome type 1 (APS-1) are —

A. monogenic disorders influencing the development of regulatory T cells or expression of peripheral antigens in the thymus.
B. inherited in an autosomal recessive manner.
C. polygenic disorders.
D. common.

Question 3. Which of the following statements concerning autoantibodies is true?

A. Anti–21-hydroxylase autoantibodies are diagnostic of Addison disease.
B. Anti-insulin autoantibodies develop in most individuals treated with subcutaneous insulin, including patients with type 2 diabetes.
C. Transglutaminase autoantibodies are the best marker of celiac disease.
D. All of the above

Question 4. APS-1 differs from autoimmune polyendocrine syndrome type 2 (APS-2) in that —

A. patients with APS-1 have a mutation of *AIRE*.
B. Addison disease and type 1 diabetes occur in both syndromes.
C. mucocutaneous candidiasis is present only in patients with APS-1.
D. All of the above

Study questions

Diagnostic testing and interpretation of tests for autoimmunity

Learning objectives: "Diagnostic testing and interpretation of tests for autoimmunity"

1. To understand the usefulness of autoantibodies and immunologic studies.
2. To understand the limitations of these studies.
3. To understand the major clinical presentations of autoimmune diseases.

Question 1. A 36-year-old woman is seen in the emergency department for new-onset shortness of breath with wheezing. After bronchodilation therapy, the patient no longer wheezes. Other than chronic sinusitis, she has been well. Examination shows her to be comfortable, afebrile, and normotensive, with a respiratory rate of 14 breaths/min. No wheezes are auscultated. Tender subcutaneous nodules are discovered on her right anterior leg. Screening laboratory tests reveal a mild anemia and slightly increased white blood cell count with increased eosinophil numbers. The Westergren erythrocyte sedimentation rate (ESR) is 88 mm, and the high-sensitivity C-reactive protein level is 46 mg/dL. Chest radiography shows patchy opacities without lobar or segmental distribution. What laboratory test would be highly suggestive that this is Churg-Strauss syndrome?

A. antinuclear antibody (ANA)
B. anti–proteinase 3
C. anti-myeloperoxidase (anti-MPO)
D. anti–extractible nuclear antigen (anti-ENA)
E. anti–double-stranded DNA (anti-dsDNA)

Question 2. What is the most sensitive serologic test for systemic lupus erythematosus (SLE)?

A. anti-dsDNA
B. ANA
C. anti-Smith
D. anti-ENA
E. ESR

Question 3. What is the most specific serologic test for SLE?

A. anti-dsDNA
B. ANA
C. anti-Smith
D. anti-ENA
E. ESR

Question 4. In immune complex deposition diseases, such as vasculitis, which is associated with rheumatoid arthritis, serum C3 levels will most commonly —

A. increase.
B. decrease.
C. remain unchanged.
D. decrease and then increase.

Study questions

Pulmonary disorders, including vocal cord dysfunction

Learning objectives: "Pulmonary disorders, including vocal cord dysfunction"

1. To explore the classification of pulmonary disorders with various immunologic processes.
2. To consider the causes of pulmonary eosinophilia syndromes or conditions.
3. To differentiate granulomatous T_H1 and T_H2 inflammatory conditions.
4. To appreciate the variable aspects of diagnosis of vocal cord dysfunction.

Question 1. In patients with pulmonary tuberculosis, the number of $CD4^+CD25^+$ regulatory T cells is —

A. decreased.
B. increased.
C. very low or absent.
D. very high.

Question 2. Antibodies in classic cases of Churg-Strauss syndrome are directed against —

A. proteinase-3.
B. myeloperoxidase.
C. single-stranded DNA.
D. Churg-Strauss syndrome protein.

Question 3. The expected finding in bronchoalveolar lavage differential count in a patient with acute hypersensitivity pneumonitis is —

A. macrophages of 95%.
B. lymphocytes of 60%.
C. eosinophils of 40%.
D. polymorphonuclear leukocytes of 60%.

Question 4. A characteristic feature of the reactive airways dysfunction syndrome (RADS) is that —

A. the period for sensitization is usually 1 to 5 years before onset.
B. respiratory symptoms resolve by 3 months after exposure.
C. bronchial hyperresponsiveness is present.
D. bronchial biopsy demonstrating eosinophilia is consistent with the diagnosis.

Study questions

Mucosal immunology, eosinophilic esophagitis, and other intestinal inflammatory diseases

Learning objectives: "Mucosal immunology, eosinophilic esophagitis, and other intestinal inflammatory diseases"

1. To identify anatomic features of the gastrointestinal mucosal immune system.
2. To recognize clinicopathologic features of common gastrointestinal diseases that are linked by perturbations in the mucosal immune system.

Question 1. Defects in which of the following cell types lead to a syndrome termed immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome?

A. B cells
B. mast cells
C. regulatory T cells
D. eosinophils

Question 2. Which of the following are the correct diagnostic features of eosinophilic esophagitis in a child or adult?

A. esophageal inflammation consisting of more than 15 eosinophils per high-power field
B. dysphagia and food impaction that persists despite acid blockade
C. symptoms consistent with esophageal dysfunction and more than 15 eosinophils per high-power field in which other causes have been ruled out
D. feeding dysfunction and esophageal inflammation consisting of more than 15 eosinophils per high-power field

Question 3. Which of the following diseases can be treated primarily with dietary exclusion?

A. Crohn disease
B. ulcerative colitis
C. celiac disease
D. gastroesophageal reflux disease

Question 4. Which of the following is a feature of defensins?

A. biochemical characterized as charge neutral molecules
B. They are primarily produced by Paneth cells.
C. They participate as an element of the acquired immune system.
D. Three different types have been identified.

Study questions

Complement disorders and hereditary angioedema

Learning objectives: "Complement disorders and hereditary angioedema"

1. To understand the role of complement in host defense.
2. To be able to identify and understand the difference between the various complement pathways.
3. To understand hereditary angioedema, its relation to complement and kinins, and the new therapeutic approaches.
4. To understand the consequences of genetic deficiency of complement proteins and regulatory proteins.

Question 1. The complement pathway initiated by the binding of a plasma protein to sugars on the surface of a microbe is —

A. the lectin pathway.
B. the alternative pathway.
C. the classical pathway.
D. the kinin pathway.

Question 2. Therapy for hereditary angioedema is directed at which of the following outcomes?

A. control of C1 esterase inhibitor levels to greater than 75% activity
B. decrease in the number of angioedema episodes to maximize quality of life with minimal adverse effects
C. control of C4 levels to normality
D. control of all episodes of angioedema

Question 3. The pathway of complement activation activated by antibody usually is —

A. the lectin pathway.
B. the alternative pathway.
C. the classical pathway.
D. the lectin pathway.

Question 4. What is the genetic defect that is frequently associated with high-grade pathogen infection, such as with pneumococci or staphylococci?

A. C8 deficiency
B. factor B deficiency
C. C3 deficiency
D. mannose-binding lectin deficiency

Study questions

Immune responses to malignancies

Learning objectives: "Immune responses to malignancies"

1. To understand the role of the immune system in control of tumor progression.
2. To recognize the effect of the tumor microenvironment on functions of immune cells.
3. To understand the molecular and cellular mechanisms used by the tumor for its escape from the host immune system.

Question 1. What type of evidence do we have that a patient with cancer makes an immune response specifically directed at his or her own tumor?

A. Tumors induce apoptosis of $CD8^+$ T cells.
B. Tumor cells release tumor-associated antigens (TAs) into the circulation.
C. Antibodies to MHC molecules are detectable in patients' sera.
D. A measurable or increased (relative to that seen in healthy control subjects) frequency in the blood of $CD8^+$ cytotoxic T lymphocytes that stain with TA-specific tetramers is present.

Question 2. Dendritic cells (DCs) are professional antigen-presenting cells that play a key role in the induction of adaptive immune responses to TAs. DCs in patients with cancer do not efficiently cross-present TAs to T cells because —

A. they do not home to the tumor or tumor-draining lymph nodes.
B. they are enriched in class I and class II MHC molecules relative to DCs in healthy control subjects.
C. they are immature because of the presence of soluble tumor-derived factors.
D. they produce excessive levels of IL-12.

Question 3. Regulatory T cells are in part responsible for down-regulation of antitumor activity in patients with cancer. These T cells —

A. mediate suppression of other immune cells through cell-to-cell contact.
B. secrete IFN-γ and IL-2.
C. are decreased in number in the peripheral blood of patients with cancer.
D. do not kill other T or B cells.

