CASE 31 Hereditary Angioedema

Regulation of complement activation.

Complement is a system of plasma proteins that participates in a cascade of reactions, generating active components that allow pathogens and immune complexes to be destroyed and eliminated from the body. Complement is part of the innate immune defenses of the body and is also activated via the antibodies produced in an adaptive immune response. Complement activation is generally confined to the surface of pathogens or circulating complexes of antibody bound to antigen.

Complement is normally activated by one of three routes: the classical pathway, which is triggered by antigen:antibody complexes or antibody bound to the surface of a pathogen; the lectin pathway, which is activated by mannose-binding lectin (MBL) and the ficolins; and the alternative pathway, in which complement is activated spontaneously on the surface of some bacteria. The early part of each pathway is a series of proteolytic cleavage events leading to the generation of a convertase, a serine protease that cleaves complement component C3 and thereby initiates the effector actions of complement. The C3 convertases generated by the three pathways are different, but evolutionarily homologous, enzymes. Complement components and activation pathways, and the main effector actions of complement, are summarized in Fig. 31.1.

The principal effector molecule, and a focal point of activation for the system, is C3b, the large cleavage fragment of C3. If active C3b, or the homologous but less potent C4b, accidentally becomes bound to a host cell surface instead of a pathogen, the cell can be destroyed. This is usually prevented by the rapid hydrolysis of active C3b and C4b if they do not bind immediately to the surface where they were generated. Protection against inappropriate activation of complement is also provided by regulatory proteins.

One of these, and the most potent inhibitor of the classical pathway, is the C1 inhibitor (C1INH). This belongs to a family of serine protease inhibitors (called serpins) that together constitute 20% of all plasma proteins. In addition to being the sole known inhibitor of C1, C1INH contributes to the

This case was prepared by Raif Geha, MD, in collaboration with Arturo Borzutzky, MD.

Topics bearing on this case:
Classical pathway of complement activation
Inhibition of C1 activation
Alternative pathway of complement activation
Inflammatory effects of complement activation
Regulation of C4b

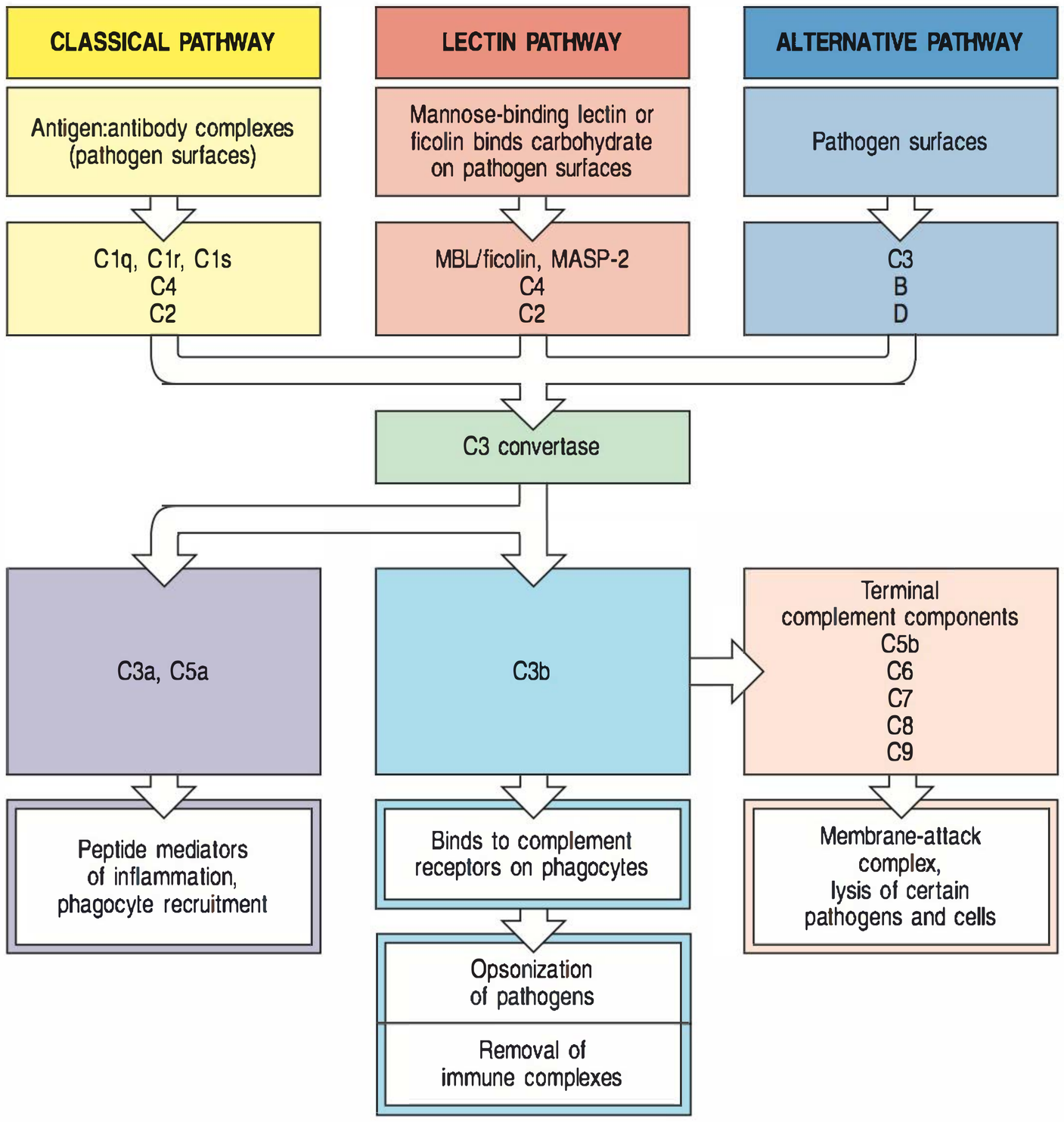


Fig. 31.1 Overview of the main components and effector actions of complement. The early events of all three pathways of complement activation involve a series of cleavage reactions that culminate in the formation of an enzymatic activity called a C3 convertase, which cleaves complement component C3 into C3b and C3a. The production of the C3 convertase is the point at which the three pathways converge and the main effector functions of complement are generated. C3b binds covalently to the bacterial cell membrane and opsonizes the bacteria, enabling phagocytes to internalize them. C3a is a peptide mediator of local inflammation. C5a and C5b are generated by the cleavage of C5b by a C5 convertase formed by C3b bound to the C3 convertase (not shown in this simplified diagram). C5a is also a powerful peptide mediator of inflammation. C5b triggers the late events in which the terminal components of complement assemble into a membrane-attack complex that can damage the membrane of certain pathogens. Although the classical complement activation pathway was first discovered as an antibody-triggered pathway, it is now known that C1q can activate this pathway by binding directly to pathogen surfaces, as well as paralleling the lectin activation pathway by binding to antibody that is itself bound to the pathogen surface. In the lectin pathway, MASP stands for mannose-binding lectin-associated serine protease.

regulation of serine proteases of the clotting system and of the kinin system, which is activated by injury to blood vessels and by some bacterial toxins. The main product of the kinin system is bradykinin, which causes vasodilation and increased capillary permeability.

C1INH intervenes in the first step of the complement pathway, when C1 binds to immunoglobulin molecules on the surface of a pathogen or antigen:antibody complex (Fig. 31.2). Binding of two or more of the six tulip-like heads of the C1q component of C1 is required to trigger the sequential activation of the two associated serine proteases, C1r and C1s. C1INH inhibits both of these proteases, by presenting them with a so-called bait-site, in the form of an arginine bond that they cleave. When C1r and C1s attack the bait-site they covalently bind C1INH and dissociate from C1q. By this mechanism, the C1 inhibitor limits the time during which antibody-bound C1 can cleave C4 and C2 to generate C4b2a, the classical pathway C3 convertase.

Activation of C1 also occurs spontaneously at low levels without binding to an antigen:antibody complex, and can be triggered further by plasmin, a protease of the clotting system, which is also normally inhibited by C1INH. In the absence of C1INH, active components of complement and bradykinin are produced. This is seen in hereditary angioedema (HAE), a disease caused by a genetic deficiency of C1INH.

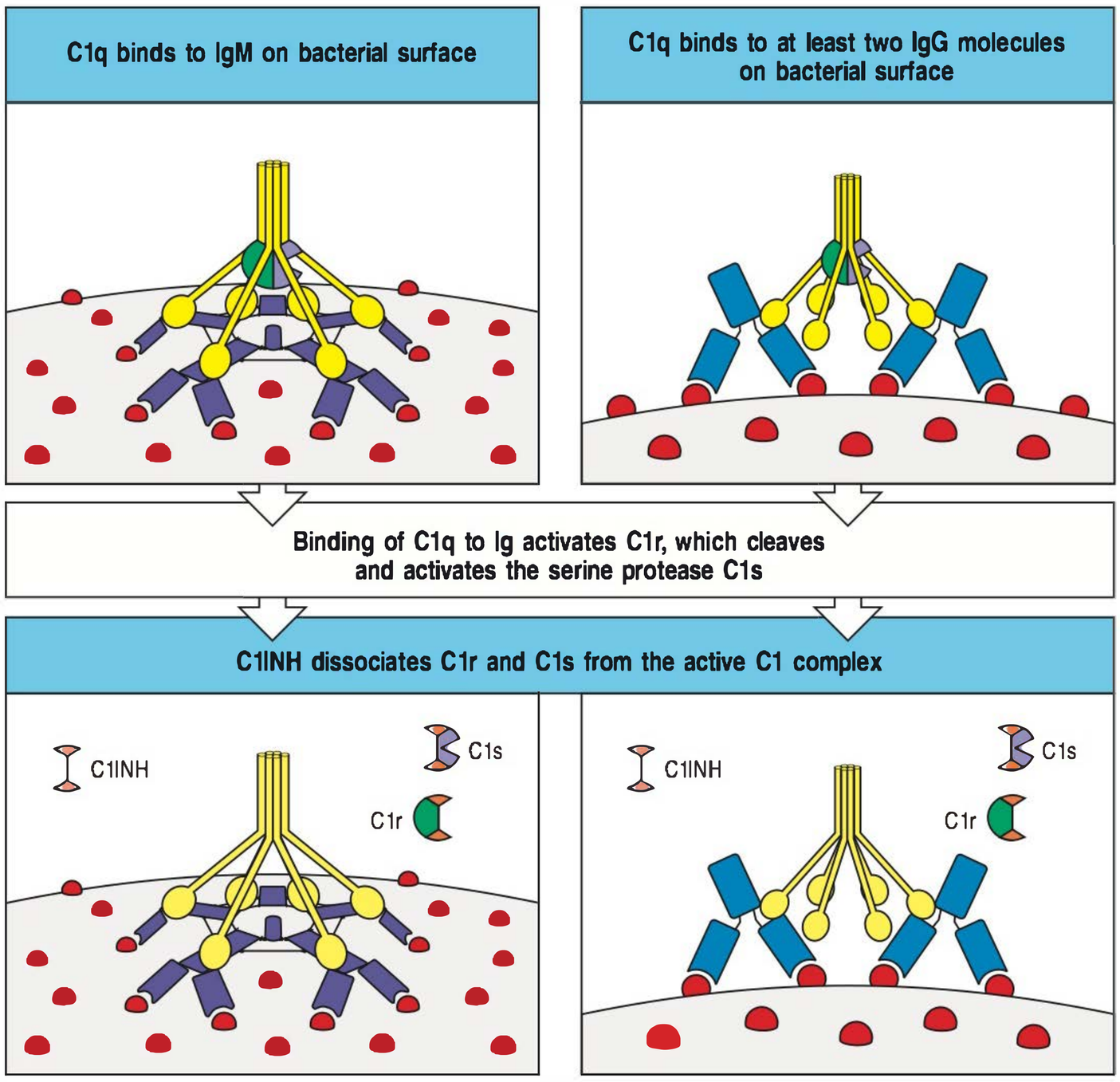


Fig. 31.2 Activation of the classical pathway of complement and intervention by C1INH. In the left panel, one molecule of IgM, bent into the 'staple' conformation by binding several identical epitopes on a pathogen surface, allows binding by the globular heads of C1q to its Fc pieces on the surface of the pathogen. In the right panel, multiple molecules of IgG bound to the surface of the pathogen allow binding by C1q to two or more Fc pieces. In both cases, binding of C1q activates the associated C1r, which becomes an active enzyme that cleaves the proenzyme C1s, a serine protease that initiates the classical complement cascade. Active C1 is inactivated by C1INH, which binds covalently to C1r and C1s, causing them to dissociate from the complex. There are in fact two C1r and two C1s molecules bound to each C1q molecule, although for simplicity this is not shown here. It takes four molecules of C1INH to inactivate all the C1r and C1s.

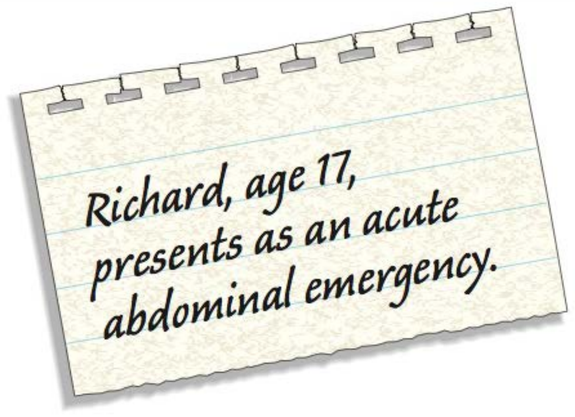
The case of Richard Crafton: a failure of communication as well as of complement regulation.

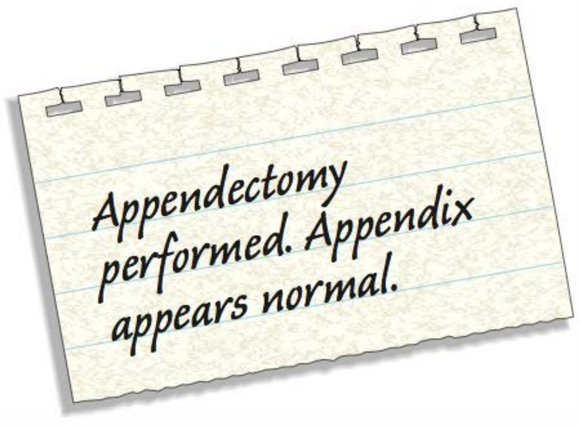
Richard Crafton was a 17-year-old high-school senior when he had an attack of severe abdominal pain at the end of a school day. The pain came as frequent sharp spasms and he began to vomit. After 3 hours, the pain became unbearable and he went to the emergency room at the local hospital.

At the hospital, the intern who examined him found no abnormalities other than dry mucous membranes of the mouth, and a tender abdomen. There was no point tenderness to indicate appendicitis. Richard continued to vomit every 5 minutes and said the pain was getting worse.

A surgeon was summoned. He agreed with the intern that Richard had an acute abdominal condition but was uncertain of the diagnosis. Blood tests showed an elevated red blood cell count, indicating dehydration. The surgeon decided to proceed with exploratory abdominal surgery. A large midline incision revealed a moderately swollen and pale jejunum but no other abnormalities were noted. The surgeon removed Richard's appendix, which was normal, and Richard recovered and returned to school 5 days later.

What Richard had not mentioned to the intern or to the surgeon was that, although he had never had such severe pains as those he was experiencing when he went to the





emergency room, he had had episodes of abdominal pain since he was 14 years old. No one in the emergency room asked him if he was taking any medication, or took a family history or a history of prior illness. If they had, they would have learned that Richard's mother, his maternal grandmother, and a maternal uncle, also had recurrent episodes of severe abdominal pain, as did his only sibling, a 19-year-old sister.

As a newborn, Richard was prone to severe colic. When he was 4 years old, a bump on his head led to abnormal swelling. When he was 7, a blow with a baseball bat caused his entire left forearm to swell to twice its normal size. In both cases, the swelling was not painful, nor was it red or itchy, and it disappeared after 2 days. At age 14 years, he began to complain of abdominal pain every few months, sometimes accompanied by vomiting and, more rarely, by clear, watery diarrhea.

Richard's mother had taken him at age 4 years to an immunologist, who listened to the family history and immediately suspected hereditary angioedema. The diagnosis was confirmed on measuring key complement components. C1INH levels were 16% of the normal mean and C4 levels were markedly decreased, while C3 levels were normal.

When Richard turned up for a routine visit to his immunologist a few weeks after his surgical misadventure, the immunologist, noticing Richard's large abdominal scar, asked what had happened. When Richard explained, he prescribed daily doses of Winstrol (stanozolol). This caused a marked diminution in the frequency and severity of Richard's symptoms. When Richard was 20 years old, purified C1INH became available; he has since been infused intravenously on several occasions to alleviate severe abdominal pain, and once for swelling of his uvula, pharynx, and larynx. The infusion relieved his symptoms within 25 minutes.

Richard subsequently married and had two children. The C1INH level was found to be normal in both newborns.

Hereditary angioedema.

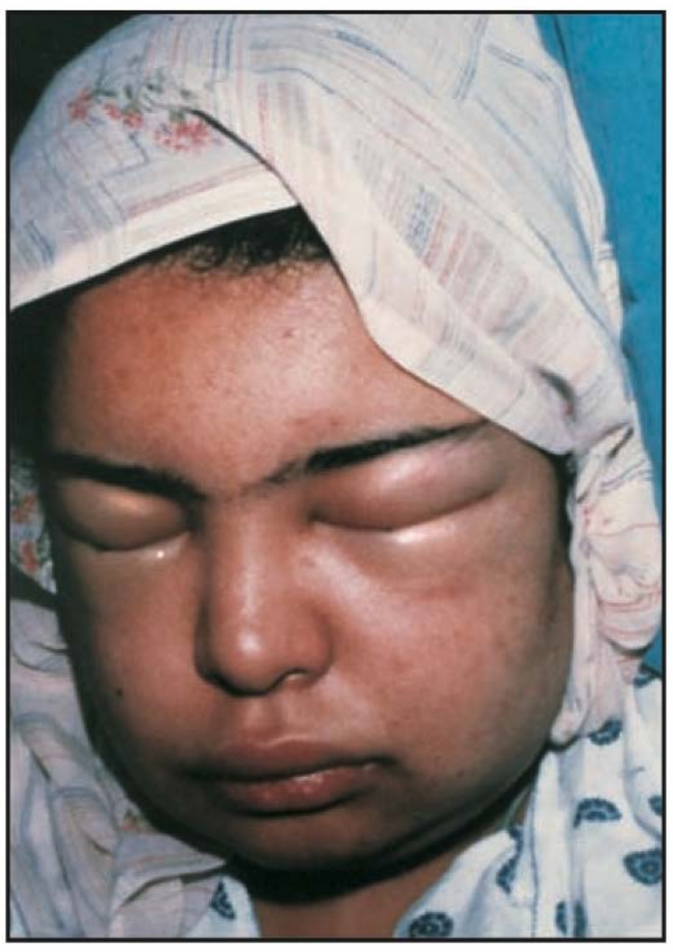


Fig. 31.3 Hereditary angioedema. Transient localized swelling that occurs in this condition often affects the face.

Individuals like Richard with a hereditary deficiency of C1INH are subject to recurrent episodes of circumscribed swelling of the skin (Fig. 31.3), intestine, and airway. Attacks of subcutaneous or mucosal swelling most commonly affect the extremities, but can also involve the face, trunk, genitals, lips, tongue, or larynx. Cutaneous attacks cause temporary disfigurement but are not dangerous. When the swelling occurs in the intestine it causes severe abdominal pain, and obstructs the intestine so that the patient vomits. When the colon is affected, watery diarrhea may occur. Swelling in the larynx is the most dangerous symptom, because the patient can rapidly choke to death. HAE attacks do not usually involve itching or hives, which is useful to differentiate this disease from allergic angioedema. However, a serpiginous, or linear and wavy, rash is sometimes seen before the onset of swelling symptoms. Such episodes may be triggered by trauma, menstrual periods, excessive exercise, exposure to extremes of temperature, mental stress, and some medications such as angiotensin-converting enzyme inhibitors and oral contraceptives.