Question 4. Inflammatory infiltrates into human solid tumors have been carefully examined because of their potential prognostic significance. These infiltrates have the following characteristic.

A. They resemble acute inflammatory infiltrates into healing wounds.
B. They are enriched in natural killer cells.
C. The ratio of $CD8^+/CD4^+$ T cells is always high because of the excess of $CD8^+$ T cells.
D. They are a major source of proinflammatory cytokines that support tumor growth.

Study questions

Clinical laboratory assessment of immediate-type hypersensitivity

Learning objectives: "Clinical laboratory assessment of immediate-type hypersensitivity"

1. To understand the principal properties of human IgE antibodies.
2. To describe the components of the diagnostic algorithm that are used in the evaluation of a patient with a suspected allergic disease.
3. To define laboratory methods for studying IgE antibody cross-reactivity with structurally similar allergens (eg, Hymenoptera venoms).
4. To define the humoral immune response parameters that determine the most effective translation of an IgE antibody response into mediator release from mast cells and basophils.
5. To understand when detection of IgG antibody responses can be diagnostically useful in the evaluation of lung-related hypersensitivity states.

Question 1. IgE is the immunoglobulin that has been called the "gatekeeper" of the allergic response. Once bound to the surface of basophils or mast cells, it serves to mediate vasoactive mediator release after cross-linking by allergenic molecules. Which of the following is a property of human IgE antibodies?

A. Its molecular weight is approximately 150,000 d.
B. It freely passes the placenta to contribute to neonatal total serum IgE levels that allow identification of a neonate's atopic predisposition.
C. Its concentration in serum is highly age-dependent.
D. It constitutes 2% of the total serum immunoglobulin.

Question 2. The diagnostic algorithm for allergic disease begins with a carefully collected clinical history. If the history indicates a highly probable association between the patient's reported upper airway allergic symptoms and a probable aeroallergen exposure, what is the next recommended step in the diagnostic process based on the practice parameters?

A. Immediately quantify mast cell α-tryptase within 30 minutes to 4 hours after symptom initiation.
B. Perform serology or skin test measurements (depending on the suspected allergen specificity) to verify that the patient is sensitized (IgE antibody positive).
C. Perform a direct allergen challenge of the indicated target organ.
D. Perform an allergen-specific IgG measurement to verify exposure.

Question 3. IgE antibody can be present in the absence of any evident clinical symptoms. Which one of the following humoral immune response–related conditions enhances the effectiveness of IgE antibody responses to induce mediator release from mast cells and basophils?

A. lower allergen-specific IgE antibody concentration in circulation
B. less mature specificity directed at the allergen's or allergens' specific epitopes
C. lower proportion of specific IgE to total IgE in a patient's serum
D. higher affinity of the IgE antibody for specific allergen

Question 4. In which one of the following situations are specific IgG antibody responses considered diagnostically useful in the workup of a patient?

A. evaluation of food allergy symptoms for the planning of food-elimination diets
B. assessment of patients with rhinitis associated with seasonal aeroallergen exposure
C. testing of a child with spina bifida who experienced anaphylaxis after the insertion of a *Hevea brasiliensis* latex catheter
D. evaluation of a patient suspected of hypersensitivity pneumonitis as a result of exposure to organic dusts

Study questions

Laboratory evaluation of primary immunodeficiencies

Learning objectives: "Laboratory evaluation of primary immunodeficiencies"

1. To recognize the clinical symptoms of the most common primary immunodeficiencies.
2. To describe the appropriate laboratory approach for investigating general categories of primary immunodeficiencies.

Question 1. Choose the alternative that best describes the most appropriate initial laboratory test to evaluate a patient with recurrent bacterial sinopulmonary infections and chronic diarrhea caused by *Giardia lamblia*.

A. lymphocyte immunophenotyping
B. mitogen-induced lymphocyte proliferation
C. oxidative burst by dihydrorhodamine 123 (DHR)
D. serum immunoglobulin levels

Question 2. Recurrent infections by a narrow range of pathogens, such as *Streptococcus pneumoniae* and *Staphylococcus aureus*, together with a poor fever response to infection is most consistent with a defect in signaling by which of the following?

A. IFN-γ receptor
B. IL-12 receptor
C. Toll-like receptors
D. GM-CSF receptor

Question 3. The results of which of the following laboratory tests would most likely be abnormal in a 2 month-old infant with failure to thrive, persistent diarrhea, and oral thrush?

A. DHR
B. CH50
C. lymphocyte count
D. serum immunoglobulin measurement

Question 4. Which of the following assays correlates most closely with CD45RA expression on CD4 T cells?

A. T-cell receptor excision circles
B. T-cell receptor diversity
C. T cell–mediated cytotoxicity
D. T-cell response to mitogens

Study questions

Allergen immunotherapy

Learning objectives: "Allergen immunotherapy"

1. To have a clear understanding of the mechanisms believed to be responsible for the beneficial effects of allergen immunotherapy.
2. To be able to explain the principles of patient selection and safe administration of immunotherapy.
3. To be aware of the scope for improving immunotherapy in the future.

Question 1. Which of these conditions is not an indication for specific immunotherapy (SIT)?

A. bee venom–induced anaphylaxis
B. aspirin-induced asthma
C. allergic rhinitis
D. cat dander–induced asthma

Question 2. Sublingual immunotherapy —

A. uses similar doses of allergen to conventional (injected) SIT.
B. has a large evidence base for use in children.
C. works by induction of local (mucosal) tolerance.
D. has been show to induce antigen-specific regulatory T cells.

Question 3. Venom immunotherapy (VIT) —

A. abolishes the risk of anaphylaxis to subsequent stings.
B. provides protection against large local reactions.
C. offers protection once the maintenance dose is reached.
D. needs to be continued indefinitely in most patients.

Question 4. Recombinant allergens —

A. are more effective than conventional extracts in clinical trials in patients with allergic rhinitis.
B. are inherently less allergenic than natural allergens.
C. could allow the development of patient-tailored therapy.
D. work better if coupled to CpG oligodeoxynucleotides.

Study questions

Immunomodulator therapy: Monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins

Learning objectives: "Immunomodulator therapy: Monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins"

1. To review potential therapeutic roles of mAbs and fusion proteins in the treatment of autoimmune conditions.
2. To review the mechanism of action for biologic agents commonly used in the treatment of inflammatory arthritis.
3. To recognize potential safety concerns related to mAbs and fusion proteins used in the treatment of autoimmune diseases.

Question 1. TNF inhibitors have been shown to be effective in the treatment of several autoimmune diseases. Which of following statements is true regarding the efficacy of TNF inhibitors?

A. All approved TNF inhibitors are effective in the treatment of rheumatoid arthritis (RA).
B. Etanercept is effective in the treatment of inflammatory bowel disease (eg, Crohn disease).
C. TNF inhibitors are effective in the treatment of congestive heart failure.
D. TNF inhibitors are effective in the treatment of demyelinating diseases (eg, multiple sclerosis).

Question 2. Biologic agents have significantly improved the clinical outcomes of many autoimmune diseases. However, they are also associated with potentially serious adverse events. Which of the following statements on safety considerations of biologic agents is true?

A. The risk of infection does not increase when TNF inhibitors are combined with other biologic agents.
B. TNF inhibitors have been associated with increased risk of tuberculosis.
C. No cases of progressive multifocal leukoencephalopathy have been reported among patients treated with rituximab.
D. Rituximab is safe to use in patients with hepatitis B.

Question 3. Autoreactive T cells, especially $CD4^+$ T_H1 T cells, serve a key role in orchestrating the immune-driven inflammatory responses in autoimmune diseases. Which of the following statements on T-cell activation is true?

A. The binding of specific antigen-associated MHC class II molecules to the T-cell receptor is sufficient to activate $CD4^+$ T cells.
B. The binding of CD28 to CD80/CD86 results in T-cell inhibition and anergy.
C. Cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) binds to CD80/CD86 with higher affinity than CD28 and inhibits T-cell costimulation.
D. The binding of lymphocyte function–associated antigen 3 to $CD2^+$ T cells activates memory T cells.

Question 4. Rituximab therapy has been approved for the treatment of non-Hodgkin lymphoma and RA. Which of the following statements on the potential mechanism of action is true?