HAE is not an allergic disease, and attacks are not mediated by histamine. HAE attacks are associated with activation of four serine proteases, which are normally inhibited by C1INH. At the top of this cascade is Factor XII, which directly or indirectly activates the other three (Fig. 31.4). Factor XII is normally activated by injury to blood vessels, and initiates the kinin cascade, activating

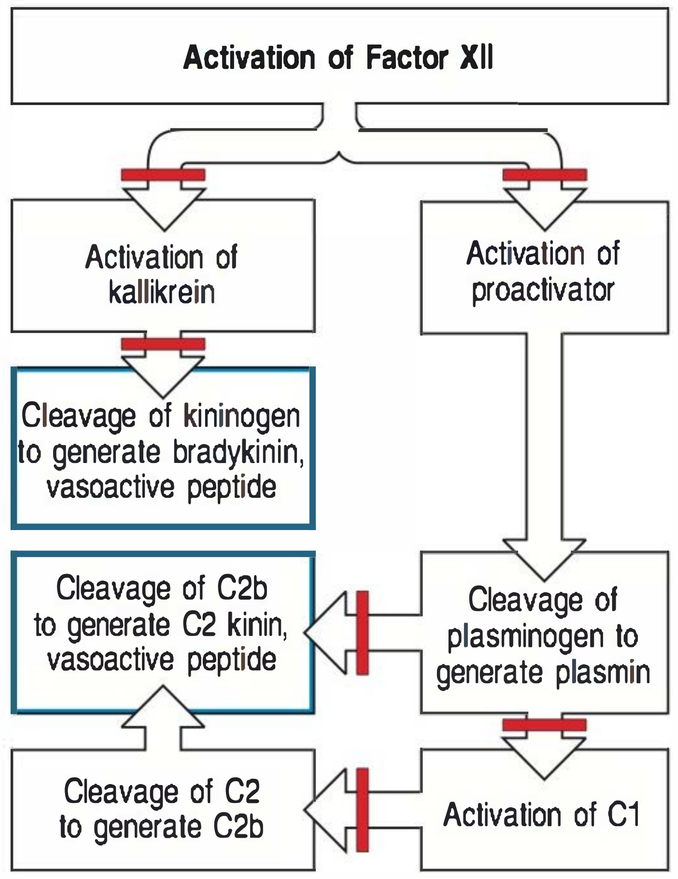


Fig. 31.4 Pathogenesis of hereditary angioedema. Activation of Factor XII leads to the activation of kallikrein, which cleaves kininogen to produce the vasoactive peptide bradykinin; it also leads to the activation of plasmin, which in turn activates C1. C1 cleaves C2, whose smaller fragment C2b is further cleaved by plasmin to generate the vasoactive peptide C2 kinin. The red bars represent inhibition by C1INH.

kallikrein, which generates the vasoactive peptide bradykinin. Factor XII also indirectly activates plasmin, which, as mentioned earlier, activates C1 itself. Plasmin also cleaves C2b to generate a vasoactive fragment called C2 kinin. In patients deficient in C1INH, the uninhibited activation of Factor XII leads to the activation of kallikrein and plasmin; kallikrein catalyzes the formation of bradykinin, and plasmin produces C2 kinin. Bradykinin is the main mediator responsible for HAE attacks by causing vasodilation and increasing the permeability of the postcapillary venules by causing contraction of endothelial cells so as to create gaps in the blood vessel wall (Fig. 31.5). This is responsible for the edema; movement of fluid from the vascular space into another body compartment, such as the gut, causes the symptoms of dehydration as the vascular volume contracts.

Treatment of HAE can focus on preventing attacks or on resolving acute episodes. Purified or recombinant C1INH is an effective therapy in both these settings. A kallikrein inhibitor and a bradykinin receptor antagonist have also been developed to target the kinin cascade and bradykinin activity.

Questions.

1 *Activation of the complement system results in the release of histamine and chemokines, which normally produce pain, heat, and itching. Why is the edema fluid in HAE free of cellular components, and why does the swelling not itch?*

2 *Richard has a markedly decreased amount of C4 in his blood. This is because it is being rapidly cleaved by activated C1. What other complement component would you expect to find decreased? Would you expect the alternative pathway components to be low, normal, or elevated? What about the terminal components?*

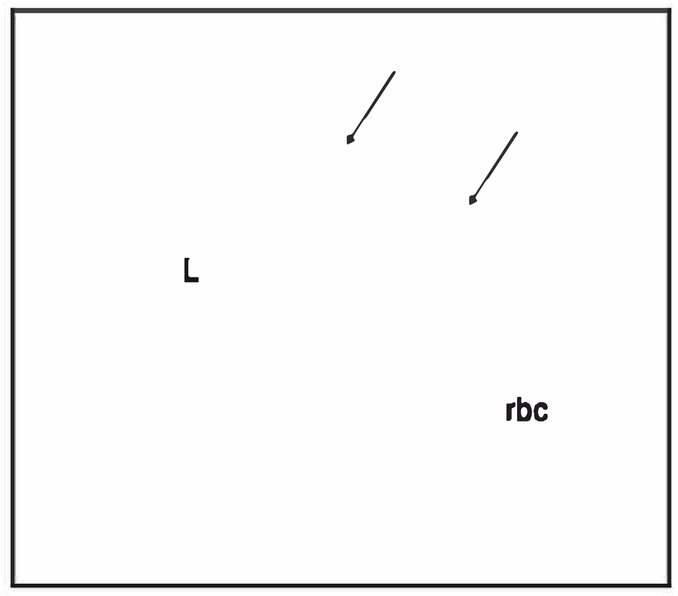


Fig. 31.5 Contraction of endothelial cells creates gaps in the blood vessel wall. A guinea pig was injected intravenously with India ink (a suspension of carbon particles). Immediately thereafter the guinea pig was injected intradermally with a small amount of activated C1s. An area of angioedema formed about the injected site, which was biopsied 10 minutes later. An electron micrograph reveals that the endothelial cells in post-capillary venules have contracted and formed gaps through which the India ink particles have leaked from the blood vessel. L is the lumen of the blood vessel; P is a polymorphonuclear leukocyte in the lumen; rbc is a red blood cell that has leaked out of the blood vessel. Micrograph courtesy of Kaethe Willms.

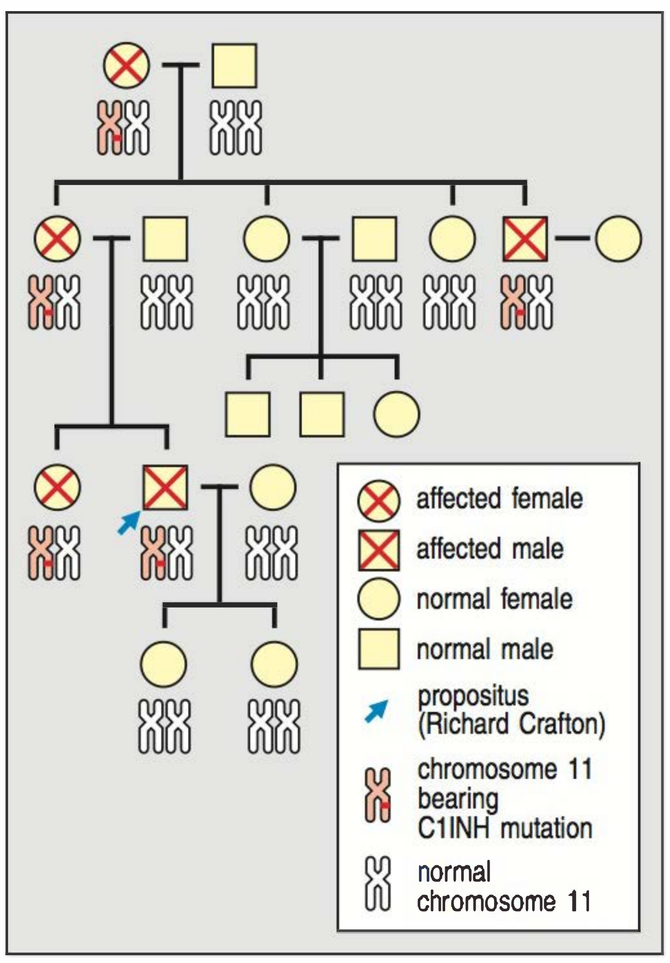


Fig. 31.6 The inheritance of hereditary angioedema in Richard's extended family.

3 Despite the complement deficiency in patients with HAE, they are not unduly susceptible to infection. Why not?

4 What is stanozolol, and why was it prescribed?

5 Emergency treatment for HAE cases is sometimes necessary because of airway obstruction. In most cases, however, a patient with obstruction of the upper airways is likely to be suffering from an anaphylactic reaction. The treatment in this case would be epinephrine. How might you decide whether to administer epinephrine or intravenous C1INH?

6 Figure 31.6 shows Richard's family tree. What is the mode of inheritance (dominant or recessive, sex-linked or not) of HAE? Can Richard's two children pass the disease onto their offspring?

CASE 7 Omenn Syndrome

A defect in V(D)J recombination results in severe immunodeficiency.

The development of B cells in the bone marrow and T cells in the thymus is initiated by the assembly of gene segments to make the variable (V) sequence that encodes the V regions of the heavy and light chains of immunoglobulins or of the α and β chains of the T-cell antigen receptors (Fig. 7.1). This process is called V(D)J recombination. A V (variable) and a J (joining) gene segment are joined to make the V-region sequences for the light chains of immunoglobulins or the α chains of T-cell receptors. An additional gene segment, D (diversity), is involved in the rearrangements that produce the V-region sequences for the immunoglobulin heavy chain and the β chain of the T-cell receptor; a D and a J gene segment are joined first, followed by joining of a V gene segment to form VDJ. In all these recombination events, the DNA between the rearranging gene segments is deleted from the chromosome. Because there are many different V, D, and J segments in the germline genome, there are several million possible combinations. This is how much of the vast diversity in the antibody and T-cell receptor repertoires is generated. Moreover, small insertions or deletions of nucleotides at the joins between V and D, and D and J segments further contribute to diversity.

The process of V(D)J recombination is initiated by enzymes encoded by the recombinase-activating genes *RAG1* and *RAG2*. The RAG-1 and RAG-2 enzymes nick double-stranded DNA. They recognize canonical DNA sequences called recombination signal sequences, which flank the coding gene segments and consist of a heptamer (CACAGTG) followed by a spacer of 12 or 23 bases and then a nonamer (ACAAAAGTG) (Fig. 7.2). RAG-1 binds to the nonamer element followed by binding of RAG-2 to the heptamer. The DNA sequence that forms the border between the heptamer and the coding segment is then nicked, and a break in the double-stranded DNA occurs. The coding ends are initially sealed by a hairpin. A series of ubiquitously expressed proteins (Ku70, Ku80, DNA-PKcs, Artemis, DNA ligase IV (LIG4), XRCC4, and Cernunnos/XLF) are then recruited and mediate DNA repair and rejoining of coding and signal ends (Fig. 7.3).

If either of the *RAG* genes is knocked out by homologous recombination in mice, the development of B cells and T cells is completely abolished and the mice have severe combined immunodeficiency. Mutations in *RAG1* and *RAG2*

Topics bearing on this case:
V(D)J recombination
RAG enzymes
Severe combined immunodeficiency

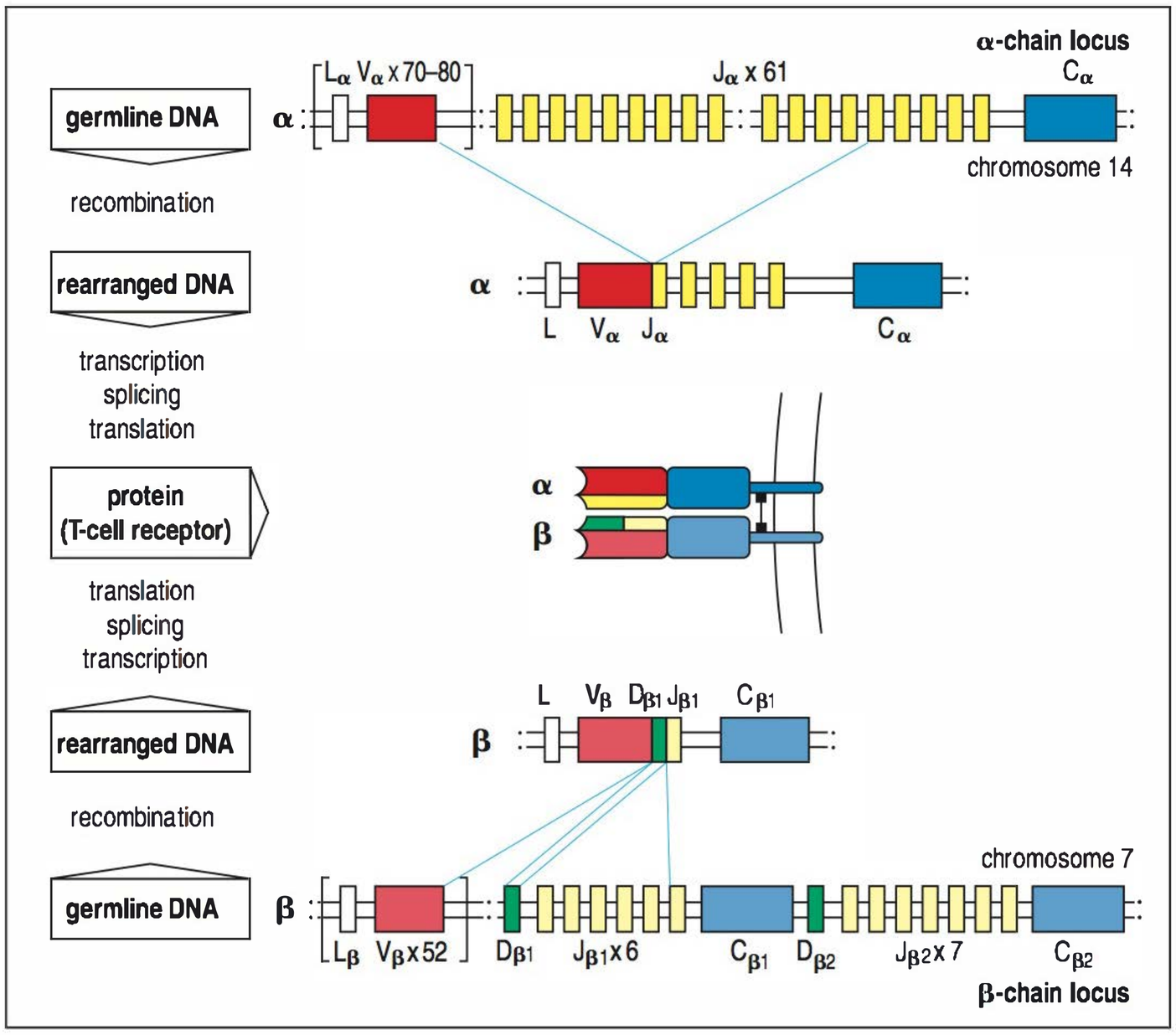


Fig. 7.1 Rearrangement of the T-cell receptor genes. The top and bottom rows of the figure show the germline arrangement of the variable (V), diversity (D), joining (J), and constant (C) gene segments at the T-cell receptor α and β loci, respectively. During T-cell development, a V-region sequence for each chain is assembled by DNA recombination. For the α chain (top), a V_α gene segment rearranges to a J_α gene segment to create a functional gene encoding the V domain. For the β chain (bottom), rearrangement of a D_β, a J_β, and a V_β gene segment creates the functional V-domain exon. A similar array of gene segments is present at the immunoglobulin loci, and immunoglobulin gene rearrangement follows an essentially similar course.

have also been found in cases of human SCID with lack of both T and B cells (T^-B^- SCID). In addition, defects of Artemis, LIG4, and DNA-PK have been also identified in patients with T^-B^- SCID, and mutations of Cernunnos/XLF cause combined immunodeficiency with markedly reduced numbers of T and B lymphocytes. However, hypomorphic mutations in these genes may allow residual protein expression and function and may result in a different phenotype, in which autoimmune manifestations associate with severe immunodeficiency. Omenn syndrome is the prototype of these conditions, and is most often due to missense mutations in the *RAG* genes.

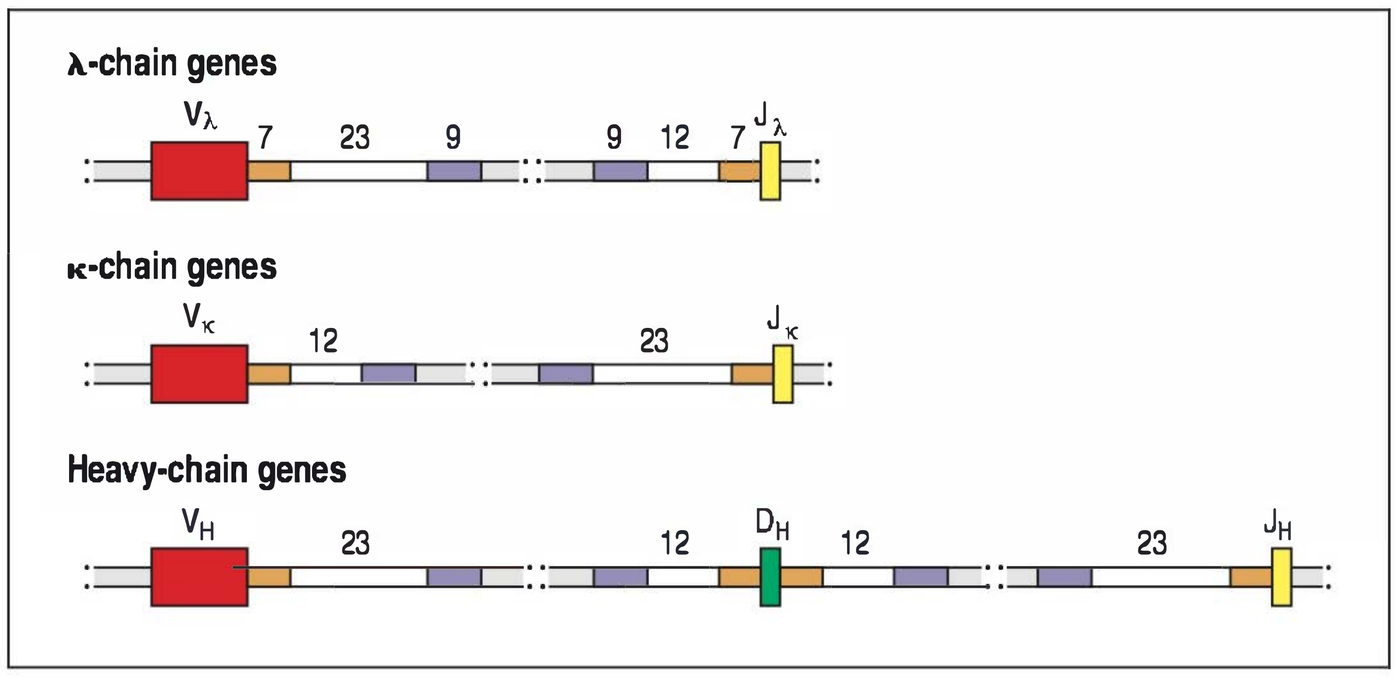


Fig. 7.2 Each V, D, or J gene segment is flanked by recombination signal sequences (RSSs). This is illustrated here with respect to the immunoglobulin genes. There are two types of RSS. One consists of a nonamer (9 nucleotides, shown in purple) and a heptamer (7 nucleotides, shown in orange) separated by a spacer of 12 nucleotides (white). The other consists of the same 9- and 7-nucleotide sequences separated by a 23-nucleotide spacer (white).

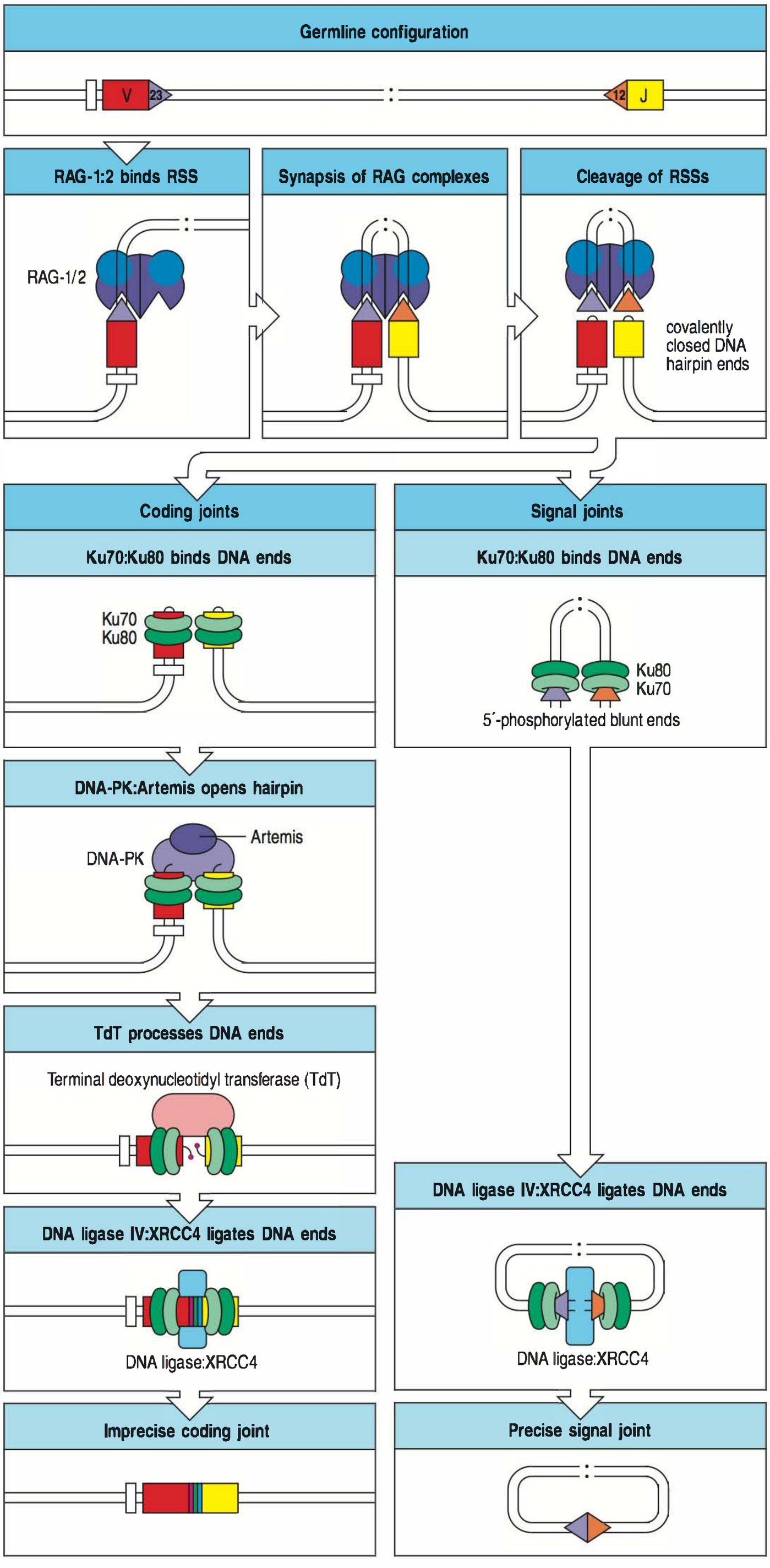


Fig. 7.3 Steps in the V(D)J recombination process. V(D)J recombination is initiated by the lymphocyte-specific RAG-1 and RAG-2 enzymes, which recognize the recombination signal sequences (RSSs) that flank the coding variable (V), diversity (D), and joining (J) elements. The DNA double-strand break at the coding ends is initially sealed by a hairpin. In the second step of the process, the ubiquitously expressed Ku70 and Ku80 are recruited both at coding ends and at signal ends. DNA-protein kinase catalytic subunit (DNA-PKcs) and Artemis are also recruited to the coding ends, and Artemis mediates opening of the coding-end hairpins. The enzyme terminal nucleotide transferase (TdT) may introduce additional nucleotides at the junction between coding elements. Finally, the enzymes DNA ligase IV and XRCC4 (involved in DNA repair and ligation) are recruited at both coding and signal ends and mediate the formation of coding and signal joints. Another enzyme, Cernunnos/XLF (not shown in the figure), also participates in the DNA repair process.

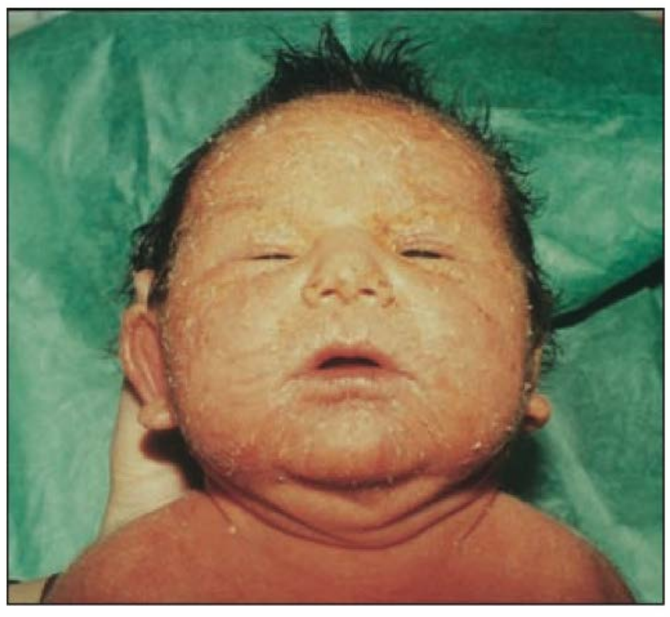


Fig. 7.4 Bright scaly red rash on the face and shoulders of an infant with Omenn syndrome.

The case of Ricardo Reis: a bright red rash betrays an immunodeficiency.

At birth, Ricardo seemed to be a normal healthy baby. He gained weight normally and cried vigorously. Soon after birth, however, his mother noticed that he had 10 loose bowel movements a day. On the 17th day after birth, he developed a rash on his legs, which over the next 7 days spread over his entire body. His parents brought him to the emergency room at Children's Hospital, and also reported that he had had a dry cough for the past week.

On physical examination, Ricardo's weight, length, and head circumference were normal. The diffuse papular scaly rash was worst on his face (Fig. 7.4) but also covered his trunk and extremities (Fig. 7.5). Small blisters were present on his palms and the soles of his feet, which were red. He had purulent conjunctivitis (yellow discharge from his eyes), his eardrums were normal, no lymph nodes could be felt, and his heart and lungs were normal. The liver and spleen were not enlarged.

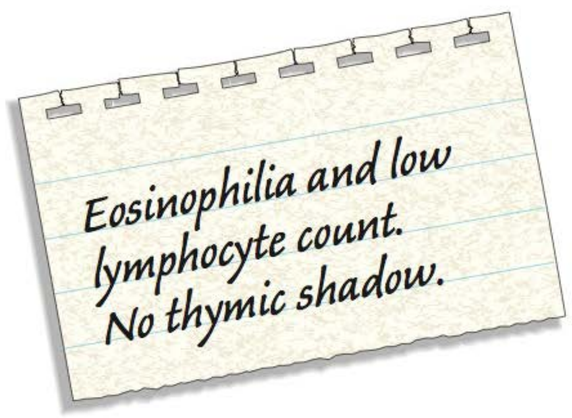
Ricardo's parents had three normal children, but had had two other children, a boy and a girl, who had died soon after the onset of a similar rash at 1 month old. The parents were first cousins of Portuguese extraction.

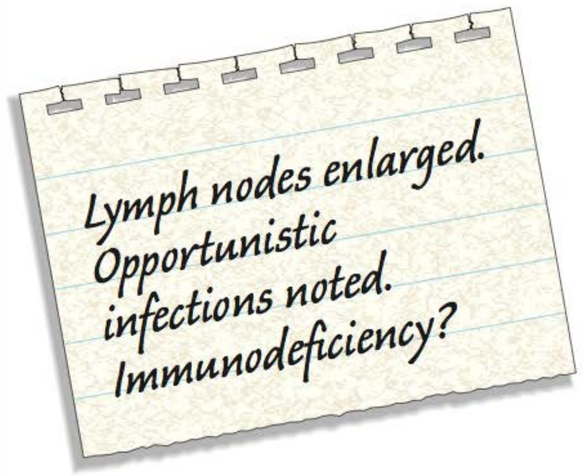
Ricardo was admitted to the hospital. Blood tests showed that his hemoglobin was 8.4 g dl^{-1} (low), his platelet count was 460,000 (slightly elevated), and his white blood cell count was 8000 μl^{-1} (normal), of which 56% were eosinophils (normal <5%), 23% monocytes (normal 10%), 15% neutrophils, and 6% lymphocytes (normal 50%). An examination of his bone marrow revealed a preponderance of eosinophil precursors. Ricardo's serum IgG level was 55 mg dl^{-1} (normal 400 mg dl^{-1}), IgA and IgM were undetectable, and IgE was 7200 IU ml^{-1} (normal <50 IU ml^{-1}). A skin biopsy showed that the dermis was infiltrated with large numbers of eosinophils, lymphocytes, and macrophages. Large numbers of cells surrounded the blood vessels. An X-ray of Ricardo's chest showed clear lungs and a normal cardiac shadow; there was no thymic shadow (see Case 6).

In the hospital, Ricardo's condition rapidly worsened. He developed enlarged lymph nodes in the neck and groin, and pus accumulated in the skin behind his ear. This was drained, and *Staphylococcus aureus* and *Candida albicans* were cultured from the drainage fluid. Thrush (*Candida albicans*) was noticed in his mouth (see Case 5).

An immunologist was consulted. He ordered blood tests that revealed an absence of B cells and a paucity of T cells. Ricardo's peripheral blood lymphocytes responded poorly to stimulation with phytohemagglutinin and with anti-CD3 monoclonal antibody. On FACS analysis, no cells were found that reacted with anti-CD19, which detects B cells (see Case 1). All the lymphocytes were $CD3^+$, of which 90% coexpressed the activation marker CD45R0, and 65% expressed major histocompatibility complex (MHC) class II molecules, another marker of T-cell activation. Eighty percent of the lymphocytes were $CD4^+$, and 15% were $CD8^+$. Flow-cytometry analysis of Ricardo's peripheral T lymphocytes, using monoclonal antibodies directed against various families of T-cell receptor V_α and V_β sequences showed that only few of them were expressed, indicating an oligoclonal T-cell receptor repertoire. The *RAG1* and *RAG2* genes were sequenced, and homozygosity for the Arg222Gln (R229Q) mutation was found in the *RAG2* gene. The T cells were definitively identified as Ricardo's (and not as transferred maternal T cells) by HLA typing.

While these studies were being carried out, Ricardo developed *Pneumocystis jirovecii* pneumonia and died of respiratory failure.





Omenn syndrome.

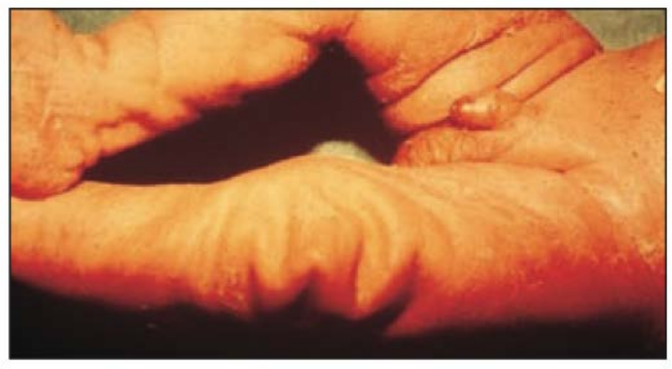


Fig. 7.5 Legs and groin of an infant with Omenn syndrome. The skin is bright red and wrinkled from edema and the infiltration of inflammatory cells.

The RAG enzymes essential for V(D)J recombination were first discovered in mice and later identified in humans. Infants with the autosomal recessive form of severe combined immunodeficiency (SCID) were screened for mutations in these genes, and several cases were identified in which RAG-1 or RAG-2 was deficient. These infants lacked T and B lymphocytes, but had a normal number of NK cells; hence they had $T^-B^-NK^+$ SCID.

Some patients were found with missense mutations in the *RAG* genes such that only partial enzyme activity was expressed. An examination of patients with a form of SCID called Omenn syndrome revealed further missense mutations in *RAG* genes. This syndrome is characterized by early onset of a generalized red rash (erythroderma), failure to thrive, protracted diarrhea, and enlargement of the liver, spleen, and lymph nodes. A high eosinophil count (eosinophilia) is usually encountered, together with a lack of B lymphocytes and a marked decrease in T cells. Immunoglobulins are also markedly decreased, but IgE levels are raised. As only partial ability to execute V(D)J recombination is retained by the mutated enzyme, in most cases no mature circulating B cells are detected and the few T cells that are found are oligoclonal; that is, they are the products of a limited number of different clones. These oligoclonal T cells infiltrate and cause significant damage in target organs.

As illustrated by this case, Omenn syndrome is usually rapidly fatal unless it is treated by bone marrow transplantation, which may result in full correction of the disease.

Genetic defects that result in a severe, but incomplete, impairment of T-cell development by interfering with mechanisms other than V(D)J recombination can also result in Omenn syndrome. These include IL-7Rα chain deficiency (IL-7 is required for lymphocyte development), γ_c deficiency (X-linked SCID; see Case 5), and mutations of the *RMRP* gene. The last of these causes cartilage hair hypoplasia, a condition characterized by dwarfism, sparse hair, a variable degree of immunodeficiency, and hematological abnormalities. As with the *RAG* genes, Omenn syndrome occurs when the defect is 'leaky'; that is, due to a missense mutation that severely impairs but does not abolish function, allowing a few T cells to develop. It is likely that in Omenn syndrome the autoimmune manifestations, with infiltration of target organs by oligoclonal T cells, reflect several mechanisms, as demonstrated by studies in patients and in animal models of the disease. Poor generation of T lymphocytes in the thymus results in impaired maturation of medullary thymic epithelial cells and reduced expression of *AIRE*, thus impinging on the deletion of self-reactive T cells (see Case 17). Furthermore, generation of regulatory T cells in the thymus is also impaired, affecting peripheral tolerance (see Case 18). Finally, the few T cells that are generated in the thymus of patients with Omenn syndrome undergo extensive peripheral expansion (homeostatic proliferation) and secrete increased amounts of cytokines, including inflammatory (IFN-γ) and TH_2 (IL-4, IL-5) cytokines.

Apart from the lymphocyte-specific RAG proteins, V(D)J recombination also involves proteins of the nonhomologous end-joining pathway that are universally used for DNA repair and recombination in human cells. In addition to $T^-B^-NK^+$ SCID, patients with defects in these genes (Artemis, DNA-PK, LIG4, and Cernunnos/XLF) present increased cellular sensitivity to ionizing radiation, because they are unable to repair radiation-induced DNA damage. These radiosensitive forms of $T^-B^-NK^+$ SCID are often associated with extraimmune clinical manifestations, such as microcephaly, neurodevelopmental problems, and growth and development defects.

Questions.

1 How do you explain the high IgE level and eosinophilia in this patient?

2 How do you explain the enlargement of the lymph nodes in this patient?

3 How does Ricardo's family history help you determine the mode of inheritance of Omenn syndrome?

4 A bright red rash (erythroderma) is characteristic of Omenn syndrome. What causes this rash?

CASE 8 MHC Class II Deficiency

An inherited failure of gene regulation.

The class II molecules of the major histocompatibility complex (MHC) are involved in presenting antigens to $CD4^+$ T cells. The peptide antigens that they present are derived from extracellular pathogens and proteins taken up into intracellular vesicles, or from pathogens such as *Mycobacterium* that persist intracellularly inside vesicles. MHC class II molecules are expressed constitutively on antigen-presenting cells, including B lymphocytes, macrophages, and dendritic cells. In humans, together with the MHC class I molecules (see Case 12), they are known as the HLA antigens. They are also expressed on the epithelial cells of the thymus and their expression can be induced on other cells, principally by the cytokine interferon-γ. T cells also express MHC class II molecules when they are activated.

MHC class II molecules are heterodimers consisting of an α chain and a β chain (Fig. 8.1). The genes encoding both chains are located in the MHC on the short arm of chromosome 6 in humans (Fig. 8.2). The principal MHC class II molecules are designated DP, DQ, and DR and, like the MHC class I molecules, they are highly polymorphic. Peptides bound to MHC class II molecules can be recognized only by the T-cell receptors of CD4 T cells and not by those of CD8 T cells (Fig. 8.3). MHC class II molecules expressed in the thymus also have a vital role in the intrathymic maturation of CD4 T cells.

Expression of the genes encoding the α and β chains of MHC class II molecules must be strictly coordinated and it is under complex regulatory control by a series of transcription factors. The existence of these transcription factors and a means of identifying them were first suggested by the study of patients with MHC class II deficiency.

Topics bearing on this case:

- Role of MHC class II molecules in antigen presentation to CD4 T cells
- Role of co-receptor molecule CD4 in antigen recognition by T cells
- Intrathymic maturation of CD4 T cells
- Mixed lymphocyte reaction
- Lymphocyte stimulation by polyclonal mitogens
- FACS analysis

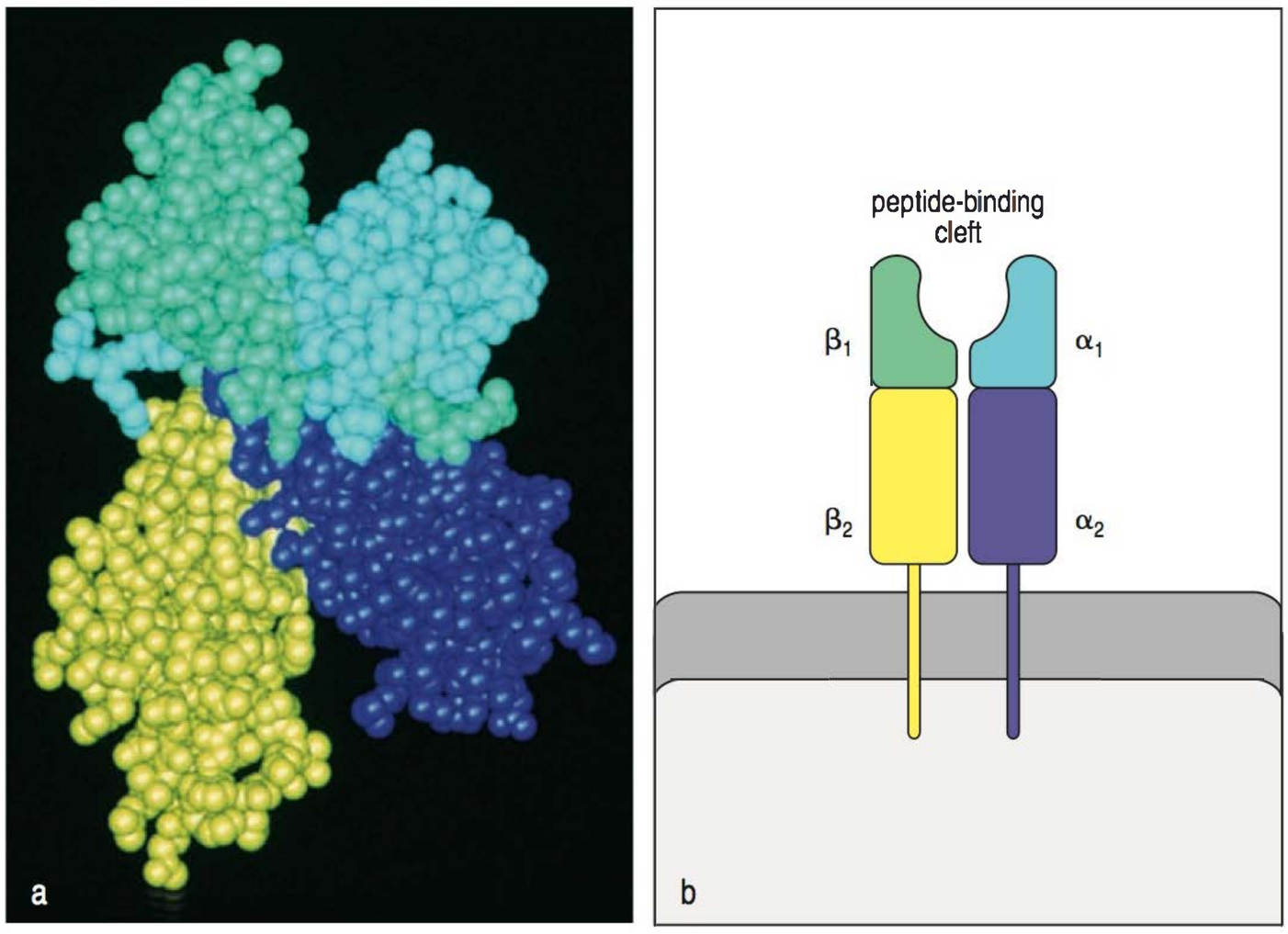
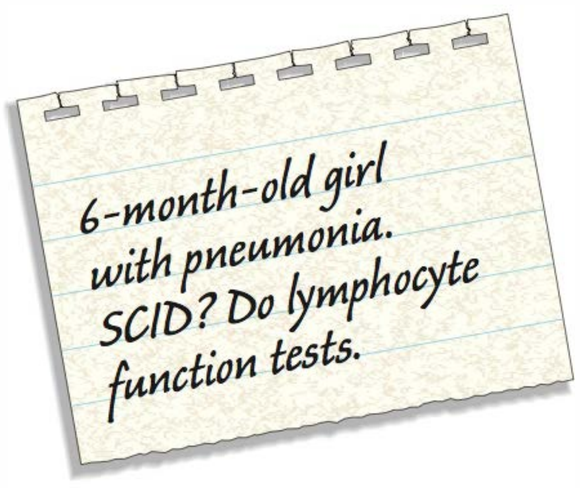


Fig. 8.1 Structure of an MHC class II molecule. Panel a shows a computer graphic representation of the MHC class II molecule HLA-DR1. Panel b is a schematic representation of the molecule. It is composed of two transmembrane glycoprotein chains, α and β, each folded into two protein domains. The antigenic peptide binds in a cleft between the two chains. Photograph courtesy of C. Thorpe.



The case of Helen Burns: a 6-month-old child with a mild form of combined immunodeficiency.

Helen Burns was the second child born to her parents. She thrived until 6 months of age when she developed pneumonia in both lungs, accompanied by a severe cough and fever. Blood and sputum cultures for bacteria were negative, but a tracheal aspirate revealed the presence of abundant *Pneumocystis jirovecii*. She was treated successfully with the *a*nti-*Pneumocystis* drug pentamidine and seemed to recover fully.