A. Rituximab binds to CD20, which is present in both mature B cells and plasma cells.
B. Rituximab can be used alone or in combination with other disease-modifying antirheumatic drugs in the treatment of RA.
C. Seropositivity for rheumatoid factor does not appear to affect the efficacy of rituximab among patients with RA.
D. B-cell depletion after a course of rituximab rarely lasts longer than 3 months.

Study questions

Transplantation immunology: Solid organ and bone marrow

Learning objectives: "Transplantation immunology: Solid organ and bone marrow"

1. To recognize the central role of donor-recipient HLA matching in transplant outcomes.
2. To know the most common diseases that benefit from hematopoietic stem cell transplantation (HSCT).

Question 1. Disparity of the HLA proteins between a transplant donor and the recipient results in —

A. immune tolerance.
B. immune activation.
C. transplant engraftment.
D. no immune effect.

Question 2. In solid-organ transplantation a characteristic of hyperacute rejection is —

A. that it usually occurs within 48 hours of transplantation.
B. that it involves the new development of anti-HLA antibodies.
C. that it is mostly mediated by T cells.
D. that treatment based on steroids is usually successful.

Question 3. Low risk of graft-versus-host disease (GVHD) in HSCT can be predicted when the graft is —

A. a cord blood unit with only 1 of 6 HLA antigens matched with the recipient.
B. bone marrow from an HLA-haploidentical related donor that has not been T-cell depleted.
C. non–T cell–depleted bone marrow from an unrelated donor with a match of 4 of 6 HLA antigens.
D. non–T cell–depleted bone marrow from an HLA-matched related donor.

Question 4. HSCT is indicated in which one of the following primary immunodeficiencies?

A. X-linked agammaglobulinemia
B. C2 complement deficiency
C. severe combined immunodeficiency
D. partial DiGeorge syndrome

Study questions

Embryonic and adult stem cell therapy

Learning objectives: "Embryonic and adult stem cell therapy"

1. To develop a basic understanding of the processes involved in stem cell development.
2. To understand the complexity of and recent advances in stem cell programming.
3. To appreciate the therapeutic possibilities and problems associated with the transplantation of manipulated stem cells.
4. To appreciate the ethical and political debate surrounding the use of human stem cells.

Question 1. Allogeneic hematopoietic stem cell transplantation is often used for the treatment of acute leukemia. The most desirable source of donor cells is from —

A. a parent.
B. an HLA-matched sibling.
C. HLA-matched umbilical cord blood.
D. an HLA-matched unrelated donor.

Question 2. When and why would one use donor lymphocyte infusion during the course of allogeneic transplantation for malignant hematopoietic disorders?

A. 1 week before to facilitate engraftment
B. 1 month after to consolidate engraftment
C. At the time of relapse to obtain remission
D. Never, because it has too many side effects

Question 3. Haploidentical hematopoietic stem cell transplantation is made possible by what manipulation of the donor cells?

A. T-cell depletion
B. B-cell depletion
C. natural killer cell depletion
D. That procedure is currently not possible in human subjects.

Question 4. Human embryonic stem cells can be derived from which of the following sources?

A. aborted fetus
B. hematopoietic stem cells
C. living embryos *in utero*
D. unused embryos made by means of *in vitro* fertilization for infertility problems

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Answers to Study questions

Chapter 1: Overview of the immune response

1. Answer: B

Explanation: In virus-infected cells, some viral proteins are degraded in the cellular proteasome, the major cellular protein degrading organelle. TAP-1 and TAP-2 are proteins that participate in the transport of peptide fragments from the proteasome into the endoplasmic reticulum where they are loaded into newly synthesized class I MHC molecules. Without TAP-1 or TAP-2, viral peptides are not loaded into class I molecules, and recognition by $CD8^+$ T cells does not occur. HLA-DM is required for peptide loading into class II MHC molecules, permitting recognition by $CD4^+$ T cells. B-lymphocytes must traffic through the germinal center in order to undergo somatic mutation and differentiation to high affinity antibody producing cells. Trafficking through the germinal center is not required for $CD8^+$ T cells development or differentiation. Some viruses down-regulate (extinguish) expression of the class I proteins of the cell they have infected. This is a strategy for avoiding the CD8 host immune response. Sustained class I molecule expression is necessary for recognition by $CD8^+$ T cells.

2. Answer: C

Explanation: Loading of peptide fragment from exogenous antigens into class II HLA molecules occurs when the acidified endosome fuses with the class II loading compartment. This fusion results in the proteolytic degradation of the invariant chain that then allows peptides to associate with the class II molecules. The invariant chain is not associated with peptide loading into class I HLA molecules. The invariant chain associates with the newly synthesized class II HLA molecules in the endoplasmic reticulum. The invariant chain is designated as 'invariant' because is shows very low sequence variability in different individuals in the population.

3. Answer: D

Explanation: β_2-Microglobulin associates with class I MHC molecules and several other molecules with class I–like structures. The MHC molecules that are the targets of T_H cells ($CD4^+$ T cells) are class II MHC molecules. These do not contain β_2-microglobulin. The CD3 complex, rearranged α and β chains of the T-cell receptor, and the CD4 molecule are all important components for recognition of peptide antigens associated with class II MHC molecules.

4. Answer: C

Explanation: TLRs are found on many somatic cells, particularly ones that are involved in early contact with microbes that invade the body through skin and mucosal tissues. The *intracellular* domains of TLRs are homologous to the corresponding domains of the IL-1 receptor. Most TLRs are cell-surface proteins, but TLR3 and TLR9 are intracellular proteins that interact with their ligands during the intracellular part of their lifecycle. TLR4 is activated by LPS. TLR9, in contrast, is activated by bacterial CpG DNA.

Chapter 2: Innate immunity

1. Answer: C

Explanation: Because TLRs are highly expressed on dendritic cells but not on T cells, the goal of TLR-based therapies in patients with allergy and asthma is to activate dendritic cells to produce a cytokine milieu (eg, IL-12 and interferons) that favors inhibition of the T_H2 immune response.

2. Answer: A

Explanation: IRAK4 deficiency is a novel primary immunodeficiency specifically affecting TLR function, which is a component of the innate immune system. IRAK4 is involved in downstream signaling from most TLRs.

3. Answer: D

Explanation: Flagellin is the ligand for TLR5.

4. Answer: B

Explanation: The NLRP3 (NALP3) inflammasome is involved in mediating the adjuvant effects of alum. This adjuvancy can occur directly through the triggering of the NALP3 inflammasome by alum crystals or indirectly through release of the endogenous danger signal uric acid, which subsequently activates NLRP3.

Chapter 3: Adaptive immunity

1. Answer: C

Explanation: T-cell receptor activation leads to release of intracellular calcium stores, as well as influx of extracellular calcium. Mature T cells express either CD4 or CD8 but not both. CD8 serves as a coreceptor for MHC class I and not MHC class II molecules. The newborn screening for severe combined immunodeficiency is performed by means of PCR quantitation of T-cell receptor excision circles on blood spots.

2. Answer: D

Explanation: Natural killer T cells express markers of both T ($\alpha\beta$) and natural killer (CD56) cells. $CD25^+$ regulatory T cells express forkhead box protein 3. RORγt is present in T_H17 cells, which arise under the influence of IL-6 and TGF-β. Target cell killing by cytolytic T lymphocytes is complement independent and involves perforin, granzymes, and Fas-mediated apoptotic mechanisms.

3. Answer: D

Explanation: The complete pre-B cell receptor is made up of the IgM heavy chain, the surrogate light chain, and the Ig-α and Ig-β signal-transducing molecules. The κ and λ light chains and IgD heavy chains are contained in mature B-cell immunoglobulin receptors. CD20 is a B-cell marker that is not a component of the immunoglobulin receptor.

4. Answer: C

Explanation: Immunoglobulin class-switching and somatic hypermutation are the critical processes of antigen-dependent B-cell development that take place in germinal centers. Immunoglobulin gene rearrangement occurs mainly during B-cell development in the bone marrow, IgD is expressed on the surfaces of mature IgM-expressing B cells in the spleen and in the circulation, and large-scale antibody production by plasma cells occurs mainly in the spleen, bone marrow, and mucosal sites.