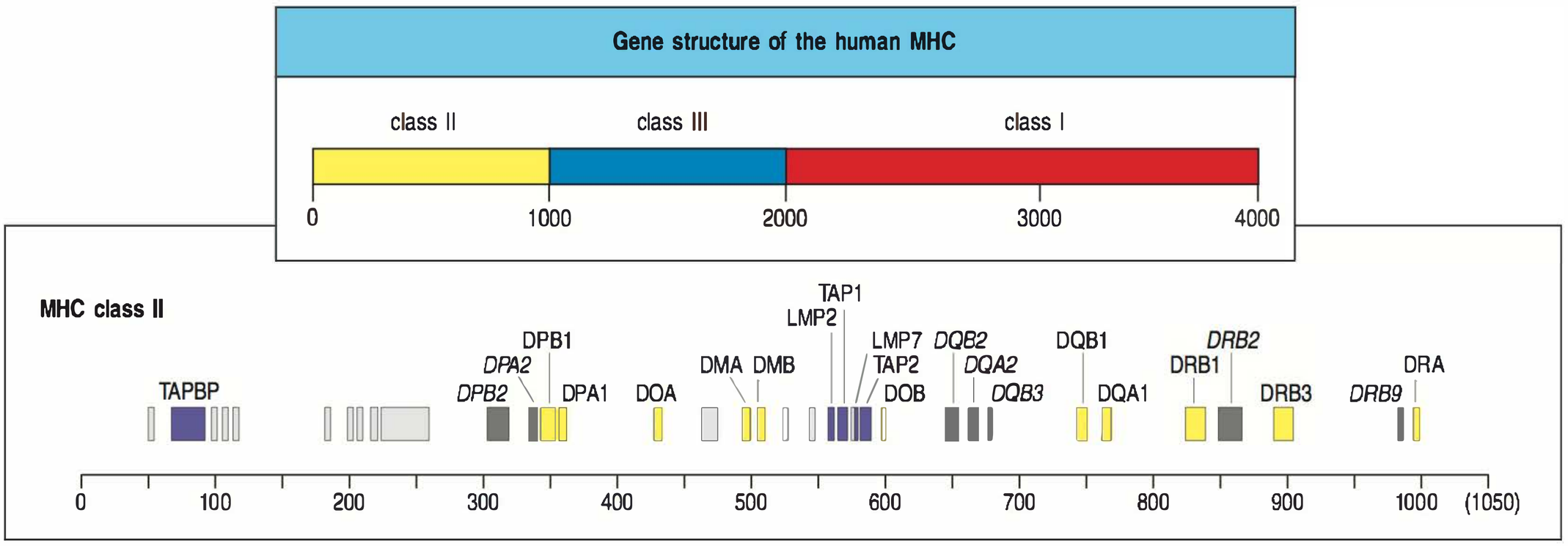


Fig. 8.2 Detailed map of the MHC class II region. The genes for the α and β chains of the HLA-DP, HLA-DR, and HLA-DQ molecules are shown as *DPA*, *DPB*, etc. The situation is complicated because there are two *DPA* genes, two *DPB* genes, and several *DRB* genes. Genes shown in gray and named in italic are pseudogenes. MHC class II genes are shown in yellow. Genes in the MHC region that have immune functions but are not related to the MHC class I and class II genes are shown in purple. Approximate genetic distances given in thousands of base pairs.

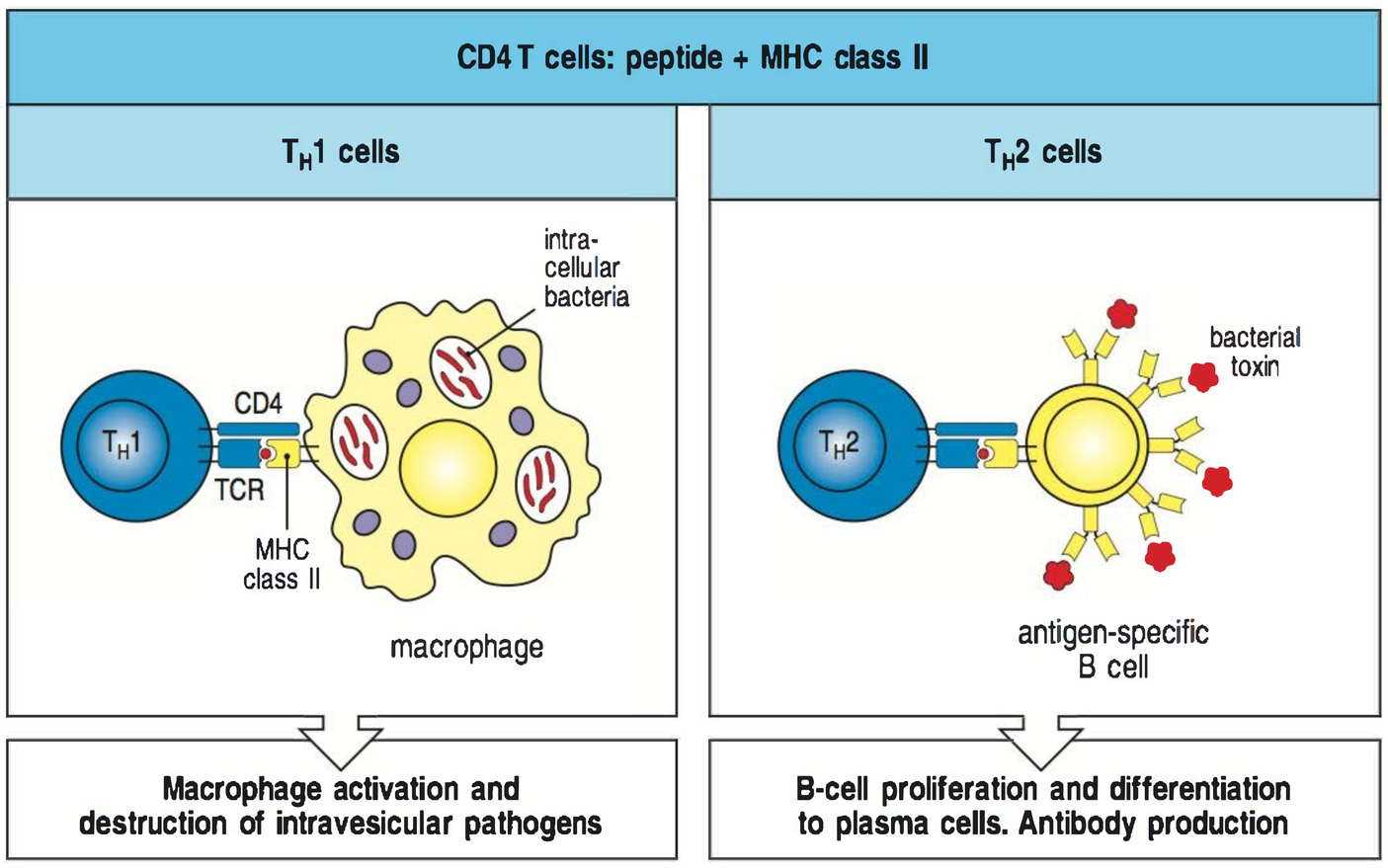
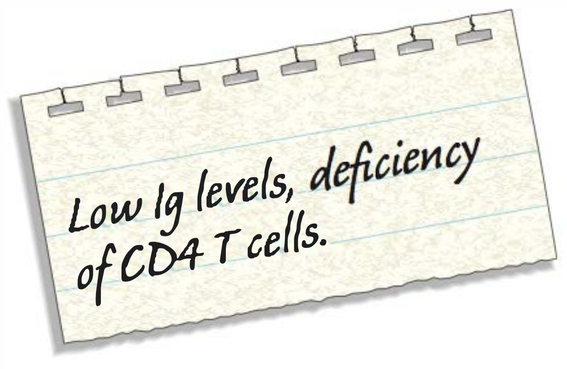


Fig. 8.3 Effector CD4 cells recognize antigens bound to MHC class II molecules. CD4 T cells carry the co-receptor molecule CD4, which binds to MHC class II molecules on the antigen-presenting cell and helps to stabilize the binding of T-cell receptor to antigen. Effector CD4 T cells fall into several different subclasses: T_H1 and T_H2 are shown here. T_H1 cells are involved mainly in responding to antigens presented by macrophages, whereas T_H2 cells respond to antigen presented by B cells, stimulating the differentiation of B cells to plasma cells and the production of antibodies.

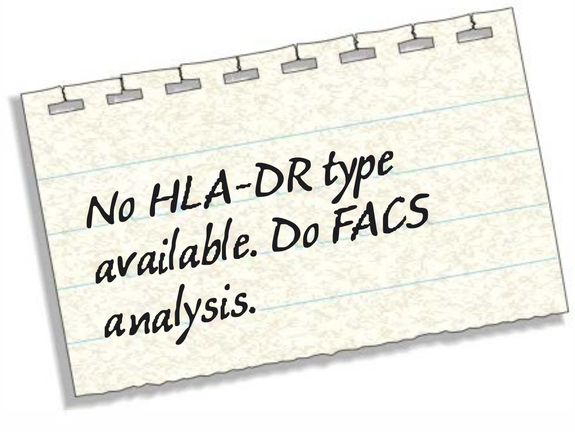
As her pneumonia was caused by the opportunistic pathogen *P. jirovecii*, Helen was suspected to have severe combined immunodeficiency. A blood sample was taken and her peripheral blood mononuclear cells were stimulated with phytohemagglutinin (PHA) to test for T-cell function by ^{3}H-thymidine incorporation into DNA. A normal T-cell proliferative response was obtained, with her T cells incorporating 114,050 counts min^{-1} of ^{3}H-thymidine (normal control 75,000 counts min^{-1}). Helen had received routine immunizations with orally administered polio vaccine and DPT (diphtheria, pertussis, and tetanus) vaccine at 2 months old. However, in further tests her T cells failed to respond to tetanus toxoid *in vitro*, although they responded normally in the ^{3}H-thymidine incorporation assay when stimulated with allogeneic B cells (6730 counts min^{-1} incorporated, in contrast with 783 counts min^{-1} for unstimulated cells).

When it was found that Helen's T cells could not respond to a specific antigenic stimulus, her serum immunoglobulins were measured and found to be very low. IgG levels were 96 mg dl^{-1} (normal 600–1400 mg dl^{-1}), IgA was 6 mg dl^{-1} (normal 60–380 mg dl^{-1}), and IgM 30 mg dl^{-1} (normal 40–345 mg dl^{-1}).

Helen's white blood cell count was elevated at 20,000 cells μl^{-1} (normal range 4000–7000 μl^{-1}). Of these, 82% were neutrophils, 10% lymphocytes, 6% monocytes, and 2% eosinophils. The calculated number of 2000 lymphocytes μl^{-1} was low for her age (normal >3000 μl^{-1}). Of her lymphocytes, 27% were B cells as determined by an antibody against CD20 (normal 10–12%), and 47% reacted with antibody to the T-cell marker CD3. In particular, 34% of Helen's lymphocytes were positive for CD8, and 10% were positive for CD4. Thus, at 680 cells μl^{-1} her number of CD8 T cells was within the normal range, but the number of CD4 T cells (200 μl^{-1}) was much lower than normal (her CD4 T-cell count would be expected to be twice her CD8 T-cell count). The presence of substantial numbers of T cells, and thus a normal response to PHA, ruled out a diagnosis of severe combined immunodeficiency (see Case 5).



Helen's pediatrician referred her to the Children's Hospital for consideration for a bone marrow transplant, despite the lack of a diagnosis. When an attempt was made to HLA-type Helen, her parents, and her healthy 4-year-old brother by serology, a DR type could not be obtained from Helen's white blood cells. Her circulating B lymphocytes were transformed with the Epstein–Barr virus (EBV) to establish a B-cell line, which was then analyzed by flow cytometry. The EBV-transformed B lymphocytes did not express HLA-DQ or HLA-DR molecules. Hence, a diagnosis of MHC class II deficiency was established (Fig. 8.4).



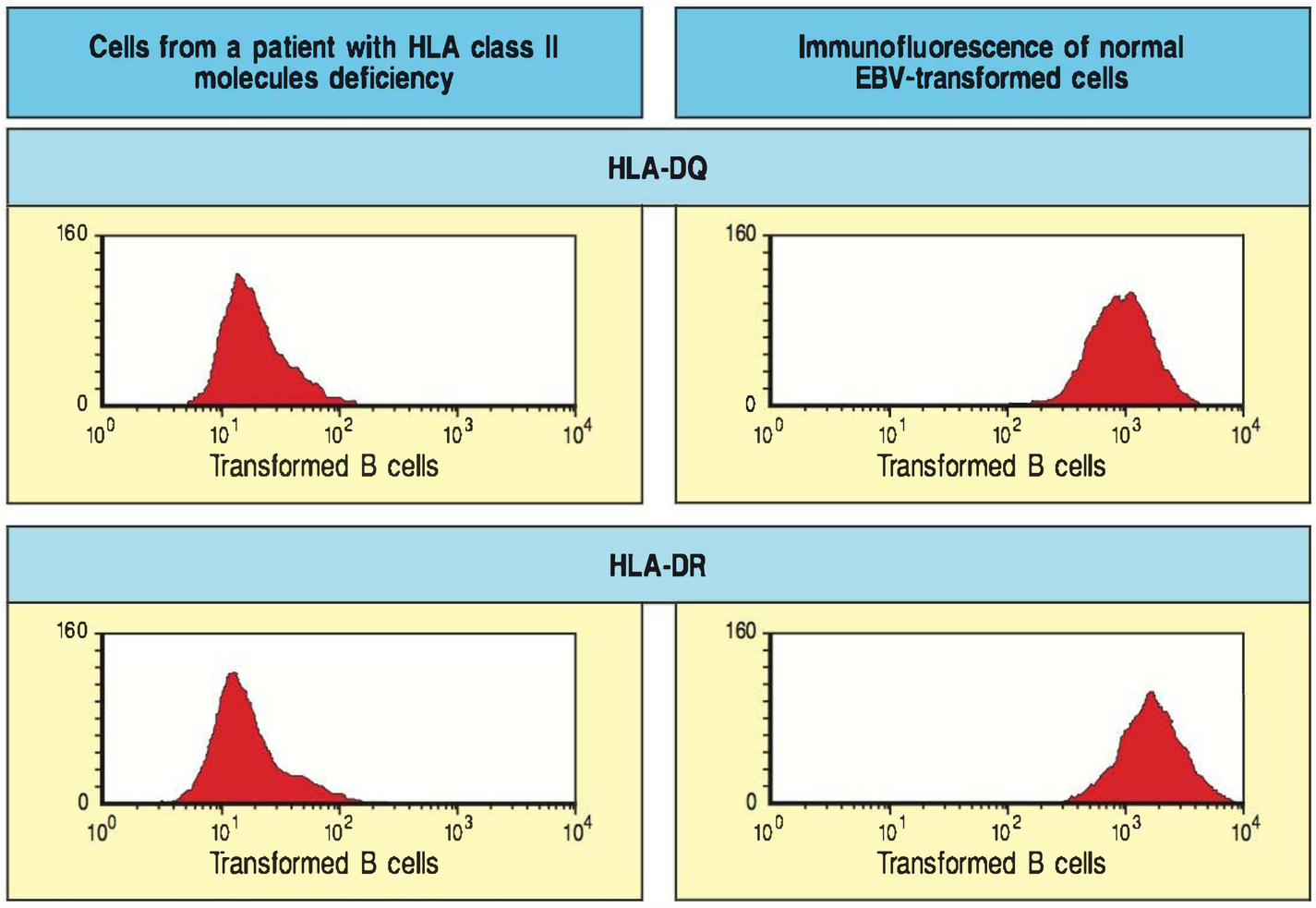
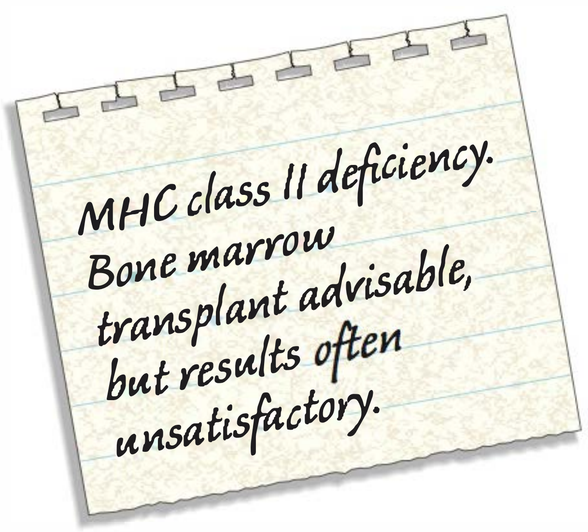


Fig. 8.4 Detection of MHC class II molecules by fluorescent antibody. Helen's transformed B-cell line was examined by using a fluorescent antibody against HLA-DQ and HLA-DR. Helen (left panels) expressed approximately 1% of the amount of MHC class II molecules compared with a transformed B-cell line from a normal control (right panels).



Her brother was found to have the same HLA type as Helen, and therefore was chosen as a bone marrow donor. Helen was given 1 mg kg^{-1} of body weight of the cytotoxic drug busulfan every 6 hours for 4 days and then 50 mg kg^{-1} cyclophosphamide each day for 4 days to ablate her bone marrow. The brother's bone marrow was administered to Helen by transfusion without any *in vitro* manipulation. The graft was successful and immune function was restored.

MHC class II deficiency.

MHC class II deficiency is inherited as an autosomal recessive trait. Health problems show up early in infancy. Affected babies present the physician with a mild form of combined immunodeficiency as they have increased susceptibility to pyogenic and opportunistic infections. However, they differ from infants with severe combined immunodeficiency (SCID; see Case 5) in that they have T cells, which can respond to nonspecific T-cell mitogens such as PHA and to allogeneic stimuli. Unlike in some other types of immunodeficiency, progressive infection with the attenuated live vaccine strain BCG has not been observed in MHC class II-deficient patients after BCG vaccination against tuberculosis (most cases of MHC class II deficiency have been observed in North African migrants in Europe, where BCG vaccination is routine). This is because mycobacterial antigens derived from BCG can be presented on MHC class I molecules and infected cells can be destroyed by cytotoxic T cells. In contrast, and for reasons that are unclear so far, patients with MHC class II deficiency are highly prone to severe viral infections.

Patients with MHC class II deficiency are deficient in CD4 T cells, in contrast with MHC class I deficiency, in which CD8 T-cell numbers are very low and

the levels of CD4 T cells are normal (see Case 12). Typically, patients with MHC class II deficiency also have moderate to severe hypogammaglobulinemia.

Hematopoietic stem cell transplantation (HSCT) is the treatment of choice for patients with MHC class II deficiency. Helen Burns was cured after a bone marrow transplant from her HLA-identical brother. However, the results of HSCT in patients with MHC class II deficiency, even when transplanted from HLA-identical donors, are often not satisfactory, and the number of circulating CD4 T cells frequently remains low. This is likely to be because positive selection of donor-derived CD4 thymocytes is compromised, owing to a lack of MHC class II molecules on the surface of the patient's thymic epithelial cells.

Genetic linkage analysis in large extended families with MHC class II deficiency has shown that this condition is not linked to the MHC locus on the short arm of chromosome 6 and that the genes encoding the MHC class II molecules at this locus are normal. Interferon-γ induces the expression of MHC class II molecules on antigen-presenting cells from normal people but fails to induce their expression on the antigen-presenting cells of patients with MHC class II deficiency. This suggested that the defect might lie in the regulation of expression of the MHC class II genes.

The search for the cause of the defect was complicated further by the discovery that MHC class II deficiency in different patients seems to have different causes. B-cell lines isolated from class II-deficient patients do not express MHC class II molecules. However, when B cells from two different patients are fused, MHC class II expression is often observed. The fusion of the two cell lines has corrected the defect. This means that one cell must be able to replace whatever is lacking in the other, and thus the two cells must carry different genetic defects causing the MHC class II deficiency. Pairwise fusions were performed on a large number of cell lines from different patients, and four complementation groups were found (Fig. 8.5).

These experiments provided clues that led eventually to the identification of the defect. The lack of MHC class II molecules turns out to result from defects in the transcription factors required to regulate their coordinated expression. All four of these transcription factors, which bind to the 5′ regulatory region of the MHC class II genes, have been identified.

	A	B	C	D
A	–	+	+	+
B	+	–	+	+
C	+	+	–	+
D	+	+	+	–

Fig. 8.5 Complementation groups of MHC class II deficiency. B-cell lines isolated from different patients were fused in all pairwise combinations to determine whether they could correct each other's defect. If two cell lines do not correct each other (–), they are in the same complementation group and have the same genetic defect. However, if the defect is corrected (+), the two cell lines belong to two different complementation groups and have two different defects. Four complementation groups, A, B, C, and D, were discovered by this technique.

Questions.

1 *Why did Helen lack CD4 T cells in her blood?*

2 *Why did Helen have a low level of immunoglobulins in her blood?*

3 *In SCID, lymphocytes fail to respond to mitogenic stimuli. Although Helen was first thought to have SCID, this diagnosis was eliminated by her normal response to PHA and an allogeneic stimulus. How do you explain these findings?*

4 *If a skin graft were to be placed on Helen's forearm do you think she would reject the graft?*

CASE 2 CD40 Ligand Deficiency

Failure of immunoglobulin class switching.

After exposure to an antigen, the first antibodies to appear are IgM. Later, antibodies of other classes appear: IgG predominates in the serum and extravascular space, while IgA is produced in the gut and in the respiratory tract, and IgE may also be produced in the mucosal tissues. The different effector functions of these different antibody classes are summarized in Fig. 2.1. The changes in the class of the antibody produced in the course of an immune response reflect the occurrence of heavy-chain isotype switching in the B cells that synthesize immunoglobulin, so that the heavy-chain variable (V) region, which determines the specificity of an antibody, becomes associated with

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	–	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.04	9	3	1	0.5	2.1	3×10^{-5}

Fig. 2.1 Each human immunoglobulin isotype has specialized functions and a unique distribution. The major effector functions of each isotype (+++) are shaded in dark red, while lesser functions (++) are shown in dark pink, and very minor functions (+) in pale pink. The distributions are similarly marked, with the actual average levels in serum shown in the bottom row. *IgG2 can act as an opsonin in the presence of Fc receptors of a particular allotype, found in about 50% of Caucasians.