Chapter 4: Structure and function of immunoglobulins

1. Answer: C

Explanation: Activation-induced cytidine deaminase deaminates cytosine to produce uracil, which in turn can be removed from DNA through the action of uracil DNA glycosylase to permit either double-stranded DNA breaks that permit class-switch recombination or substitution of nucleotide sequence to advance somatic hypermutation. κ Light chains can be replaced by λ light chains; hence although the repertoire is restricted in their absence, rearrangement can still occur in the heavy chain and λ locus to permit immunoglobulin formation. RAG1 and RAG2 directly catalyze V(D)J recombination. In their absence VDJ recombination does not occur. The result is a complete deficiency of B and T cells. Surrogate light chain plays a key role in checking the function of new heavy chains after they have rearranged. In the absence of surrogate light chain, B-cell development is blocked at the pre–B-cell stage, creating agammaglobulinemia. However, this occurs after heavy chain rearrangement. TdT adds nucleotides at random to rearranging gene segments, but it is not necessary for the rearrangement process itself. Indeed, fetal mice lack TdT expression entirely.

2. Answer: D

Explanation: Of the isotypes, IgM is the most potent at activating complement, followed by IgG. Within IgG subclasses, IgG2 and IgG4 are very ineffective at activating complement, with considerable activity by IgG1 and IgG3.

3. Answer: A

Explanation: Effector cells differ in their expression of Fcε receptors, and IgA receptors are not present on mast cells.

4. Answer: C

Explanation: Proper glycosylation of immunoglobulin is very important to immunoglobulin function, metabolism, and half-life. Aberrant glycosylation can result in altered clearance and/or be recognized as foreign by the immune system and lead to autoimmune manifestations.

Chapter 5: Immunologic messenger molecules: Cytokines, interferons, and chemokines

1. Answer: A

Explanation: IL-6 signals through a ligand-binding IL-6 receptor α chain (CD126) and the signal-transducing component glycoprotein 130 (CD130). CD130 is the common signal transducer for several cytokines in the IL-6 family and is ubiquitously expressed.

2. Answer: C

Explanation: As a unique regulator of T_H17 development, retinoic acid receptor–related orphan receptor γt through stimulation with IL-6 acting in the additional presence of TGF-β is responsible for differentiation of T_H17 cells.

3. Answer: B

Explanation: Unlike many cytokines that use the shared γ chain, TSLP receptor is a heterodimer composed of a unique TSLP-specific receptor and the IL-7 receptor α chain (CD127).

4. Answer: D

Explanation: Expression of CCL17, which can be induced by IL-4 and IL-13, promotes T_H2 cell development.

Chapter 6: IgE, mast cells, basophils, and eosinophils

1. Answer: D

Explanation: The tetrameric form of the FcεRI receptor ($\alpha\beta\gamma2$) is present on mast cells and basophils, whereas the trimeric form ($\alpha\gamma2$), lacking the β chain, is present on antigen-presenting cells. The β chain stabilizes the receptor and amplifies signaling. The α subunit of FcεRI binds the c3 domain of the Fc region of IgE, the same domain recognized by omalizumab.

2. Answer: D

Explanation: Unlike mast cells, prostaglandin D_2 is not produced in significant quantities by basophils. Production of GM-CSF, IL-4, and granzyme B by basophils has been reported.

3. Answer: B

Explanation: Peripheral eosinophilia is present in hypoadrenalism (Addison disease); some primary immunodeficiency diseases, including Omenn syndrome; and Kimura disease. Eosinopenia is common in the setting of acute bacterial or viral infections, such as sepsis.

4. Answer: C

Explanation: Anaphylaxis to parenteral agents is usually associated with increased serum tryptase levels, whereas anaphylaxis to oral agents frequently is not associated with increased serum tryptase levels. Baseline serum tryptase levels are composed primarily of protryptases, whereas mature β-tryptase is the form stored in secretory granules and secreted after mast cell activation. Tryptase is stabilized in the secretory granules by heparin.

Chapter 7: Genetics of allergic disease

1. Answer: B

Explanation: The most efficient approach to studying whether genetic variation affecting the expression level of a candidate gene or function of the encoded protein alters susceptibility to allergic disease would be to use a panel of genetic variations across the gene selected on the basis of linkage disequilibrium patterns to tag all common variations in the gene region to genotype a case-control cohort. Genome-wide association study approaches, or genome-wide positional cloning in families, are hypothesis-independent approaches in which the entire genome is assessed for gene regions/genes that are associated/linked to the phenotype being assessed. Hence these are best suited to finding novel genes whose encoded proteins by definition must play important roles in disease pathophysiology, or genetic variation affecting their expression, function, or both would not be associated with disease.

2. Answer: A

Explanation: *CHRNA3* is a candidate gene identified for lung cancer, chronic obstructive pulmonary disease, and smoking behavior. Genetic variation in the promoter and coding region of the gene encoding IL-13 has been shown to be associated with atopy and asthma phenotypes in a number of case-control candidate gene studies. *SH2B3* was identified as a gene associated with blood eosinophil levels in a genome-wide association approach but was not associated with asthma; rather, it was strongly associated with risk of myocardial infarction. The genetic region around the *ORMDL3* gene was the first locus identified for asthma susceptibility by using a genome-wide association approach.

3. Answer: B

Explanation: *ADRB2* polymorphisms have been associated with bronchodilator responses in asthma. Polymorphisms in the genes encoding IL-5 and CD14 have been associated with asthma and atopy phenotypes in case-control studies. The cytochrome P450 gene *CYP1A1* might potentially modify responses to therapeutics metabolized by this isoenzyme but has not been identified as playing an important role in modulating responses to asthma therapy.

4. Answer: B

Explanation: *IL13* can regulate both atopic inflammation through its effect on B-cell IgE production and tissue responses through effects on structural cells, such as promoting mucus hypersecretion by airway epithelial cells and collagen production by airway fibroblasts. *FLG* polymorphisms do modulate epidermal barrier function and are the strongest genetic risk factor for atopic dermatitis, although they are not expressed in the lung, and association with asthma can occur through increased allergen sensitization as a result of a poor epidermal barrier. *ORMDL3*, although of unknown function, is expressed in epithelial cells and might be important in epithelial barrier function. *PHF11* is a candidate gene encoding a transcription factor that is likely to be involved with the atopic immune response.

Chapter 8: Asthma: Clinical expression and molecular mechanisms

1. Answer: C

Explanation: Assessment of the risk domain involves an evaluation of the following over time: rates of exacerbations, loss of lung function, and side effects from medications. In contrast, an assessment of pulmonary function, symptoms, and albuterol use are factors that one evaluates in assessing current impairment.

2. Answer: B
Explanation: In both children and adults, the virus most frequently found to be associated with asthma exacerbations is rhinovirus.

3. Answer: C
Explanation: The only pain medication not in the class of COX pathway inhibitors is acetaminophen.

4. Answer: D
Explanation: The use of long-acting β-agonists as monotherapy has been demonstrated in a number of studies to increase risk for loss of control, exacerbations, and perhaps mortality from asthma.

Chapter 9: Rhinitis and sinusitis

1. Answer: C
Explanation: The dust mite antigen has proteolytic activity that cleaves tight junctions in the airway epithelium. Activated epithelial cells produce thymic stromal lymphopoietin, a protein that interacts with interepithelial and subepithelial dendritic cells to skew T-cell development toward T_H2 allergic sensitization. The house dust mite allergen Der p 2 has a unique property, namely that it mimics MD-2, the LPS-binding component of the TLR4 signaling complex, and facilitates TLR4 signaling and airway T_H2-type inflammation. In the nose allergens are processed by antigen-presenting cells (dendritic cells expressing CD1a and CD11c and macrophages) in the nasal epithelial mucosa, with subsequent presentation of allergenic peptides by MHC class II molecules to T-cell receptors on resting $CD4^+$ T lymphocytes in regional lymph nodes.

2. Answer: D
Explanation: Nonallergic rhinitis often occurs without eosinophilia. The terms *nonallergic rhinitis without eosinophilia* and *idiopathic rhinitis* are used interchangeably. Irritant-induced rhinitis, cold-induced rhinitis, and vasomotor rhinitis are all considered subsets of this condition. *Vasomotor rhinitis* is sometimes used synonymously with *nonallergic rhinitis without eosinophilia*, but it sometimes can more specifically connote nasal symptoms that occur in response to environmental conditions, such as changes in temperature or relative humidity, odors (eg, perfume or cleaning materials), passive tobacco smoke, alcohol, sexual arousal, and emotional factors. Nonallergic rhinitis with aspirin sensitivity is usually associated with marked tissue eosinophilia (ie, nonallergic rhinitis with eosinophilia).