Topics bearing on this case:

- Isotype or class switching
- Antibody isotypes and classes
- CD40 ligand and class switching
- Antibody-mediated bacterial killing

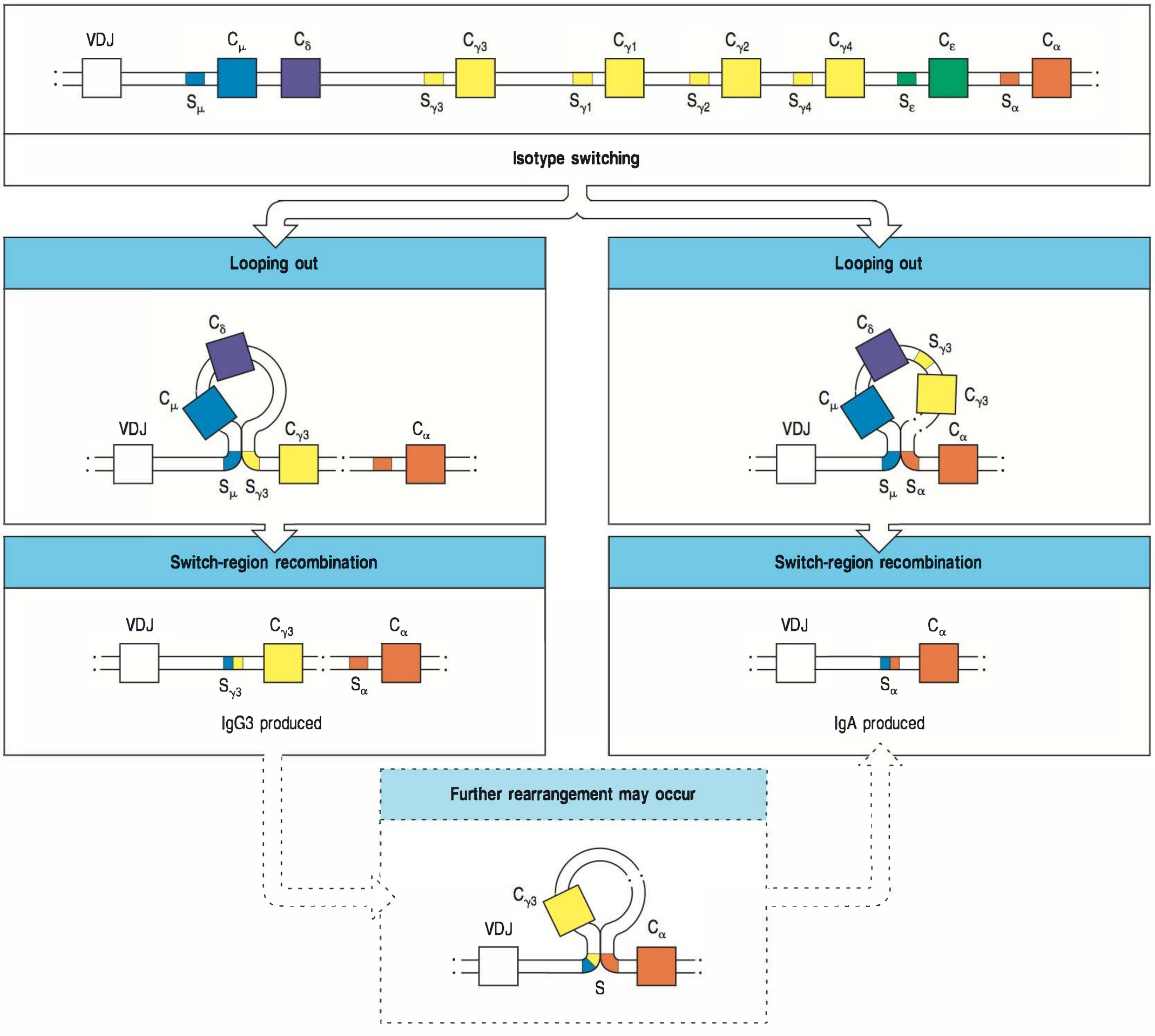


Fig. 2.2 Isotype switching involves recombination between specific switch signals. Repetitive DNA sequences that guide isotype switching are found upstream of each of the immunoglobulin C-region genes, with the exception of the C_δ gene. Switching occurs by recombination between these repetitive sequences or switch signals as a result of the repair of double-strand breaks (see Case 3), with deletion of the intervening DNA.The initial switching event takes place from the μ switch region (S_μ): switching to other isotypes can take place subsequently from the recombinant switch region formed after μ switching. S, switch region.

heavy-chain constant (C) regions of different isotypes, which determine the class of the antibody, as the immune response progresses (Fig. 2.2).

Class switching in B cells, also known as isotype switching and class-switch recombination, is induced mainly by T cells, although it can also be induced by T-cell independent Toll-like receptor (TLR)-mediated signaling. T cells are required to initiate B-cell responses to many antigens; the only exceptions are responses triggered by some microbial antigens or by certain antigens with repeating epitopes. This T-cell 'help' is delivered in the context of an antigen-specific interaction with the B cell (Fig. 2.3). The interaction activates the T cell to express the cell-surface protein CD40 ligand (CD40L, also known as CD154), which in turn delivers an activating signal to the B cell by binding CD40 on the B-cell surface. Activated T cells secrete cytokines, which are required at the initiation of the humoral immune response to drive the proliferation and differentiation of naive B cells, and are later required to induce class switching (Fig. 2.4). In humans, class switching to IgE synthesis is best understood, and is known to require interleukin-4 (IL-4) or IL-13, as well as stimulation of the B cell through CD40.

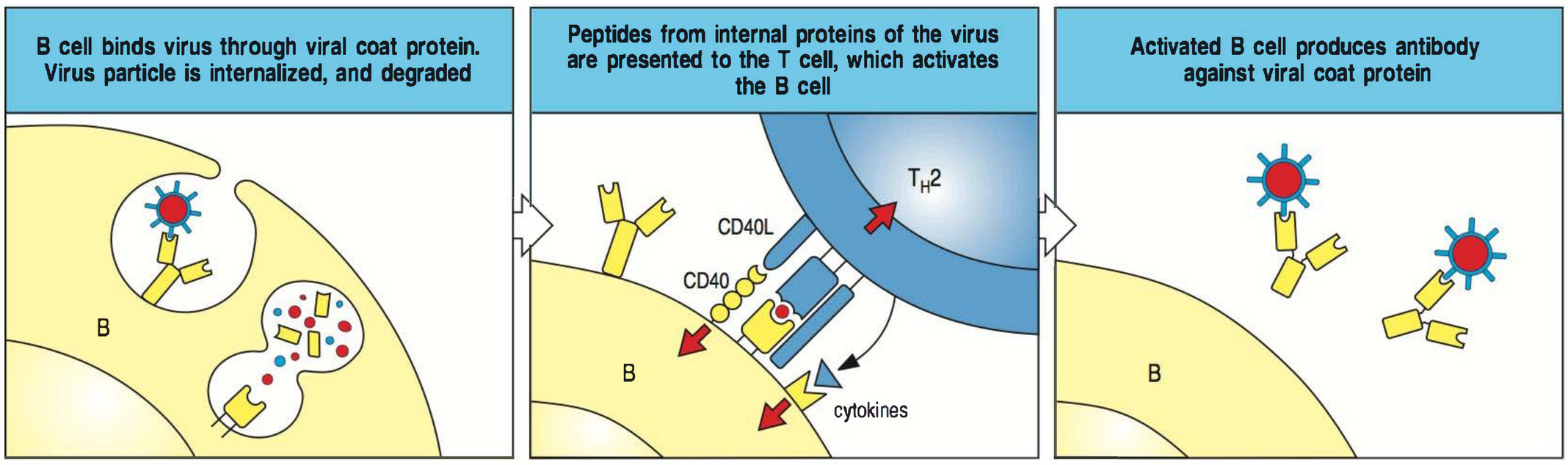


Fig. 2.3 B cells are activated by helper T cells that recognize antigenic peptide bound to class II molecules on their surface. An epitope on a viral coat (spike) protein is recognized by the surface immunoglobulin on a B cell, and the virus is internalized and degraded. Peptides derived from viral proteins are returned to the B-cell surface bound to MHC class II molecules, where they are recognized by previously activated helper T cells that activate the B cells to produce antibody against the virus.

The gene for CD40L (*CD40LG*) is located on the X chromosome at position Xq26. In males with a defect in this gene, isotype switching fails to occur; such individuals make only IgM and IgD and are severely impaired in their ability to switch to IgG, IgA, or IgE synthesis. This phenotype is known generally as 'hyper IgM syndrome,' and can also be due to defects other than the absence of CD40L (see Case 3). Similarly, defective class switching is also observed in patients with CD40 deficiency, a rare autosomal recessive condition. Defects in class switching can be mimicked in mice in which the genes for CD40 or CD40L have been disrupted by gene targeting; B cells in these animals fail to undergo switching. The underlying defect in patients with CD40L deficiency can be readily demonstrated by isolating their T cells and challenging them with soluble, fluorescently labeled CD40 (made by engineering the extracellular domain of CD40 onto the constant region (Fc) of IgG) or with monoclonal antibodies that recognize the CD40-binding epitope of CD40L. *In vitro* activated T cells from patients with CD40L deficiency fail to bind the soluble CD40–Fc (Fig. 2.5).

CD40 is expressed not only on B cells but also on the surfaces of macrophages, dendritic cells, follicular dendritic cells (FDCs), mast cells, and some epithelial and endothelial cells. Macrophages and dendritic cells are antigen-presenting

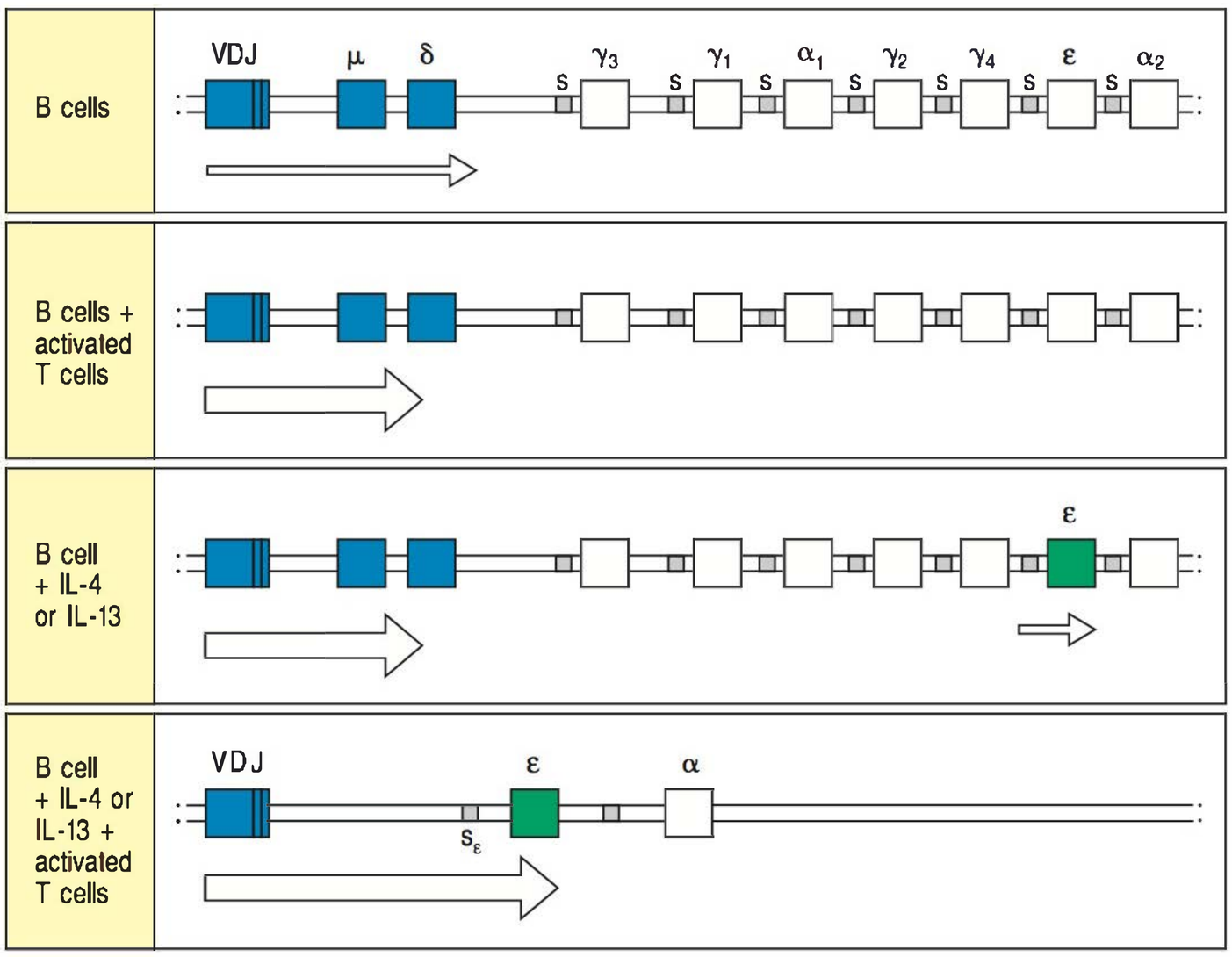


Fig. 2.4 Class switching to IgE production by human B cells. Purified human B cells in culture transcribe the μ and δ loci at a low rate, giving rise to surface IgM and IgD. On co-culture with T cells activated with ionomycin and phorbol myristate acetate (PMA), IgM is secreted. The presence of IL-4 or IL-13 stimulates an isotype switch to IgE. Purified B cells cultured alone with these cytokines transcribe the Cε gene at a low rate, but the transcripts originate in the switch region preceding the gene and do not code for protein. On co-culture with activated T cells in addition to IL-4 or IL-13, an isotype switch occurs, mature ε RNA is expressed, and IgE is synthesized.

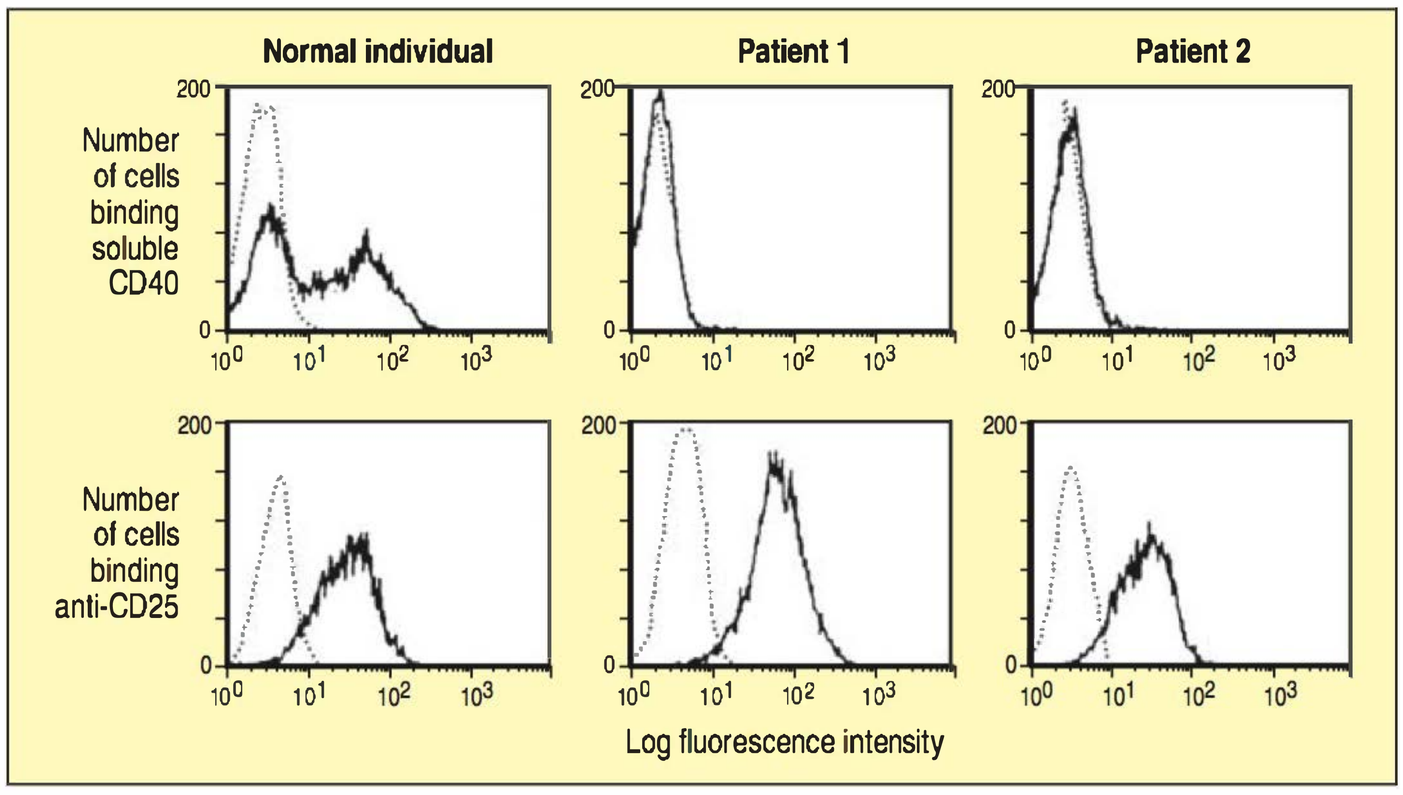


Fig. 2.5 Flow cytometric analysis showing that activated T cells from hyper IgM patients do not express the CD40 ligand. T cells from two patients and one healthy donor were activated *in vitro* with a T-cell mitogen, incubated with soluble CD40 protein, and analyzed by flow cytometry (see Fig. 1.3). The results are shown in the top three panels. In the normal individual, there are two populations of cells: one that does not bind CD40 (the peak to the left, with low-intensity fluorescence) and one that does (the peak to the right, with high-intensity fluorescence). The dotted line is the negative control, showing nonspecific binding of a fluorescently labeled protein to the same cells. In the patients with CD40L deficiency (center and right-hand panels), CD40 fluorescence exactly coincides with the nonspecific control, showing that there is no specific binding to CD40 by these cells. The bottom panels show that the T cells have been activated by the mitogen, because the T cells of both the normal individual and the two patients have increased expression of the IL-2 receptor (CD25), as expected after T-cell activation. The negative control is fluorescent goat anti-mouse immunoglobulin.

cells that can trigger the initial activation and expansion of antigen-specific T cells at the start of an immune response. Experiments in CD40L- or CD40-deficient patients and in gene-targeted CD40L-deficient mice indicate a role for the CD40–CD40L interaction in this early priming event, because in the absence of either CD40L or CD40 the initial activation and expansion of T cells in response to protein antigens is greatly reduced. The impairment of T-cell activation is the basis of some severe clinical features that distinguish CD40L and CD40 deficiency from other conditions characterized by a pure antibody deficiency.

The case of Dennis Fawcett: a failure of T-cell help.

Dennis Fawcett was 5 years old when he was referred to the Children's Hospital with a severe acute infection of the ethmoid sinuses (ethmoiditis). His mother reported that he had had recurrent sinus infections since he was 1 year old. Dennis had pneumonia from an infection with *Pneumocystis jirovecii* when he was 3 years old. These infections were treated successfully with antibiotics. While he was in the hospital with ethmoiditis, group A β-hemolytic streptococci were cultured from his nose and throat. The physicians caring for Dennis expected that he would have a brisk rise in his white blood cell count as a result of his severe bacterial infection, yet his white blood cell count was 4200 μl^{-1} (normal count 5000–9000 μl^{-1}). Sixteen percent of his white blood cells were neutrophils, 56% were lymphocytes, and 28% were monocytes. Thus his neutrophil number was low, whereas his lymphocyte number was normal and the number of monocytes was elevated.

Seven days after admission to the hospital, during which time he was successfully treated with intravenous antibiotics, his serum was tested for antibodies against streptolysin O, an antigen secreted by streptococci. When no antibodies against the streptococcal antigen were found, his serum immunoglobulins were measured. The IgG level was 25 mg dl^{-1} (normal 600–1500 mg dl^{-1}), IgA was undetectable (normal 150–225 mg dl^{-1}), and his IgM level was elevated at 210 mg dl^{-1} (normal 75–150 mg dl^{-1}). A lymph-node biopsy showed poorly organized structures with an absence of secondary follicles and germinal centers (Fig. 2.6).

Dennis was given a booster injection of diphtheria toxoid, pertussis antigens, and tetanus toxoid (DPT) as well as typhoid vaccine. After 14 days, no antibody was detected against tetanus toxoid or against typhoid O and H antigens. Dennis had red blood

cells of group O. People with type O red blood cells make antibodies against the A substance of type A red cells and antibodies against the B substance of type B red cells. This is because bacteria in the intestine have antigens that are closely related to A and B antigens. Dennis's anti-A titer was 1:3200 and his anti-B titer 1:800, both very elevated. His anti-A and anti-B antibodies were of the IgM class only.

His peripheral blood lymphocytes were examined by fluorescence-activated cell sorting analysis, and normal results were obtained: 11% reacted with an antibody against CD19 (a B-cell marker), 87% with anti-CD3 (a T-cell marker), and 2% with anti-CD56 (a marker for natural killer (NK) cells). However, all of his B cells ($CD19^+$) had surface IgM and IgD and none were found with surface IgG or IgA. When his T cells were activated *in vitro* with phorbol ester and ionomycin (a combination of potent polyclonal T-cell activators), they did not bind soluble CD40.

Dennis had an older brother and sister. They were both well. There was no family history of unusual susceptibility to infection.

Dennis was treated with intravenous gamma globulin, 600 mg kg^{-1} body weight each month, and subsequently remained free of infection until 15 years of age, when he developed severe, watery diarrhea. Cultures of the stools grew *Cryptosporidium parvum*. Within a few months, during which his diarrhea persisted in spite of treatment with the antibiotic azithromycin, he developed jaundice. His serum total bilirubin level was 8 mg dl^{-1}, and the serum level of conjugated bilirubin was 7 mg dl^{-1}. In addition, levels of γ-glutamyl transferase (γ-GT) and of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were elevated at 93 IU l^{-1}, 120 IU ml^{-1} and 95 IU ml^{-1}, respectively, suggesting cholestasis. A liver biopsy showed abnormalities of biliary ducts (vanishing bile ducts) that progressed to sclerosing cholangitis (chronic inflammation and fibrosis of the bile ducts). In spite of supportive treatment, Dennis died of liver failure at 21 years of age.

Lymph node from a patient with CD40L deficiency (no germinal centers)

Lymph node with germinal centers

Fig. 2.6 Comparison of lymph nodes from a patient with CD40L deficiency (upper panel) and a normal individual (lower panel). Lower photograph courtesy of A. Perez-Atayde.

CD40 ligand deficiency (CD40L deficiency).

Males with a hereditary deficiency of CD40L exhibit consequences of a defect in both humoral and cell-mediated immunity. As we saw in Case 1, defects in antibody synthesis result in susceptibility to so-called pyogenic infections. These infections are caused by pyogenic (pus-forming) bacteria such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*, which are resistant to destruction by phagocytic cells unless they are coated (opsonized) with antibody and complement. On the other hand, defects in cellular immunity result in susceptibility to opportunistic infections. Bacteria, viruses, fungi, and protozoa that often reside in our bodies and only cause disease when cell-mediated immunity in the host is defective are said to cause opportunistic infections.

Dennis revealed susceptibility to both kinds of infection. His recurrent sinusitis, as we have seen, was caused by *Streptococcus pyogenes*, a pyogenic infection. He also had pneumonia caused by *Pneumocystis jirovecii* and diarrhea caused by *Cryptosporidium parvum*, a fungus and a protozoan, respectively, that are ubiquitous and cause opportunistic infections in individuals with defects in cell-mediated immunity.

Patients with a CD40L deficiency can make IgM in response to T-cell independent antigens but they are unable to make antibodies of any other isotype, and they cannot make antibodies against T-cell dependent antigens, which leaves the patient largely unprotected from many bacteria. They also have a defect in cell-mediated immunity that strongly suggests a role for CD40L in the T cell-mediated activation of macrophages. *Cryptosporidium* infection

can cause persistent inflammation in the liver, and ultimately sclerosing cholangitis and liver failure. In addition, individuals with CD40L deficiency have severe neutropenia, with a block at the promyelocyte/myelocyte stage of differentiation in the bone marrow. Although the mechanisms underlying the neutropenia in these patients remain unclear, the lack of neutrophils accounts for the presence of severe sores and blisters in the mouth. The neutropenia and its consequences can often be overcome by administering recombinant granulocyte-colony stimulating factor (G-CSF).

Treatment of CD40L deficiency is based on immunoglobulin replacement therapy, prophylaxis with trimethoprim-sulfamethoxazole to prevent *Pneumocystis jirovecii* infection, and protective measures to reduce the risk of *Cryptosporidium* infection (such as avoiding swimming in lakes or drinking water with a high concentration of *Cryptosporidium* cysts). In spite of this, many patients with CD40L deficiency die in late childhood or adulthood of infections, liver disease, or tumors (lymphomas and neuroectodermal tumors of the gut). The disease can be cured by hematopoietic cell transplantation, and this treatment should be considered when HLA-identical donors are available and when the first signs of severe complications become manifest.

Few cases of CD40 deficiency have been reported. Its clinical and immunological features, and its treatment, are very similar to CD40L deficiency, but the disease is inherited as an autosomal recessive trait.

Questions.

1 *Dennis's B cells expressed IgD as well as IgM on their surface. Why did he not have any difficulty in isotype switching from IgM to IgD?*

2 *Normal mice are resistant to Pneumocystis jirovecii. SCID mice, which have no T or B cells but have normal macrophages and monocytes, are susceptible to this microorganism. In normal mice, Pneumocystis jirovecii organisms are taken up and destroyed by macrophages. Macrophages express CD40. When SCID mice are reconstituted with normal T cells they acquire resistance to Pneumocystis infection. This can be abrogated by antibodies against the CD40 ligand. What do these experiments tell us about this infection in Dennis?*

3 *Why did Dennis make antibodies against blood group A and B antigens but not against tetanus toxoid, typhoid O and H, and streptolysin antigens? Would he have made any antibodies in response to his Streptococcus pyogenes infection?*

4 *Most IgM is circulating in the blood, and less than 30% of IgM molecules get into the extravascular fluid. On the other hand, more than 50% of IgG molecules are in the extravascular space. Furthermore we have 30–50 times more IgG than IgM in our body. Why are IgG antibodies more important in protection against pyogenic bacteria?*

5 *Newborns have difficulty in transcribing the gene for CD40L. Does this help to explain the susceptibility of newborns to pyogenic infections? Cyclosporin A, a drug widely used for immunosuppression in graft recipients, also inhibits transcription of the gene for CD40L. What does this imply for patients taking this drug?*

CASE 30 Congenital Asplenia

The role of the spleen in immunity.

The adaptive immune response occurs mainly in the peripheral lymphoid tissues—the lymph nodes, the gut-associated lymphoid tissue, and the spleen (Fig. 30.1). Pathogens and their secreted antigens are trapped in these tissues and presented to the naive lymphocytes that constantly pass through. Microorganisms that enter the body through the skin or the lungs drain to regional lymph nodes, where they stimulate an immune response. Microorganisms and food antigens that enter the gastrointestinal tract are collected in the gut-associated lymphoid tissue. Microbes that enter the bloodstream stimulate an immune response in the spleen.

The spleen is organized to accomplish two functions (Fig. 30.2). In addition to being a peripheral lymphoid organ, it acts as a filter of the blood to remove aged or abnormal red cells and other extraneous particles that may enter the bloodstream, including microorganisms. In the absence of a functioning spleen, these aged and abnormal red blood cells can be seen in a peripheral blood smear in the form of pitted red blood cells and Howell–Jolly bodies (nuclear remnants in red blood cells that are usually removed by the spleen) (Fig. 30.3).

The lymphoid function of the spleen is performed in the white pulp, and the filtration function by the red pulp. Many microorganisms are recognized directly and engulfed by the phagocytes of the red pulp. Others are not removed efficiently until they are coated by antibodies generated in the white pulp. In experimental animals, an immune response (as measured by

This case was prepared by Raif Geha, MD, in collaboration with Itai Pessach, MD.

Topics bearing on this case:

- Circulation of lymphocytes through peripheral lymphoid tissues
- Toxoid vaccines
- Hemagglutination tests

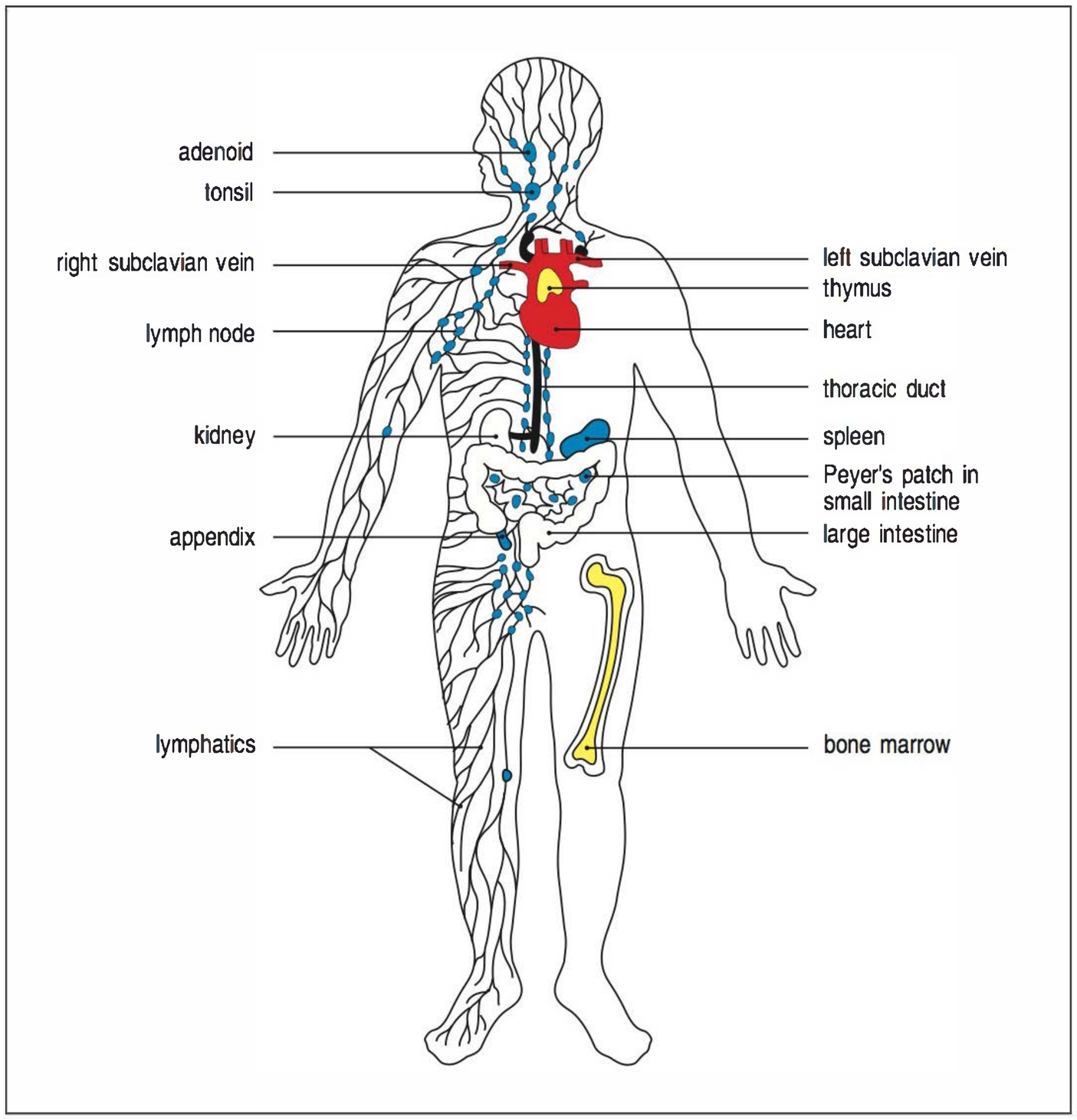


Fig. 30.1 The distribution of lymphoid tissues in the body. Lymphocytes arise from stem cells in bone marrow, and differentiate in the central lymphoid organs (yellow)—B cells in bone marrow and T cells in the thymus. They migrate from these tissues through the bloodstream to the peripheral lymphoid tissues (blue)—the lymph nodes, spleen, and mucosa-associated lymphoid tissues such as tonsils, Peyer's patches, and appendix. These are the sites of lymphocyte activation by antigen. Lymphatics drain extracellular fluid as lymph through the lymph nodes and into the thoracic duct, which returns the lymph to the bloodstream by emptying into the left subclavian vein. Lymphocytes that circulate in the bloodstream enter the peripheral lymphoid organs, and are eventually carried by lymph to the thoracic duct, where they reenter the bloodstream.

antibody formation) can be detected in the white pulp of the spleen about 4 days after the intravenous injection of a dose of microorganisms. The clearance of antibody- and complement-coated bacteria or viruses by the phagocytic cells of the red pulp of the spleen is very rapid. Rapid clearance from the blood is important because it prevents these bacteria from disseminating and causing infections of the meninges (meningitis), the kidney (pyelonephritis), the lung (pneumonia), or other distant anatomical sites.

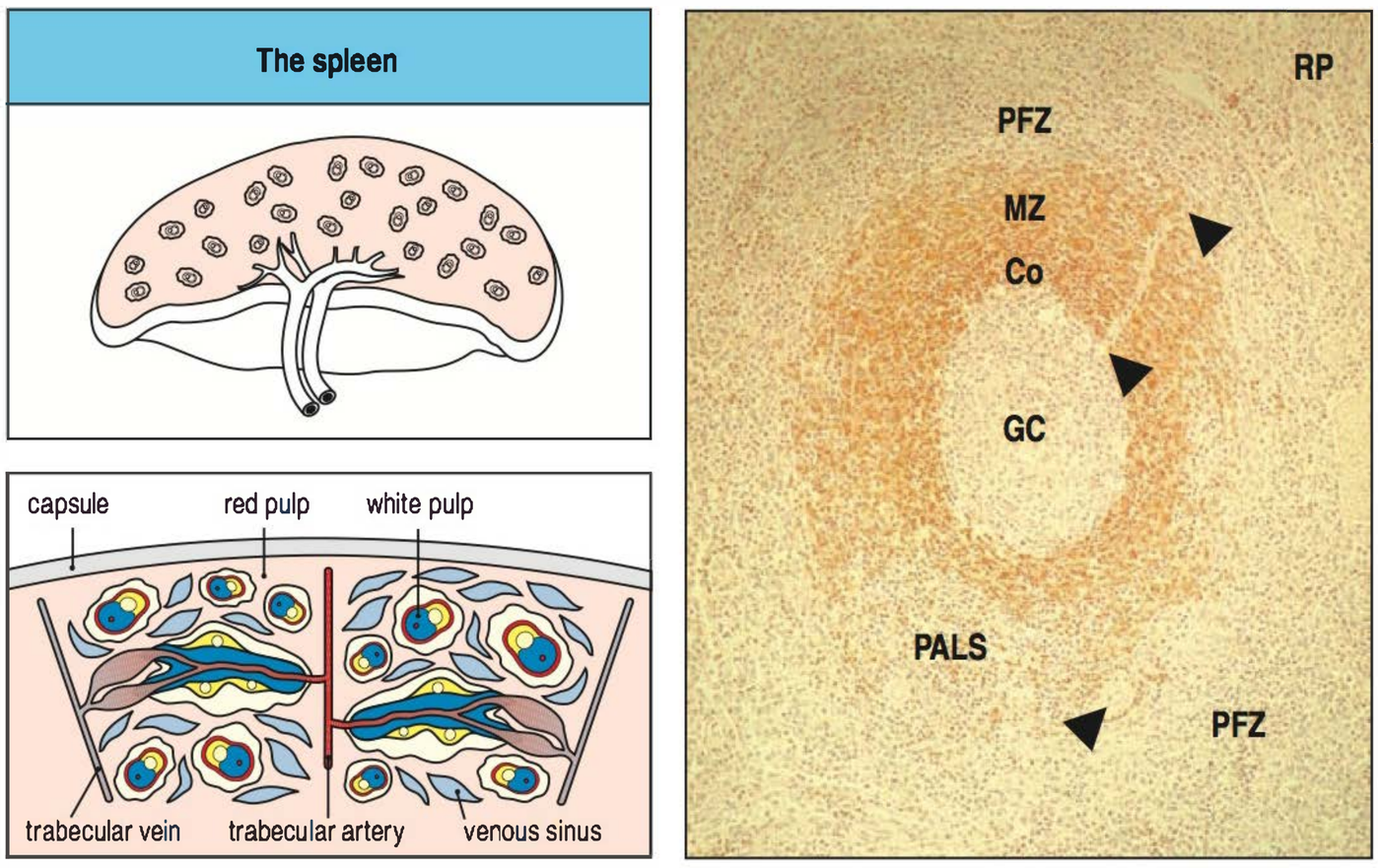


Fig. 30.2 Schematic views and light micrograph of a section of human spleen. The schematic at top left shows that the spleen consists of red pulp (pink areas), which is a site of red blood cell destruction, interspersed with the lymphoid white pulp. An enlargement of an area of white pulp is shown below. The follicle (yellow in the middle panel) and T-cell areas (blue) are surrounded by the perifollicular zone (PFZ) (palest yellow). The light micrograph on the right shows a transverse section of white pulp immunostained for mature B cells. The follicular arteriole emerges in the periarteriolar lymphoid sheath (PALS) of T cells (lower arrowhead in the bottom panel), traverses the follicle, goes through the marginal zone and opens into the perifollicular zone (upper arrowheads). Co, follicular B-cell corona; GC, germinal center (activated B cells); MZ, marginal zone (B cells); RP, red pulp; arrowheads, central arteriole. Photograph courtesy of N.M. Milicevic.

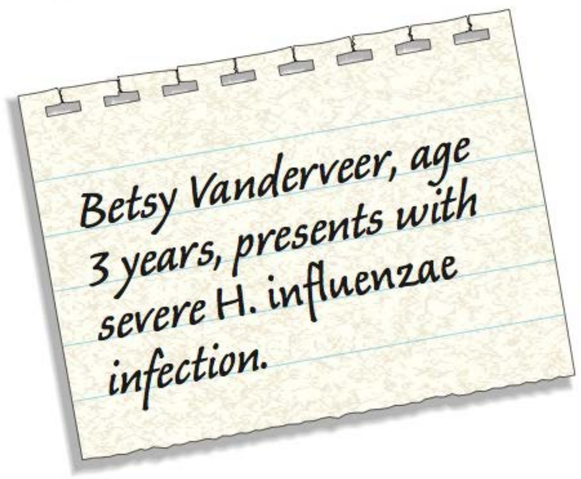
Fig. 30.3 The arrow marks a red blood cell with Howell–Jolly body. These inclusions are formed by the retention of nuclear remnants in red cells, which are usually removed by the spleen but can be found in patients with asplenia or significantly decreased splenic function.

Bacteria enter the bloodstream all the time, such as when we brush our teeth or when we have a local infection, for example of the skin or middle ear. Normally, these bacteria are disposed of efficiently by the spleen. When, for one reason or another, the spleen is not present, serious, even fatal, infections occur.

The case of Susan Vanderveer: a fatality because of an absent spleen.

Mr and Mrs Vanderveer owned a farm in the Hudson Valley in lower New York State. They were both descended from Dutch settlers who came to the Hudson Valley in the mid-17th century. There were multiple consanguineous marriages among their ancestors, and Mr and Mrs Vanderveer were distantly related to each other. At the time of this case, they had five children—three girls and two boys. Their youngest daughter, Susan, was 10 months old when she developed a cold, which lasted for 2 weeks. On the 14th day of her upper respiratory infection, she became sleepy and felt very warm. Her mother found that her temperature was 41.7°C. When Susan developed convulsive movements of her extremities, she was rushed to the emergency room but she died on the way to the hospital. Post-mortem cultures of blood were obtained, and also from her throat and cerebrospinal fluid. All the cultures grew *Haemophilus influenzae*, type b. At autopsy Susan was found to have no spleen.

At the time of Susan's death her 3-year-old sister, Betsy, also had a fever, of 38.9°C. She complained of an earache, and her eardrums were found to be red. She had no other complaints and no other abnormalities were detected on physical examination. Her white blood count was 28,500 cells μl^{-1} (very elevated). Cultures from her nose, throat, and blood grew out *H. influenzae*, type b. She was given ampicillin intravenously for 10 days in the hospital and was then sent home in good health. Her cultures were negative at the time of discharge from the hospital. She was seen by a pediatrician on three occasions during the following year for otitis media (inflammation of the middle ear), pneumonia, and mastoiditis (inflammation of the mastoid bone behind the ear).



David, Susan's 5-year-old brother, had been admitted to the hospital at 21 months of age with meningitis caused by *Streptococcus pneumoniae*. He had responded well to antibiotic therapy and had been discharged. Another occurrence of pneumococcal meningitis at 27 months of age had also been followed by an uneventful recovery after antibiotics. He had had pneumonia at age 3½ years. At the time of Susan's death he was well.

The two other children of the Vanderveers, a girl aged 8 years and a newborn male, were in good health.