3. Answer: D
Explanation: T_H2-type immune hyperresponsiveness in sinus tissue is an important feature of CRS without distinction for the presence of nasal polyps. Patients with CRS typically have fungi, such as *Alternaria* species, in the mucus secretions and *in vitro* hyperresponsiveness to *Alternaria* species, with production of IL-5 and IL-13. Local production of IgE against staphylococcal enterotoxins (superantigens) has been found in homogenates of nasal polyps and is regarded as specific for CRS with nasal polyps. Production of bacterial biofilm on sinus mucosal tissue has been demonstrated in several studies without distinction for the presence of nasal polyps. Glandular hyperplasia is a feature of CRS without nasal polyps.

4. Answer: D
Explanation: Opacified sinus cavities might contain inspissated mucus that produces an inhomogeneous hyperdense pattern on sinus CT scanning. Hyperdensities suggest the presence of allergic mucin. They are a classic feature of allergic fungal rhinosinusitis (in which case the allergic mucin also contains fungal hyphae), but they can be seen in both patients with CRS without nasal polyps and patients with CRS with nasal polyps.

Chapter 10: Food allergy

1. Answer: B
Explanation: Studies of a referral population in the United States indicated that only 11% resolved egg and 19% resolved milk allergy by age 4 years; however, about 80% resolved these allergies by age 16 years. Allergy to fish/shellfish is reported more often in adults compared with children. Although several studies showed an increase, approximately doubling, in peanut allergy among children in the past 10 to 15 years, there are no data to indicate a general doubling of food allergy. Peanut allergy resolves for about 20% of young children by school age.

2. Answer: C
Explanation: The symptom complex of having mild oral pruritis to raw apple but tolerating cooked apple is consistent with a diagnosis of oral allergy syndrome/pollen-food syndrome, in which initial sensitization to pollen results in reactions to homologous proteins in a raw food. Here there was likely sensitization to birch pollen protein in this "atopic" man; the birch pollen protein Bet v 1 is homologous to Mal d 1 in apple. Lipid transfer protein is more stable to heat and less likely to result in mild symptoms. Although he might have a positive skin test result to commercial apple extract, the birch-related protein is less stable, and testing with fresh raw juice of an apple is more likely to show a positive result in this scenario. Although heating apple reduces the Mal d 1 protein level and

generally results in a form of the food that does not trigger symptoms in persons with birch pollen–related allergy, this is not a Maillard reaction. High heat resulting in a Maillard reaction, a chemical reaction between an amino acid and a reducing sugar, has been proposed to increase the allergenicity of some foods (roasted peanut) by increasing the stability of allergens.

3. Answer: B

Explanation: Food allergy requires an adverse immune response. This description fits rice-induced enterocolitis syndrome. This is a non–IgE-mediated food allergy, and results of skin testing are expected to be negative. Choice A describes auriculotemporal syndrome, which is a neurologic response to the spicy or tart triggers for the child described. Choice C describes lactose intolerance, which is dose dependent. Choice D most likely describes an episode of scombroid fish poisoning.

4. Answer: C

Explanation: Increasingly larger food-specific skin test results and increasingly higher food-specific serum IgE levels are associated with higher risks of clinical allergy. However, false-negative test results are possible, and the history is important in assessing the prior probability of allergy. This child had repeated allergic responses to egg, including a significant reaction 6 months before the most recent serum testing that was "undetectable" by using this assay. Therefore performing a food challenge next might be a poor choice given a relatively recent reaction. Seeing a decrease in serum IgE levels to egg is an encouraging indication that the egg allergy might be resolving. However, some egg-reactive children (approximately 20%) might have negative test results on the serum test and still react clinically on challenge. In this setting a skin test might be helpful as additional information before deciding on an oral food challenge.

Chapter 11: Drug allergy

1. Answer: c

Explanation: A detailed history is essential to the management of patients with drug allergy. Skin testing and *in vitro* testing might be helpful in a limited number of drug-induced allergic reactions. Some, but not all, drug-induced allergic reactions can be classified by using the Gell and Coombs system.

2. Answer: B

Explanation: Skin testing has a very high negative predictive value in patients with penicillin allergy, and resensitization to penicillin is rare, especially with oral courses of penicillin. Although the history is suggestive, it is usually not adequate for confirming or negating a history of penicillin allergy. Cross-reactivity between cephalosporins and penicillin is low.

3. Answer: C

Explanation: Induction of drug tolerance procedures can involve IgE-mediated and non–IgE-mediated processes and cause temporary tolerance to the drug. These procedures can take days or weeks to complete for non–IgE-mediated reactions and are generally contraindicated in patients with life-threatening cutaneous drug reactions.

4. Answer: D

Explanation: This patient's history is consistent with anaphylaxis. Anaphylactic reactions to nonsteroidal anti-inflammatory drugs are drug-specific reactions, and therefore he should tolerate aspirin.

Chapter 12: Allergic skin diseases

1. Answer: D

Explanation: It is clear that sera from patients with chronic urticaria have a biologic activity that can activate donor mast cells, and most of this activity is found in the IgG fraction. However, there is no gold standard for measuring these antibodies, and the value of this finding for predicting prognosis or decisions regarding management is unclear.

2. Answer: B

Explanation: Although there are many case reports and case series suggesting efficacy of a variety of immunomodulatory drugs, only cyclosporin A has been studied in double-blind placebo-controlled trials.

3. Answer: B

Explanation: Answer A is false. Irritant contact dermatitis commonly presents as a localized dermatitis without vesicles more common in the palms and ventral surfaces of the hands and rarely extending beyond the area of contact. Answer B is true. Answer C is false. Atopic dermatitis is an important factor in susceptibility to persistent postoccupational dermatitis. Answer D is false. "Unscented" might erroneously suggest absence of fragrance when, in fact, a masking fragrance is present. "Fragrance-free" products are typically free of classic fragrance ingredients and are generally acceptable for the patient with allergic contact dermatitis.

4. Answer: C

Explanation: Answer A is false. Patch test results are affected by oral corticosteroids, cancer chemotherapy, and immunosuppressive drugs but not by antihistamines. Answer B is false. Allergens not found on commercially available screening series in the United States

frequently produce relevant reactions, and personal products are a useful supplement, especially in facial or periorbital dermatitis. Answer C is true. Answer D is false. Glycerol thioglycolate is the active ingredient in permanent wave solution. Unlike PPD, the thioglycolates can remain allergenic in the hair long after it has been rinsed out. Answer E is false. Medicaments containing lanolin are more sensitizing than lanolin-containing cosmetics. It is a weak sensitizer in normal skin but a stronger sensitizer in damaged skin. Thus patients with chronic dermatitis, especially stasis dermatitis, are at higher risk of lanolin sensitivity.

Chapter 13: Environmental and occupational allergies

1. Answer: A

Explanation: This is important not only because control of indoor humidity is important in reducing mite exposure but also because the presence of house dust mites is less in arid climates or at higher altitudes.

2. Answer: B

Explanation: Because cat allergens are widespread throughout the home, it is important to end the generation of the contamination. Even so, it takes several weeks to eradicate the allergen from the home.

3. Answer: C

Explanation: The prevalence of allergy and asthma is higher in subjects who live near highly traveled roads.

4. Answer: C

Explanation: Onset of symptoms can be delayed for several hours after exposure. Bronchial provocation tests are appropriate only in research centers. Many low-molecular-weight agents do not elicit an IgE response.

Chapter 14: Anaphylaxis

1. Answer: D

Explanation: Accurate community-based population estimates are difficult to obtain because of underdiagnosis, underreporting, and miscoding; however, lifelong prevalence of anaphylaxis from all triggers in the general population is estimated at 0.05% to 2%.

2. Answer: B

Explanation: In patients with newly diagnosed idiopathic anaphylaxis, the serum total tryptase level should be measured. This test reflects the increased burden of mast cells in all forms of mastocytosis and is therefore an important screening test for mastocytosis. If the total tryptase level is greater than 11.4 ng/mL, the new upper limit of normal, meticulous examination for cutaneous mastocytosis is indicated, and if the level is greater than 20 ng/mL, a bone marrow biopsy is indicated, even if cutaneous manifestations are absent.