All the Vanderveer children had received routine immunization at ages 3, 4, and 5 months with tetanus and diphtheria toxoids and killed *Bordetella pertussis* to protect against tetanus, diphtheria, and whooping cough, which are three potentially fatal diseases caused by bacterial toxins (Fig. 30.4). Serum agglutination tests were used to test their antibody responses to these and other immunogens. Samples of serum from both Betsy and David caused hemagglutination (the clumping of red blood cells) when added to red blood cells (type O) coated with tetanus toxoid. Hemagglutinating antibodies against tetanus toxoid were seen at serum dilutions of 1:32 for both Betsy

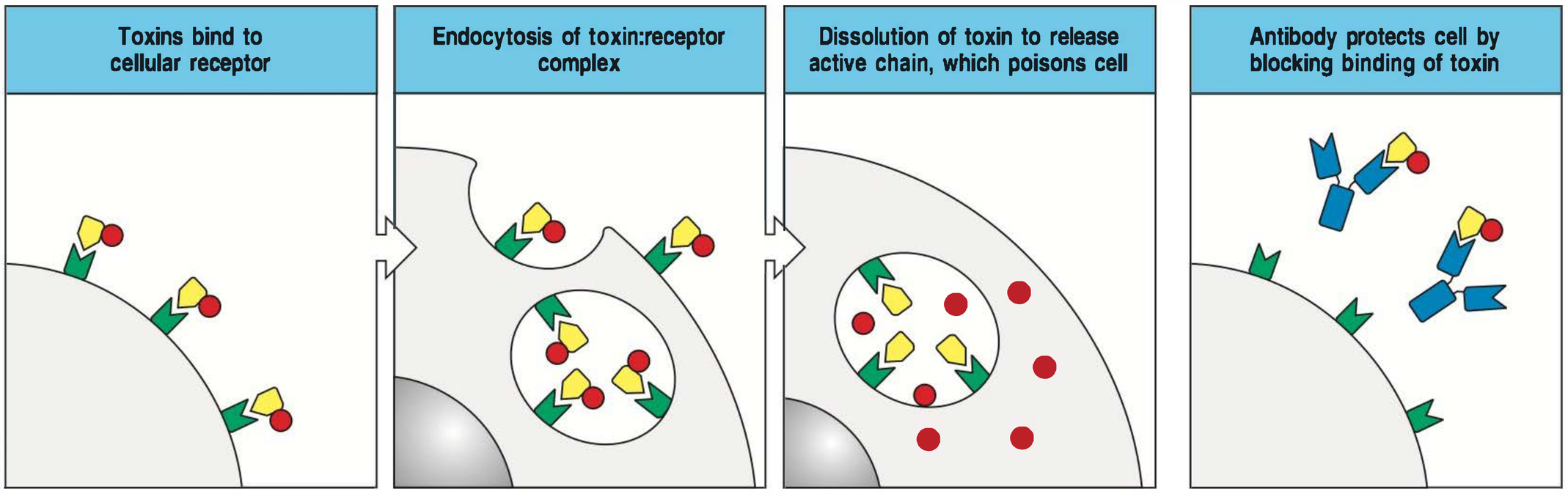
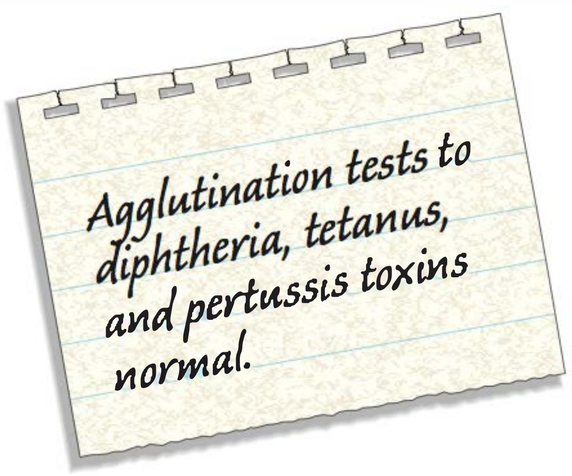


Fig. 30.4 Neutralization by antibodies protects cells from toxin action. Secreted bacterial toxins usually contain several distinct moieties. One piece of the toxin must bind a cellular receptor, which allows the molecule to be internalized. A second part of the toxin molecule then enters the cytoplasm and poisons the cell. In some cases, a single molecule of toxin can kill a cell. Antibodies that inhibit toxin binding can prevent, or neutralize, these effects. Protective antibodies can be generated by subcutaneous immunization with toxoids. Toxoids are toxins rendered harmless by treatment with denaturing agents, such as formalin, which destroy their toxicity but not their ability to generate neutralizing antibodies. In the case of the DPT vaccine, the killed *Bordetella pertussis* cells act as an adjuvant, which enhances the immune response to all components of the vaccine by delivering activating signals to antigen-presenting cells.



and David, and were found at a similar titer in their 8-year-old sister. All three children were given typhoid vaccine subcutaneously, and 4 weeks later samples of their sera were tested for the ability to agglutinate killed *Salmonella typhosa*. The results indicated a normal immune response. David had an agglutination titer of 1:16, Betsy 1:32, and their normal 8-year-old sister 1:32. All three children were given 1 ml of a 25% suspension of sheep red cells intravenously. David had a titer of 1:4 for hemagglutinating antibodies against sheep red blood cells before the injection. He was tested again 2 and 4 weeks later and there was no increase in titer. Betsy had an initial titer of 1:32 and her titer did not increase either. The 8-year-old normal sister had a preimmunization titer of 1:32. She was tested 2 and 4 weeks after the immunization, when she was found to have a hemagglutinating titer of 1:256 against sheep red blood cells.

All the children and their parents were injected intravenously with radioactive colloidal gold (^{198}Au), which is taken up by the reticuloendothelial cells of the liver and spleen within 15 minutes after the injection. A scintillation counter then scans the abdomen for radioactive gold. The pattern of scintillation revealed that Betsy and David had no spleens (Fig. 30.5).

Asplenia and splenectomy.

Significant impairment of splenic function can be either congenital, where the spleen is absent or dysfunctional at birth, or acquired as a result of conditions that damage the spleen such as trauma or sickle-cell anemia, and which often lead to its surgical removal (splenectomy). Congenital asplenia is further divided into two main categories. The more prevalent is syndromic asplenia, in which the lack of splenic tissue is part of a more complex genetic syndrome affecting other systems as well. In these syndromes splenic defects are usually associated with significant heart defects and heterotaxia, a condition in which malformations arise as a result of lateralization defects of organs in the thorax and the abdomen. Several human genes, including *ZIC3*, *LEFTYA*, *CRYPTIC*,

ACVR2B, and *CFC1*, all of which have important roles in directing lateralization, have been shown to be associated with these syndromes.

The second category is isolated congenital asplenia. This is a group of conditions in which the only abnormality is the lack of splenic tissue. Only a relatively small number of cases of true isolated congenital asplenia have so far been described. Most of these cases were diagnosed after episodes of overwhelming pneumococcal infections, either *post mortem* or while screening family members of affected individuals, as in the Vanderveer family. The genetic defect causing asplenia has not yet been identified. Most familial cases described so far follow an autosomal dominant pattern of inheritance, although families with an autosomal recessive or X-linked pattern of inheritance have also been reported.

The Vanderveer family is unusual in that three of their first four children were born without a spleen. After the events described in this case, the Vanderveers had three more children. One of the boys and the girl were also born without a spleen; the other boy had a normal spleen. This family provides us with an uncomplicated circumstance in which to examine the role of the spleen. The major consequence of its absence is a susceptibility to bacteremia, usually caused by the encapsulated bacteria *Streptococcus pneumoniae* or *Haemophilus influenzae*. This susceptibility is caused by a failure of the immune response to these common extracellular bacteria when they enter the bloodstream.

Surgical removal of the spleen is quite common. The capsule of the spleen may rupture from trauma, for example in an automobile accident. In such cases, the spleen has to be removed very quickly because of blood loss into the abdominal cavity. The spleen may also be removed surgically for therapeutic reasons in certain autoimmune diseases, or because of a malignancy in the spleen. After splenectomy, patients, particularly children, are susceptible to bloodstream infections by microorganisms against which they have no antibodies. Microorganisms against which the host has antibodies are removed quickly from the bloodstream by the liver, where the Kupffer cells complement the role of the red pulp of the spleen. Antibodies against the encapsulated bacteria that commonly cause bloodstream infections persist for a very long time in the bloodstream of exposed individuals, even in the absence of a spleen (for reasons that are not fully understood). Adults who already have antibodies against these microorganisms are therefore much less vulnerable to bacteremia than children who have not yet developed such antibodies. Fortunately, effective vaccines against both *S. pneumoniae* and *H. influenzae*, type b, have been developed, and are now part of the routine vaccinations given to many children worldwide, thus protecting asplenic children from some of the severe infections to which they are prone. Nevertheless, specific precautions, including prophylactic antibiotic treatment, are recommended to most individuals with an absent or non-functional spleen.

Fig. 30.5 A scintillation scan of the abdomen after intravenous injection with radioactive colloidal gold (^{198}Au) reveals that Betsy and David Vanderveer have no spleens. The top panel shows an abdominal scan of Betsy's mother. The large mass on the left is the liver and the small mass on the right is the spleen. The reticuloendothelial cells of both liver and spleen take up the labeled gold within 15 minutes after the injection. No spleen is seen in either Betsy (middle panel) or David (bottom panel).

Questions.

1 *Nicholas Biddleboy, a 5-year-old boy, has had his spleen removed after a sledding accident, during which both he and his sled struck a tree trunk. In the emergency room of a nearby hospital, it was determined that his spleen had ruptured. The surgeon, after removal of a spleen that had indeed ruptured, calls you for an immunology consultation. What do you advise?*

2 Why did David and Betsy have normal responses to the typhoid vaccine but not to the sheep red blood cells?

3 The Vanderveer family is unique in the medical literature. The parents, who were distantly related, were normal and had normal spleens. Five of their eight children were born without spleens. Of these, only Betsy subsequently had children—four boys and one girl. They are all normal and have spleens. What is the inheritance pattern of congenital asplenia in this family? According to Mendelian laws how many of the eight Vanderveer children would be expected to have no spleen?

CASE 19 Autoimmune Lymphoproliferative Syndrome (ALPS)

Increased survival of lymphocytes as a result of a mutation in Fas.

When antigen-specific lymphocytes are activated through their antigen receptors, they undergo blast transformation and then begin to increase their numbers exponentially by cell division. This clonal expansion can continue for up to 7 or 8 days, so that lymphocytes specific for the infecting antigen increase vastly in number and can come to predominate in the population. In the response to certain viruses, 50% or more of the CD8 T cells at the peak of the response are specific for a single virus-derived peptide:MHC class I complex. After clonal expansion, the activated lymphocytes undergo their final differentiation into effector cells; these remove the pathogen from the body and so terminate the antigenic stimulus.

When an infection has been overcome, activated effector T cells are no longer needed, and cessation of the antigenic stimulus prompts them to undergo programmed cell death, or apoptosis (Fig. 19.1). Apoptosis is widespread in the immune system and can be induced by several mechanisms; for example, the granule proteins released by cytotoxic T cells kill their target cells by inducing apoptosis. Another well-defined apoptotic pathway is that triggered by the interaction of the receptor molecule Fas with its ligand, called Fas ligand (FasL) (Fig. 19.2), which induces apoptosis in the Fas-bearing cell. FasL is a member of the tumor necrosis factor (TNF) family of membrane-associated cytokines, and Fas is a member of the TNF receptor (TNFR) family. Both Fas and FasL are normally induced on lymphocytes and other cell types during the course of an adaptive immune response. Apoptosis induced by cytotoxic T cells bearing FasL is a minor mechanism of cytotoxicity, whereas apoptosis in lymphocytes themselves, induced through Fas, seems to be an important mechanism of lymphocyte homeostasis, as this case shows. Finally, apoptosis can also be induced through a mitochondria-dependent mechanism (the so-called 'intrinsic pathway' of apoptosis), in which cell damage, cytokine deprivation, and other mechanisms result in an increased release of cytochome *c* contained in mitochondria, and the activation of caspase 9.

Topics bearing on this case:
Lymphocyte survival
Fas–Fas ligand interactions
Apoptosis
Lymphocyte activation
Autoimmune disease
TUNEL staining

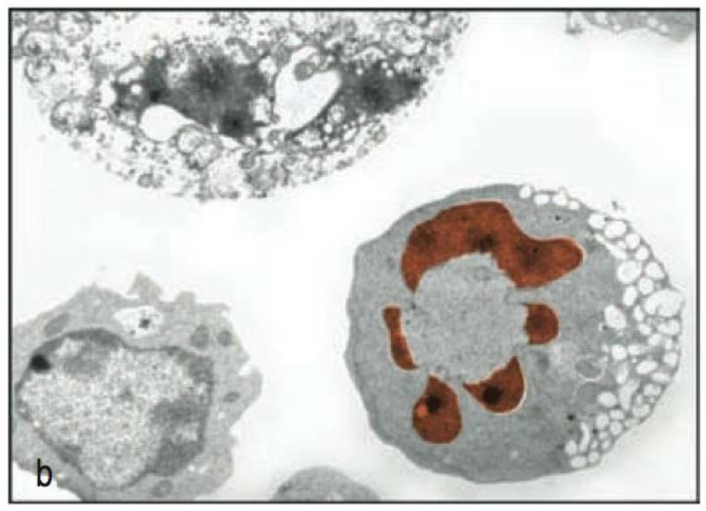
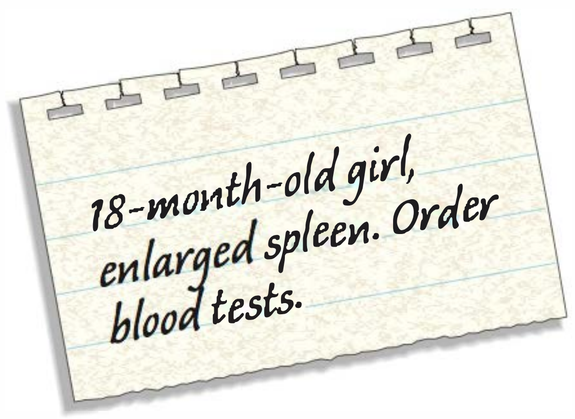


Fig. 19.1 Apoptosis. Apoptosis is a form of induced 'cell suicide' in which the cell undergoes chromatin compaction and DNA fragmentation, followed by cell shrinkage and internal degradation. Panel (a) shows a healthy cell with a normal nucleus. Early in apoptosis (panel b), the chromatin in the nucleus becomes condensed (red) and, although the cell sheds membrane vesicles, the integrity of the cell membrane is retained, in contrast to the necrotic cell in the upper part of the same field. In late stages of apoptosis (panel c), the cell nucleus (middle cell) is very condensed, no mitochondria are visible, and the cell has lost much of its cytoplasm and membrane through the shedding of vesicles. Photographs (× 3500) courtesy of R. Windsor and E. Hirst.



The case of Ellen O'Hara: uncontrolled lymphocyte proliferation in the absence of infection or malignancy.

Ellen O'Hara was born after a normal and uncomplicated pregnancy, was breast fed, and received her routine immunizations without any adverse reactions. At 18 months old, during a routine check-up by her pediatrician, she was found to have an enlarged spleen (splenomegaly) and extensive enlargement of her lymph nodes (lymphadenopathy) (Fig. 19.3). According to her parents, she had had no unusual infections and seemed to be growing and developing normally.

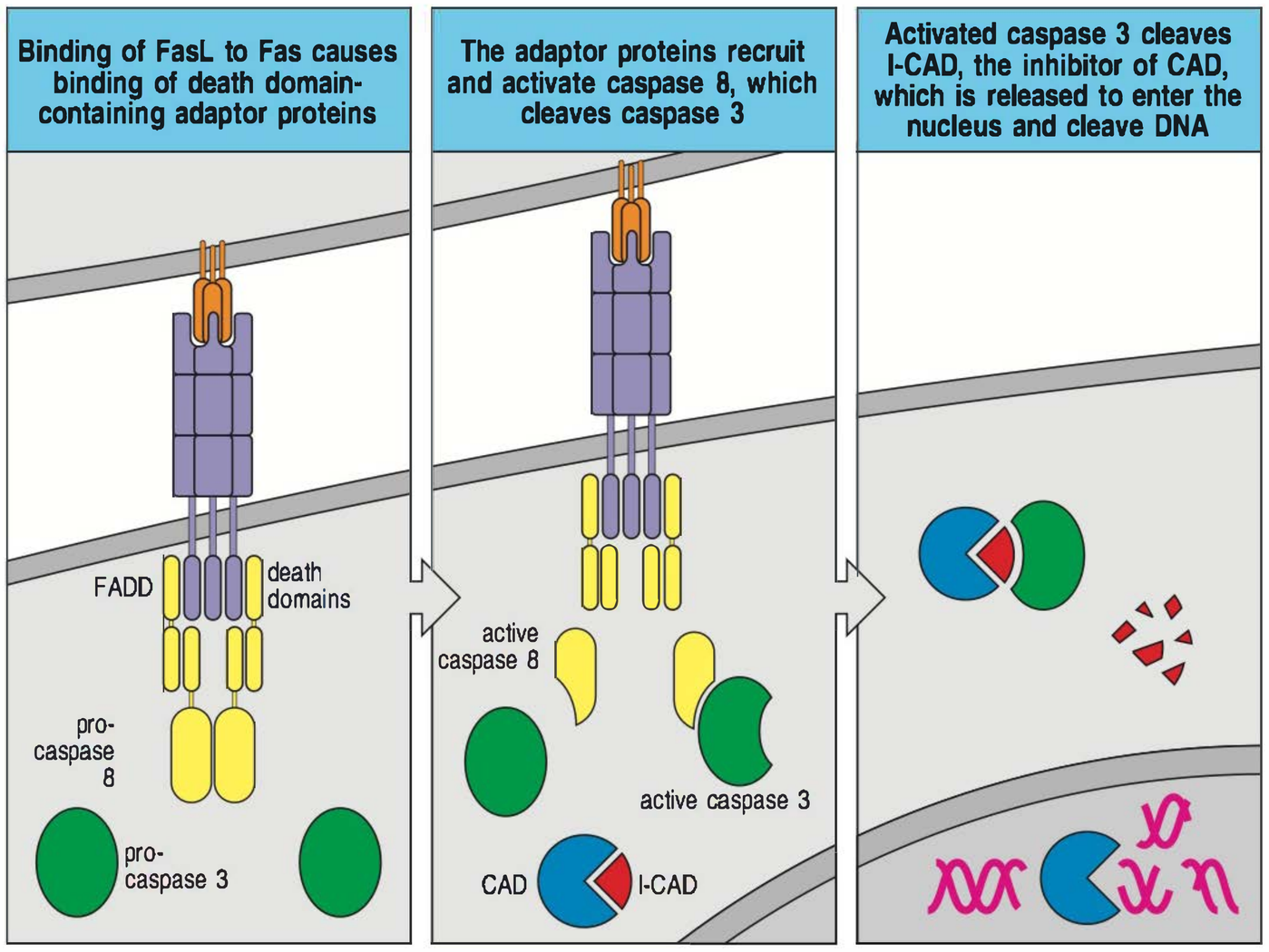


Fig. 19.2 Binding of FasL to Fas initiates the process of apoptosis in the Fas-bearing cell. Binding of trimeric FasL to trimeric Fas brings the death domains in the Fas cytoplasmic tails together. A number of adaptor proteins containing death domains bind to the death domains of Fas, in particular the protein FADD, which in turn interacts through a second death domain with the protease caspase 8. Clustered caspase 8 can transactivate, cleaving caspase 8 itself to release an active caspase domain that in turn can activate other caspases. The ensuing caspase cascade culminates in the activation of the caspase-activatable DNase (CAD), which is present in all cells in an inactive cytoplasmic form bound to an inhibitory protein called I-CAD. When I-CAD is broken down by caspases, CAD can enter the nucleus, where it cleaves DNA into the 200 bp fragments that are characteristic of apoptosis.

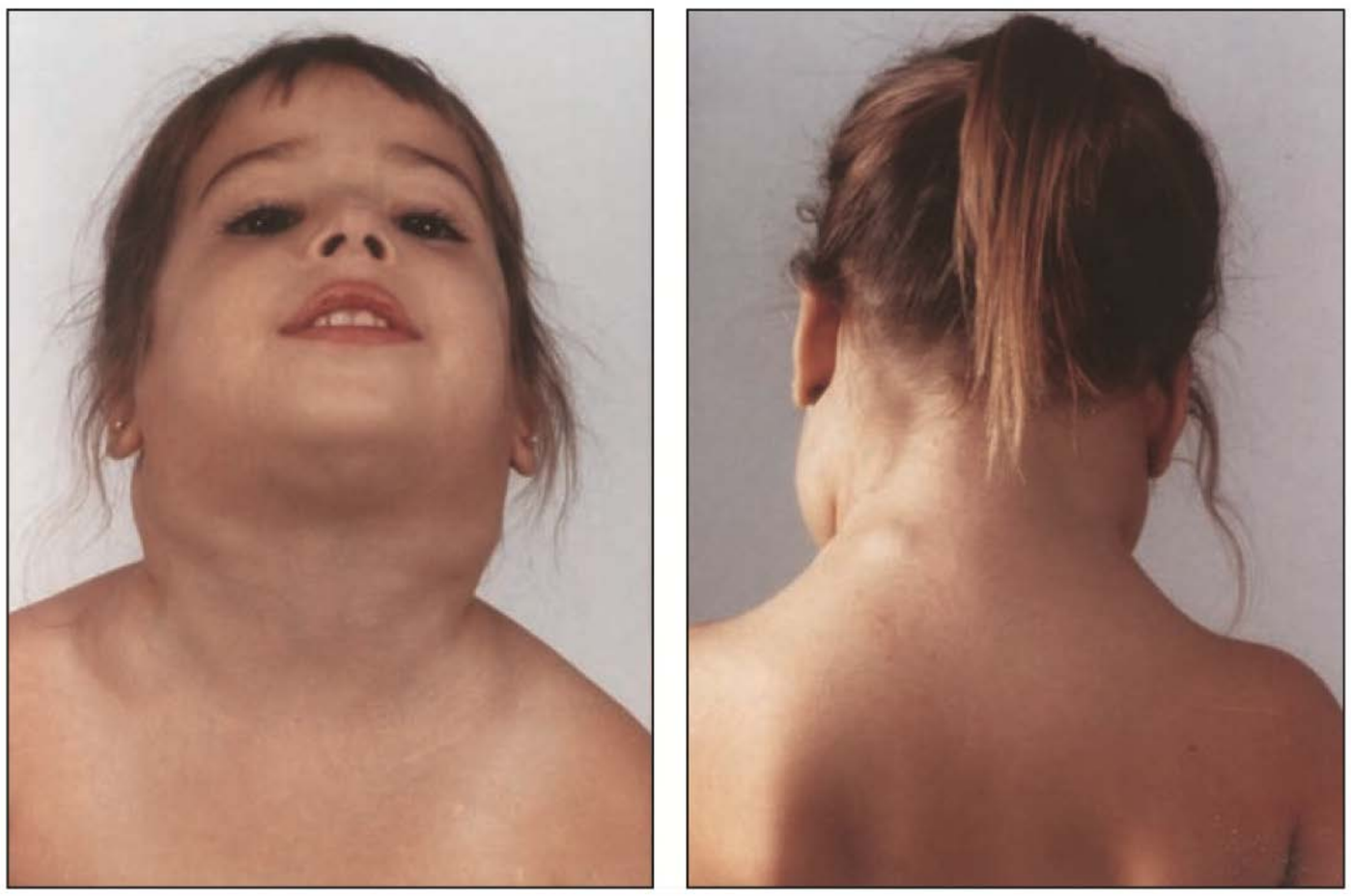
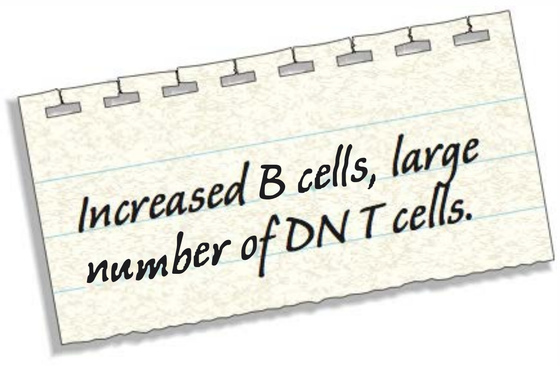
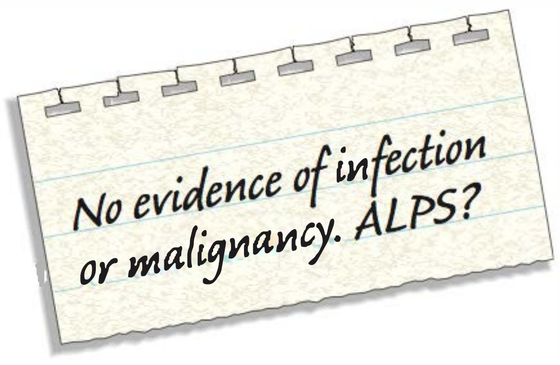


Fig. 19.3 Lymphadenopathy in ALPS. Young girl with ALPS with very enlarged lymph nodes in her neck. Photograph courtesy of Jennifer Puck.