3. Answer: C

Explanation: A 3- to 5-year course of subcutaneous injections of the relevant standardized insect venom or venoms reduces the risk of anaphylaxis from a subsequent sting, based on randomized, double-blind, placebo-controlled trials. In children, a 98% protection rate can be achieved.

4. Answer: A

Explanation: Epinephrine's multiple pharmacologic effects in many organ systems are useful in anaphylaxis; however, its α_1-adrenergic vasoconstrictor effects in the small arterioles and precapillary sphincters are unique among medications used in the prehospital treatment of anaphylaxis. By decreasing mucosal edema, it prevents and relieves upper airway obstruction. It also prevents and relieves hypotension and shock. When used in first-aid treatment, prompt injection is important. The epinephrine doses currently available in autoinjectors for outpatient use are too low for use in cardiopulmonary resuscitation.

Chapter 15: Primary immunodeficiencies

1. Answer: A

Explanation: SCID includes a heterogeneous group of disorders characterized by severe defects in T-cell development. Some (but not all) forms of SCID also have defects in B-cell development, natural killer cell development, or both, whereas impaired myeloid differentiation is restricted to a few rare forms of SCID. Regardless of the presence or absence of B cells, patients with SCID have a severe defect in antibody production, reflecting a lack of T lymphocytes.

2. Answer: B

Explanation: XLA and all other forms of congenital agammaglobulinemia are caused by genetic defects that affect signaling through the pre–B-cell receptor in the bone marrow. Therefore patients with congenital agammaglobulinemia typically lack circulating mature B cells.

3. Answer: C

Explanation: Neutrophils are important in the defense against bacteria and fungi. Patients with neutrophil defects often present with severe infections, among which purulent lymphadenitis is common.

4. Answer: C

Explanation: It is important that patients with antibody deficiency receive appropriate replacement treatment. This is usually achieved with 400 mg/kg/mo intravenous immunoglobulins or with weekly injections of subcutaneous immunoglobulins at a dose of 100 mg/kg/wk. This regimen applies to patients of any age.

Chapter 16: Secondary immunodeficiencies, including HIV infection

1. Answer: B

Explanation: From the 4 options, option B is the most likely answer. HIV infection can be considered as a cause of immunodeficiency at any age. Options A, C, and D are primary immunodeficiencies that present clinically in infancy or early childhood.

2. Answer: A

Explanation: Secondary immunodeficiencies have a variable clinical presentation. T-cell, B-cell, or innate immunity components, including phagocyte function, might or might not be affected. Management should include immunoglobulin supplementation only if humoral responses are not restored despite optimal control of the primary disease.

3. Answer: C

Explanation: Calcineurin inhibitors suppress IL-2–induced T-cell activation and proliferation, by binding immunophilin proteins in the cytoplasm. They do not affect the oxidative burst, complement activity, or calcium receptors.

4. Answer: C

Explanation: HIV infects its target cell by using the CD4 molecule in the cell membrane and the chemokine receptors CCR5 and CXCR4. Cells presenting with CCR5 deletions are not permissive for HIV infection. AIDS develops when there is severe depletion of T cells. Although an adenovirus-based anti-HIV vaccine has been shown to elicit specific immunologic responses, it did not demonstrate protection in a large trial of 3,000 subjects.

Chapter 17: Immunologic rheumatic disorders

1. Answer: B

Explanation: Anti-CCP antibodies can be present years before the onset of clinical disease. Patients with RA who have anti-CCP antibodies tend to have more aggressive erosive disease. Anti-CCP antibodies are more specific but less sensitive than rheumatoid factor for diagnosing RA.

2. Answer: A

Explanation: DMARDs should be initiated within 3 months of diagnosis, but traditional DMARDs are currently used in most cases before initiating biologic DMARDs. Nonsteroidal anti-inflammatory drugs have been shown to increase cardiovascular risk in patients with RA, but DMARDs have not. Adding a biologic DMARD to a traditional DMARD generally increases efficacy.

3. Answer: B

Explanation: Many immunologic based rheumatic diseases such as SLE, RA and SS are more common in women than men but the seronegative spondyloarthropathies are a prominent exception. The biologic basis for these findings is unknown.

4. Answer: D

Explanation: Patients with a low titer of ANA are less likely to have SLE than those with high-titer ANA, and the absence of typical clinical features of SLE makes the diagnosis even more unlikely. ANA-negative SLE is rare as long as the testing is done by means of indirect immunofluorescence. Anti-Ro (SSA) antibody is present in about 25% of patients with SLE, although it is seen in up to 75% of patients with Sjögren syndrome.

Chapter 18: Vasculitis

1. Answer: C

Explanation: The diagnosis of Wegener granulomatosis is usually made by means of biopsy, with nonrenal tissues demonstrating the presence of granulomatous inflammation and necrosis with necrotizing or granulomatous vasculitis. Surgically obtained biopsy specimens of abnormal pulmonary parenchyma demonstrate diagnostic changes in 91% of cases, which provides the highest diagnostic yield. Biopsy of the upper airways is less invasive but demonstrates diagnostic features only 21% of the time. The gastrointestinal tract is involved in less than 5% of patients with Wegener granulomatosis, with biopsy specimens of mucosa rarely revealing vasculitis.

2. Answer: A

Explanation: Vasculitis of small- to medium-sized vessels is a prominent feature of Churg-Strauss syndrome and is typically accompanied by prior or concurrent allergic rhinitis, asthma, and eosinophilia. The organ site most commonly affected by vasculitis is the peripheral nerve, which is involved in 70% to 80% of patients and manifests as a mononeuritis multiplex. Glomerulonephritis can lead to renal failure but only develops in 10% to 40% of patients. Gastrointestinal involvement occurs in 30% to 50% of patients and can be associated with mortality. The highest rate of mortality is seen with cardiac involvement, which occurs in 10% to 40% of patients.

3. Answer: B

Explanation: Two main antigen associations are seen in conjunction with ANCAs in patients with vasculitis. ANCAs directed against the neutrophil serine protease proteinase 3, which causes a cytoplasmic immunofluorescence pattern (cANCA) on ethanol-fixed neutrophils, are seen in 75% to 90% of patients with active generalized Wegener granulomatosis. ANCAs directed against the neutrophil enzyme myeloperoxidase that produce a perinuclear pattern (pANCA) are seen in 5% to 20% of patients with Wegener granulomatosis and are more common in microscopic polyangiitis. ANCAs directed against human neutrophil elastase can be seen in patients with cocaine-induced sinonasal destructive disease, which can be a mimic of Wegener granulomatosis. Bactericidal permeability–increasing protein is a target antigen for pANCA that has been described in patients with cystic fibrosis and ulcerative colitis.

4. Answer: D

Explanation: GCA is the most common form of systemic vasculitis that affects human subjects. GCA can be thought of as having 4 phenotypes that include cranial disease, PMR, systemic inflammatory disease, and large-vessel involvement. Large-vessel involvement of the aorta or its primary branches occurs in 27% of cases. The most dreaded complication of cranial disease is vision loss, which can occur in 14% of patients and is caused by optic nerve ischemia from arteritis involving vessels of the ocular circulation. A marker of systemic inflammation is an increased erythrocyte sedimentation rate, which occurs in more than 80% of patients. PMR can occur in conjunction with other features of GCA or in isolation. Although cranial or large-vessel GCA should be treated with 40 to 60 mg/d prednisone, isolated PMR can be treated with 10 to 20 mg/d prednisone.

Chapter 19: Immunologic endocrine disorders

1. Answer: B

Explanation: DR3/4 is the highest-risk genotype for type 1 diabetes. Insulin autoantibodies are remarkably inversely related to age of onset of type 1 diabetes, with levels being highest in the youngest children presenting with diabetes. Transglutaminase autoantibodies occur in approximately 10% of patients with type 1 diabetes, and half of these patients have high levels associated with a positive intestinal biopsy result for celiac disease.

2. Answer: A

Explanation: IPEX syndrome results from mutation of the forkhead box protein 3 gene *(FOXP3)*, which controls regulatory T cells and is X-linked recessive. APS-1 results from mutation of the autoimmune regulator gene *(AIRE)*, which controls peripheral antigen expression in the thymus and is almost always autosomal recessive (1 autosomal dominant family has been described). Both disorders are rare.

3. Answer: D

Explanation: We measure transglutaminase and 21-hydroxylase autoantibodies to screen for Addison disease and celiac disease. A major caveat with testing for insulin autoantibodies to aid in the diagnosis of type 1A diabetes (immune mediated) is that essentially everyone treated with subcutaneous insulin for more than 1 to 2 weeks had insulin antibodies that cannot be distinguished from the autoantibodies.

4. Answer: D

Explanation: APS-1 is a monogenic disorder, whereas APS-2 is a polygenic disorder, even though Addison disease occurs in both disorders. Mucocutaneous candidiasis is characteristic of APS-1, as is hypoparathyroidism, both of which rarely occur in patients with APS-2.

Chapter 20: Diagnostic testing and interpretation of tests for autoimmunity

1. Answer: C

Explanation: MPO is a serine protease that constitutes approximately 5% of the total protein content of a neutrophil. The autoantibodies directed against MPO are more often seen in patients with Churg-Strauss syndrome. The combination of the perinuclear antineutrophil cytoplasmic antibody pattern and MPO or MPO–antineutrophil cytoplasmic antibody is strongly associated with Churg-Strauss syndrome.

2. Answer: B

Explanation: ANA is seen in more than 90% of patients with SLE. However, it is not specific for SLE. ANA can also be seen in a variety of other autoimmune diseases, such as scleroderma, mixed connective tissue disease, polymyositis/dermatomyositis, and rheumatoid arthritis. Once an increased ANA level is documented, it cannot be used to measure disease activity.

3. Answer C

Explanation: Anti-Smith antibodies are highly specific for SLE (approximately 55% to 100%), but they are not very sensitive. These antibodies can remain positive when titers of anti-dsDNA antibodies are within a normal range and clinical activity of SLE has decreased. Therefore the anti-Smith titers can be useful diagnostically when anti-dsDNA antibodies are not detectable.

4. Answer: B

Explanation: In patients with rheumatoid arthritis, serum complement levels are generally normal or even increased during active disease because this is a reflection of the acute-phase response. However, in patients with rheumatoid vasculitis, hypocomplementemia is common. The combination of increased rheumatoid factor and decreased C3 levels favors rheumatoid vasculitis. There is a high prevalence of IgA immune complex deposits plus C3 deposits in the affected skin of patients with rheumatoid vasculitis.

Chapter 21: Pulmonary disorders, including vocal cord dysfunction

1. Answer: B

Explanation: In pulmonary TB lesions, there are reduced numbers of cytolytic T cells expressing low levels of perforin and granulysin. In addition, there are increased numbers of CD4+CD25+ Tregs, suggesting that an imbalance in the proportion of effector T cells to Treg cells may contribute to establishment of granulomas in TB infection.

2. Answer: B

Explanation: Approximately one third of CSS patients have antineutrophil cytoplasmic antibodies (ANCAs). Myeloperoxidase (MPO) is the antigen against which the antibodies are directed.

3. Answer: B

Explanation: In BAL from normal individuals, the usual cell percentages are 83-88% macrophages; 7-12% lymphs, 1-2% PMNs; rare basophils, eosinophils or ciliated cells. In patients with acute HP lymphocytes represent 40-60% of total cells, usually with a CD8+ predominance. Eosinophils can be significantly increased in diseases such as CSS, APBA and acute eosinophilic pneumonia.

4. Answer: C

Explanation: The clinical criteria for the diagnosis of RADS, as published by Brooks in 1985 include onset of symptoms occurred after a single specific exposure incident, onset of symptoms occurred within 24 hours after exposure and persisted for at least 3 months, methacholine challenge testing was positive, symptoms simulating asthma, and other types of pulmonary disease were ruled out.

Chapter 22: Mucosal immunology, eosinophilic esophagitis, and other intestinal inflammatory diseases

1. Answer: C

Explanation: The mucosal immune system consists of a variety of immune cells that orchestrate a complex series of tightly controlled responses that protect the host from luminal triggers. Mutations in the gene encoding the forkhead box protein 3 regulatory T cell–specific transcription factor lead to a syndrome in which patients have the gastrointestinal manifestations of diarrhea and intestinal inflammation. Defects in other cell types are not of immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome.

2. Answer: C

Explanation: Eosinophilic esophagitis is a clinicopathological disease characterized by upper intestinal symptoms and dense esophageal eosinophilia with normal gastric and duodenal mucosa. Other causes for these findings must be ruled out, especially gastroesophageal reflux disease, a more common condition, for which eosinophilic esophagitis is commonly mistaken. The acronym EE is commonly mistaken for erosive esophagitis, hence the adaptation of EoE for eosinophilic esophagitis in the gastrointestinal specialty.

3. Answer: C

Explanation: Celiac disease is treated with the complete exclusion of gluten from the diet. A body of experience and literature supports the use of dietary modifications/exclusions in the treatment of inflammatory bowel diseases. Current treatments include the use of corticosteroids, aminosalicylates, immunosuppressive agents, and biological agents. Patients with gastroesophageal reflux disease might benefit from limiting certain foods and weight loss, but antacid medications form the primary mode of treatment.

4. Answer: B

Explanation: Defensins are synthesized primarily by Paneth cells and function as one part of the innate immune system. Six different subtypes of these highly charged molecules have been thus far identified.

Chapter 23: Complement disorders and hereditary angioedema

1. Answer: A

Explanation: The mannose-binding lectin pathway is initiated by binding of mannose-binding lectin to a variety of sugars on the surface of microbes.

2. Answer: B

Explanation: Therapy is directed at controlling the disease, improving quality of life, and minimizing side effects to minimize side effects and cost. Often the patients will continue to have some attacks.

3. Answer: C

Explanation: The classical pathway is the usual pathway activated by antibody. IgM and IgG subclasses 1 and 3 are best at activating the classical pathway.

4. Answer: C

Explanation: C3 is a critical opsonin and is particularly important with high-grade pathogens.

Chapter 24: Immune responses to malignancies

1. Answer: D

Explanation: Although human solid tumors can induce apoptosis of $CD8^+$ T cells and release TAs into the circulation, the only evidence that a tumor-specific immune response is made to these TAs comes from the presence in the circulation or lymphoid tissues of effector T cells capable of binding tetramers, which are reagents containing the TA-derived peptide sitting in the groove of MHC molecules.

2. Answer: C

Explanation: In tumor-bearing hosts DCs are found at the tumor site and in tumor-draining lymph nodes. However, these DCs are immature, have low expression levels of MHC molecules, and produce immunosuppressive cytokines, such as IL-10. Tumor-derived factors, including vascular endothelial growth factor, GM-CSF, and IL-10, recruit myeloid-derived suppressor cells from the bone marrow, which migrate to lymph nodes or tumor sites and block DC maturation.

3. Answer: A

Explanation: Regulatory T cells accumulate in the peripheral blood and tumor tissues of patients with cancer and suppress functions of other T cells by secreting the immunosuppressive cytokines IL-10, TGF-β1, or both or by producing cytolysins, granzyme B, and perforin, which mediate death of effector T or B cells. This type of suppression requires cell-to-cell contact.

4. Answer: D

Explanation: Inflammatory infiltrates seen in human solid tumors are chronic in nature. They are characterized by the paucity of natural killer cells and usually contain variable proportions of $CD8^+$ and $CD4^+$ T cells. These infiltrating cells produce the proinflammatory cytokines IL-6, TNF-α, and IL-8, which can promote tumor growth.

Chapter 25: Clinical laboratory assessment of immediate-type hypersensitivity

1. Answer: C

Explanation: IgE's molecular weight is approximately 190,000 d, and it is known to not readily pass the placenta. Thus low levels of IgE are found in cord blood, with final total serum IgE concentrations representing approximately 0.004% of the total immunoglobulin in circulation. Because of the wide overlap in total serum IgE levels between atopic and nonatopic populations, IgE levels in serum are not considered a definitive discriminator for the presence of atopy. Total serum IgE levels are known to be highly age dependent, and thus evaluation of total serum IgE levels should be judged in relation to an age-adjusted mean from a clearly nonatopic population.

2. Answer: B

Explanation: Once a history indicates a high probability of allergic disease, allergen-specific IgE antibody in the skin or blood is measured to confirm sensitization and verify the specificity of the IgE antibody response. The precise method chosen as the primary confirmatory test (serology or puncture or intradermal skin testing) depends on the allergen specificity (eg, suspected food vs Hymenoptera venom sensitivity will require the use of different primary confirmatory tests). If there is a history of an anaphylactic event, a β-tryptase measurement can be useful as a subsequent measurement after IgE antibody testing. α-Tryptase is less useful as an

indicator of an immediate release of mast cell mediators. It is correct that it should be collected between 30 minutes and 4 hours after a systemic allergic reaction. Provocation tests are only done as a last resort because they tend to be risky and difficult to standardize. Allergen-specific IgG antibody measurements are not considered diagnostic for human allergic disease and thus are contraindicated in the diagnostic process.

3. Answer: D

Explanation: A higher allergen-specific IgE concentration, more mature IgE antibody specificity, higher specific IgE/total IgE molar ratio in serum, and higher IgE antibody affinity directed at the specific allergen all contribute to an enhanced translation of an IgE antibody's response into more effective basophil mediator release.

4. Answer: D

Explanation: IgG antibody is generally viewed as a marker of antigen exposure and is not diagnostic of an immediate-type hypersensitivity response. Specific IgG antibody responses are contraindicated in the assessment of food allergy because they are not diagnostic. They are also not useful in the evaluation of rhinitic conditions associated with aeroallergen exposure and the evaluation of latex allergy questions. Precipitating IgG antibody has been used as a diagnostic indicator for the evaluation of patients suspected of hypersensitivity pneumonitis after inhalation of organic dusts (molds: farmer's lung; fecal material dust from bird droppings: pigeon breeder's disease).

Chapter 26: Laboratory evaluation of primary immunodeficiencies

1. Answer: D

Explanation: The clinical symptoms of recurrent sinopulmonary infections and chronic diarrhea point to an antibody deficiency syndrome, which should be initially screened by means of measurement of serum immunoglobulin levels. Lymphocyte immunophenotyping and mitogen proliferation assays are particularly useful in the evaluation of cellular immunodeficiencies, although B-cell immunophenotyping has utility as a secondary test in evaluating humoral immunodeficiencies. DHR is used to evaluate phagocyte defects in oxidative burst, which are primarily seen in chronic granulomatous disorder.

2. Answer: C

Explanation: Toll-like receptor pathway defects, such as IL-1 receptor–associated kinase 4 and MYD88 defects, are associated almost exclusively with pyogenic bacterial infections and poor inflammatory responses. IFN-γ and IL-12 defects result in infections by mycobacterial species, whereas GM-CSF defects are associated with pulmonary alveolar proteinosis.

3. Answer: C

Explanation: The clinical symptoms in this patient are suggestive of a cellular immune defect, most likely severe combined immunodeficiency, and a lymphocyte count would likely demonstrate significant lymphopenia. DHR is directed at evaluating oxidative burst, and results are abnormal in patients with chronic granulomatous disease; the clinical picture is not consistent with this diagnosis. The CH50 assay is focused on classical component complement defects that typically would not present in infancy and usually involve bacterial infections. Immunoglobulin levels would primarily reflect maternal IgG, and defects in antibody production typically present later in infancy and show primarily bacterial infections of the sinopulmonary tract.

4. Answer: A

Explanation: T-cell receptor excision circles are present at high levels in naive $CD45RA^+$ T cells, which are not yet antigen experienced. T-cell receptor diversity is dependent on normal thymic function, and therefore it might be altered in settings of abnormal T-cell development but is not directly linked to CD45RA expression. T-cell functional capacity (cytotoxicity and mitogen proliferation) is also linked to normal T-cell development but cannot be specifically correlated with CD45RA expression.

Chapter 27: Allergen immunotherapy

1. Answer: B

Explanation: Patients with aspirin-exacerbated respiratory disease can be tolerized by repeated administration of aspirin, but this is not SIT. The other indications are appropriate.

2. Answer: D

Explanation: Sublingual immunotherapy uses high doses of allergen (up to 400 times higher than conventional SIT). There is relatively little evidence for its use in children. The exact mechanism is not known, but regulatory T cells have been demonstrated.

3. Answer: C

Explanation: VIT offers protection quite early on, during the build-up phase but certainly by the time the maintenance dose is achieved. Large local reactions are not an indication, and moreover, there is no hard evidence that they are relieved by VIT. Most patients can stop after 3 to 5 years, but a low risk of anaphylaxis remains, although the reactions are likely to be mild.

4. Answer: C

Explanation: Although it is not clear how this would be regarded by the regulatory authorities, using recombinant allergens will definitely allow us to dissect out patients' profiles of IgE response and then put together a treatment cocktail. However, in the medium term, it is more likely they will improve standardization of vaccines. They are as allergenic as natural allergens (unless genetically modified), and there is no evidence that they work better (or worse) if coupled to CpG.

Chapter 28: Immunomodulator therapy: Monoclonal antibodies, fusion proteins, cytokines, and immunoglobulins

1. Answer: A

Explanation: All TNF inhibitors have been shown to improve the signs and symptoms of RA. Although anti-TNF mAbs have been effective in the treatment of Crohn disease, the fusion protein etanercept has not been effective. Despite increased levels of TNF in patients with congestive heart failure and multiple sclerosis, TNF inhibitors have not improved and have sometimes worsened clinical outcomes.

2. Answer: B

Explanation: TNF inhibitors are generally well tolerated but have been associated with an increased risk of infections, including tuberculosis and opportunistic infections. The risk of infection is increased when combined with another biologic agent. Rituximab has been associated with rare but fatal cases of progressive multifocal leukoencephalopathy and reactivation of hepatitis B.

3. Answer: C

Explanation: Productive $CD4^+$ T-cell responses require 2 signals: binding of specific antigen-associated MHC class II molecules to the T-cell receptor complex and a second signal from costimulatory molecules (CD80 and CD86). CD28 and its natural inhibitor, CTLA-4 (CD152), are present on T cells and bind to CD80 and CD86 on antigen-presenting cells. CD28 ligation results in stimulation of T cells, whereas CTLA-4 serves an inhibitory role. CTLA-4, which binds CD80 and CD86 with substantially higher affinity than CD28, inhibits the stimulatory effects of CD28 by competitively binding to CD80 and CD86.

4. Answer: C

Explanation: Rituximab binds to CD20 on the surface of pre-B through activated mature B cells only and can deplete B cells up to 9 months or longer after a single course. Rituximab can be used alone or in combination with disease-modifying antirheumatic drugs and yields better clinical outcomes in patients with RA who are seropositive for rheumatoid factor.

Chapter 29: Transplantation immunology: Solid organ and bone marrow

1. Answer: B

Explanation: When the transplant donor HLA antigens are different from the recipient, the graft is recognized as "nonself" by the immune system, which gets activated and develops an immune response. This response eventually destroys the graft.

2. Answer: A

Explanation: Graft rejection can be classified according to the time it takes to develop. Hyperacute rejections usually occur within 48 hours of transplantation, and the injury is mediated by preformed alloantibodies and complement targeting the vascular endothelium. Treatment is generally unsuccessful.

3. Answer: D

Explanation: The lowest risk of GVHD in patients undergoing HSCT is when the donor and the recipient are HLA-matched siblings. Cord blood transplantation can be performed with an HLA mismatch of up to 4 of 6 antigens. HLA-haploidentical bone marrow transplant results in a high percentage of GVHD if T cells are not depleted from the graft. Although peripherally isolated $CD34^+$ cells have low T-cell concentrations, this small number would produce GVHD if there were no HLA compatibility.

4. Answer: C

Explanation: HSCT is the treatment of choice for patients with severe combined immunodeficiency, who otherwise would succumb early to severe and opportunistic infections. The balance of risk and benefits of HSCT is not favorable for patients with X-linked agammaglobulinemia. Partial DiGeorge syndrome and complement deficiencies might not be corrected by HSCT.

Chapter 30: Embryonic and adult stem cell therapy

1. Answer: B

Explanation: Parents are haploidentical with their children. One fourth of siblings can be HLA genoidentical to the patient. A matched unrelated donor would be HLA phenoidentical. The risk of graft-versus-host disease increases with differences in minor antigens, leading to increased morbidity/mortality in nonrelated recipients.

2. Answer: C

Explanation: Donor lymphocyte infusion is a therapy that might induce or enhance a graft-versus-leukemia effect and thus reinduce the patient into remission.

3. Answer: A

Explanation: Graft-versus-host disease is a consequence of alloreactive T cells.

4. Answer: D

Explanation: Human embryonic stem cells are currently derived from the blastocyst or sometimes earlier stages of unused embryos made by means of *in vitro* fertilization for infertility problems, with the written informed consent of the parents.