Laboratory tests revealed that Ellen's white blood cell count was 12,500 μl^{-1}, of which 9175 were lymphocytes (normal 3000–7500). Her serum immunoglobulins were all elevated: IgG, 4000 mg dl^{-1} (normal 520–1500); IgM, 400 mg dl^{-1} (normal 40–200); and IgA, 1660 mg dl^{-1}. Flow cytometry analysis of her lymphocytes revealed that 29% were CD19-positive B cells (normal 5–15%; CD19 is a component of the B-cell co-receptor complex) and 65% were CD3-positive T cells (normal 61–84%; CD3 is a component of the T-cell receptor complex). Of the CD3-positive T cells, 14% carried the co-receptor protein CD4 and 18% the co-receptor CD8. She thus had many $CD3^+4^-8^-$ T cells. Of these, the vast majority expressed TCR$\alpha\beta$ (the $\alpha\beta$ form of the T-cell-receptors) and hence were TCR$\alpha\beta^+$ double-negative (DN) T cells (normally, TCR$\alpha\beta^+$ DN T cells are either absent or constitute less than 2% of circulating T cells). A biopsy of a lymph node from Ellen's neck showed extensive enlargement of the follicles (hyperplasia) and a marked increase in the numbers of immunoblasts and plasma cells in the paracortical area. No infectious agents were cultured from the lymph node, despite the fact that the observed changes resembled those caused by a viral infection. Although more than 50% of the T cells in the lymph node were double negatives, no chromosomal abnormality was found on karyotyping, and there was no evidence of oligoclonality of the T-cell receptor, thus ruling out a malignancy.

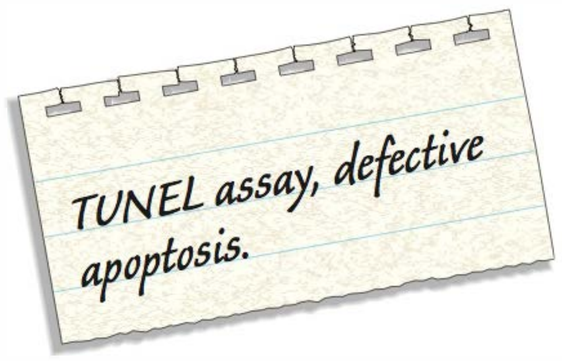


In the absence of evidence of infection or malignant disease, autoimmune lymphoproliferative disease was diagnosed and Ellen received the anti-inflammatory steroid prednisone and the immunosuppressant drug cyclosporin A. Her lymph nodes rapidly reduced in size after this therapy, but enlarged again when therapy was discontinued.



Ellen continued to grow and develop normally, and when she reached adolescence the size of her lymph nodes decreased spontaneously. At 18 years of age, repeat blood counts revealed that her platelet count was 75,000 μl^{-1} (normal 150,000–250,000). An autoantibody against platelets was found in her serum. A diagnosis of idiopathic thrombocytopenic purpura (low platelet numbers accompanied by red or purplish-red spotty skin discoloration due to local hemorrhages) was made. She was treated with the steroid dexamethasone, and the condition resolved. At age 32, Ellen's blood neutrophil count fell to <1000 μl^{-1} (normal 2500–5000). She was found to have developed an autoantibody against granulocytes.

Ellen's family history was informative in that her paternal grandfather had splenomegaly and generalized lymphadenopathy as a child, and his spleen was removed at age 25. At age 60, he developed a B-cell lymphoma. Ellen's father also had



splenomegaly and lymphadenopathy but no clinical symptoms. When blood lymphocytes from Ellen's father and paternal grandfather were examined by flow cytometry, a large number of double-negative T cells were found. In contrast, her brother, mother, and maternal grandparents had normal T cells. The TUNEL assay for apoptotic cells (Fig. 19.4) was performed on blood mononuclear cells from Ellen, her parents, and her paternal grandfather. The cells were first stimulated *in vitro* with phytohemagglutinin for 3 days, and growth of the resulting T-cell blasts was continued for 3 weeks by the addition of IL-2 to the cultures. The cultures were then divided; half were exposed to an antibody to Fas, which mimics the function of FasL. The percentage of cells undergoing apoptosis was then counted. Sixty percent of her mother's T cells underwent apoptosis, whereas only 2% of Ellen's cells, <1% of her father's cells, and 1.4% of her paternal grandfather's cells demonstrated programmed cell death (normal controls 35–70%). The *FAS* and *FASL* genes were examined in DNA samples from Ellen, her father, and her paternal grandfather. An identical single-base transversion, causing a premature termination codon, was found in one of the alleles of the *FAS* gene in these DNA samples. The *FAS* genes in Ellen's mother and brother were normal.

Autoimmune lymphoproliferative syndrome (ALPS).

ALPS is characterized by splenomegaly and lymphadenopathy from early childhood, and, frequently, autoimmunity. Affected individuals can develop autoimmune hemolytic anemia, neutropenia, thrombocytopenia, and hepatitis (inflammation of the liver) and are at increased risk of developing lymphoma. Most patients with ALPS are heterozygous for a dominant mutation in the *FAS* gene, and their activated T cells do not undergo Fas-mediated apoptosis *in vitro*, as is the case for Ellen and her father and grandfather. Patients with ALPS due to *FAS* mutations also have elevated serum levels of FasL, IL-10, and vitamin B_{12}; these can be used as reliable biomarkers, along with the increase in DN T lymphocytes. In some cases, ALPS is due to somatic mutations of *FAS* that occur in an early lymphoid progenitor. Because of the impairment of apoptosis, the proportion of lymphocytes carrying the somatic mutations may increase over time, and is particularly high among DN T cells.

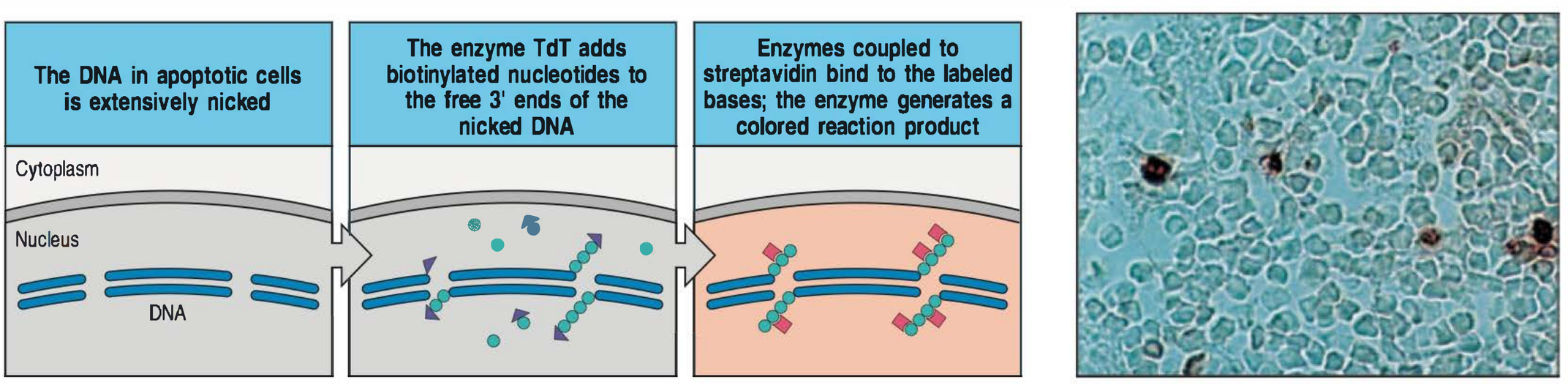


Fig. 19.4 The TUNEL assay. When cells undergo apoptosis, their DNA becomes fragmented and they can be revealed by labeling the fragmented DNA by using the enzyme terminal deoxynucleotidyltransferase (TdT). TdT adds nucleotides to the ends of DNA fragments; biotin-labeled nucleotides (usually dUTP) are most commonly added in this assay (second panel). The biotinylated DNA can be detected by using streptavidin, which binds to biotin, coupled to enzymes that convert a colorless substrate into a colored insoluble product (third panel). Cells stained in this way can be detected by light microscopy, as shown in the photograph of apoptotic cells (stained red) in the thymic cortex. Photograph courtesy of R. Budd and J. Russell.

Other patients with ALPS have been found to have mutations in the genes encoding FasL or caspase 10, an enzyme involved in triggering apoptosis via the Fas pathway. In one case, a gain-of-function mutation of the *NRAS* gene was identified that resulted in impaired induction of apoptosis in response to IL-2 deprivation. The *NRAS* mutation in this patient resulted in impaired induction of the pro-apoptotic molecule Bim, which controls mitochondrial stability upon cytokine deprivation.

Treatment of ALPS is mostly based on immune suppression and the surveillance of tumors. Splenectomy should be reserved for severe cases, because of the risk of infections by encapsulated bacteria (see Case 16).

The clinical and immunologic features of ALPS bear a striking resemblance to a lymphoproliferative disease observed in mice with *lpr* or *gld* mutations. The *lpr* phenotype results from the absence of Fas, whereas the *gld* phenotype is caused by a mutation in FasL. A progressive accumulation of DN T cells is observed in both these strains of mice (note that these circulating $CD3^+$ DN T cells should not be confused with the immature $CD3^-$ $CD4^-$ $CD8^-$ 'double-negative' thymocytes that are a normal stage of T-cell development in the thymus). The mice make antibodies against double-stranded DNA, similar to the situation in human systemic lupus erythematosus (see Case 37). Consistent with these findings in mice, patients with ALPS have defective T-cell apoptosis and abnormal accumulations of DN T cells. When B cells are activated, they also express Fas and become susceptible to Fas-mediated apoptosis. Thus, activated B cells in ALPS are not properly eliminated. The serum concentrations of immunoglobulins increase (hypergammaglobulinemia), the number of B cells is increased (B-cell lymphocytosis), and pathological autoantibody production ensues. Because T cells and B cells are not eliminated normally, patients with ALPS are predisposed to develop lymphomas. Autoimmunity may result because Fas-mediated killing is a mechanism for removing autoreactive B cells.

Questions.

1 *Patients with ALPS are heterozygous for the mutation in FAS or FASL; they have one normal allele and one mutant allele. How do you explain the dominant inheritance?*

2 *Ellen's great-aunt (her paternal grandfather's sister) was found to have the same FAS mutation as Ellen, yet she had no symptoms. How can this be explained?*

3 *It is advantageous for viruses to inhibit apoptosis so that the host cells in which they thrive do not get eliminated by apoptosis induced by recognition by cytotoxic T cells. How might a virus accomplish this?*

4 *When Fas is activated by FasL it associates with and activates caspase 8 (see Fig. 19.2). When the gene encoding caspase 8 is knocked out in mice, this proves to be lethal at the fetal stage. Would it be worthwhile to search for caspase 8 mutations in patients with ALPS when there is no mutation in FAS or FASL?*

CASE 49 Acute Systemic Anaphylaxis

A life-threatening immediate hypersensitivity reaction to peanuts.

Adaptive immune responses can be elicited by antigens that are not associated with infectious agents. Inappropriate immune responses to otherwise innocuous foreign antigens result in allergic or hypersensitivity reactions, and these unwanted responses can be serious. Allergic reactions occur when an already sensitized individual is reexposed to the same innocuous foreign substance, or allergen. The first exposure generates allergen-specific antibodies and/or T cells; reexposure to the same allergen, usually by the same route, leads to an allergic reaction.

Acute systemic anaphylaxis is a type I IgE-mediated hypersensitivity reaction (Fig. 49.1) that is rapid in onset and can cause death. There is typically involvement of at least two organ systems, including the skin, respiratory, gastrointestinal, cardiovascular, or central nervous systems. As with any type I hypersensitivity reaction, the first exposure to the allergen generates allergen-specific IgE antibodies, which become bound to Fc receptors (FcεRI) on the surface of mast cells. On repeat exposure to allergen, cross-linking of IgE bound to FcεRI on mast cells and basophils leads to degranulation, with the release of preformed mediators such as histamine and tryptase and the synthesis and release of other mediators such as prostaglandins and leukotrienes. Histamine is a major mediator of the immediate effects of anaphylaxis, causing multiple symptoms including increased permeability of blood vessels, which can cause life-threatening hypotension. The mast-cell mediators important for anaphylaxis, and the clinical consequences of their release, are illustrated in Fig. 49.2.

Allergens introduced systemically are most likely to cause a serious anaphylactic reaction through the activation of sensitized connective tissue mast cells. The disseminated effects on the circulation and on the respiratory system are the most dangerous, and localized swelling of the upper airway can cause suffocation. Ingested antigens cause a variety of symptoms through their action on mucosal mast cells.

Any protein allergen can provoke an anaphylactic reaction, but those that most commonly cause acute systemic anaphylaxis are foods, medications, and insect venoms (Fig. 49.3). Proteins in food, most commonly milk, soy beans, eggs, wheat, peanuts, tree nuts, and shellfish, can also cause systemic anaphylaxis. Contact with protein antigens found in latex, a common

This case was prepared by Raif Geha, MD, in collaboration with Lisa Bartnikas, MD.

Topics bearing on this case:
Class I hypersensitivity reactions
Allergic reactions to food
Mast-cell activation via IgE

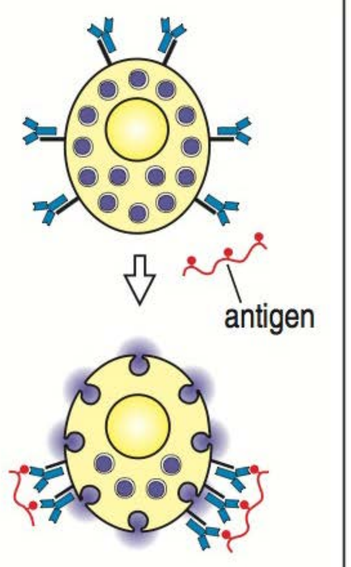
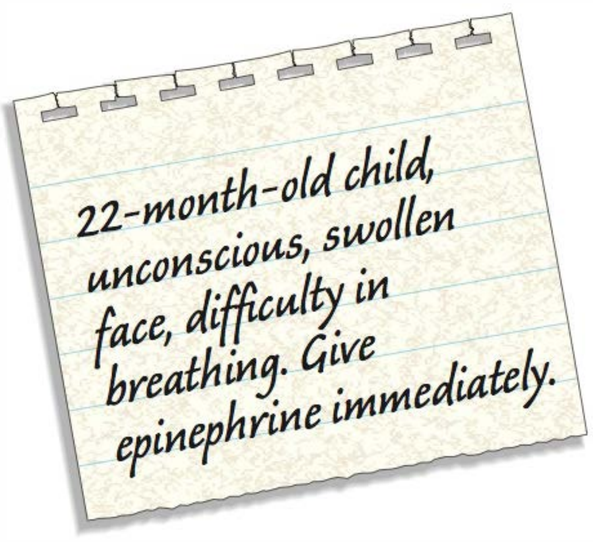
Type I immune-mediated tissue damage	
Immune reactant	IgE antibody
Antigen	Soluble antigen
Effector mechanism	Mast-cell activation
	
Example of hypersensitivity reaction	Allergic rhinitis, allergic asthma, systemic anaphylaxis

Fig. 49.1 Type I immunological hypersensitivity reactions. Type I hypersensitivity reactions involve IgE antibodies and the activation of mast cells (see also Case 50).



constituent of rubber gloves, is also known to cause anaphylaxis. In addition, small-molecule antibiotics such as penicillin can act as haptens, binding to host proteins.

Type I allergic responses are characterized by the activation of allergen-specific CD4 helper cells (T_H2 cells) and the production of allergen-specific IgE antibody. The allergen is captured by B cells through their antigen-specific surface IgM and is processed so that its peptides are presented by MHC class II molecules to T-cell receptors of antigen-specific T_H2 cells. The interleukins IL-4 and/or IL-13 produced by the activated T_H2 cells induce a switch to the production of IgE, rather than IgG, by the B cell (see Fig. 2.4). However, allergen-specific IgE antibodies can exist without the occurrence of anaphylaxis, suggesting that factors other than IgE may be required.

This case concerns a child who suffered from life-threatening systemic anaphylaxis caused by an allergy to peanuts.

The case of John Mason: a life-threatening immune reaction.

John was healthy until the age of 22 months, when he developed swollen lips while eating cookies containing peanut butter. The symptoms disappeared in about an hour. A month later, while eating the same type of cookies, he started to vomit, became hoarse, had great difficulty in breathing, started to wheeze and developed a swollen face. He was taken immediately to the emergency room of the Children's Hospital, but on the way there he became lethargic and lost consciousness.

On arrival at hospital, his blood pressure was catastrophically low at 40/0 mmHg (normal 80/60 mmHg). His pulse was 185 beats min^{-1} (normal 80–90 beats min^{-1}), and his respiratory rate was 76 min^{-1} (normal 20 min^{-1}). His breathing was labored. An anaphylactic reaction was diagnosed and John was immediately given an intramuscular injection of 0.15 ml of a 1:1000 dilution of epinephrine (adrenaline). An intravenous solution of normal saline was infused as a bolus. The antihistamine Benadryl (diphenhydramine hydrochloride) and the anti-inflammatory corticosteroid Solu-Medrol (methylprednisolone) were also administered intravenously. A blood sample was taken to test for histamine and the enzyme tryptase.

Mediators of anaphylaxis		
Mediator	**Action**	**Signs/symptoms**
Histamine	Vasodilation, bronchoconstriction	Pruritus, swelling, hypotension, diarrhea, wheezing
Leukotrienes	Bronchoconstriction	Wheezing
Platelet-activating factor*	Bronchoconstriction, vasodilation	Wheezing, hypotension
Tryptase	Proteolysis	Unknown

Fig. 49.2 Mediators released by mast cells during anaphylaxis and their clinical consequences. *Platelet-activating factor is not released by mast cells but by neutrophils, basophils, platelets, and endothelial cells.

IgE-mediated allergic reactions			
Syndrome	**Common allergens**	**Route of entry**	**Response**
Systemic anaphylaxis	Drugs Serum Venoms	Intravenous (either directly or following oral absorption into the blood)	Edema Vasodilation Tracheal occlusion Circulatory collapse Death
Acute urticaria (wheal-and-flare)	Insect bites Allergy testing	Subcutaneous	Local increase in blood flow and vascular permeability
Allergic rhinitis (hay fever)	Pollens (ragweed, timothy, birch) Dust-mite feces	Inhaled	Edema of nasal mucosa Irritation of nasal mucosa
Allergic asthma	Danders (cat) Pollens Dust-mite feces	Inhaled	Bronchial constriction Increased mucus production Airway inflammation
Food allergy	Shellfish Milk Eggs Fish Wheat	Oral	Vomiting Diarrhea Pruritus itching Urticaria (hives) Anaphylaxis (rarely)

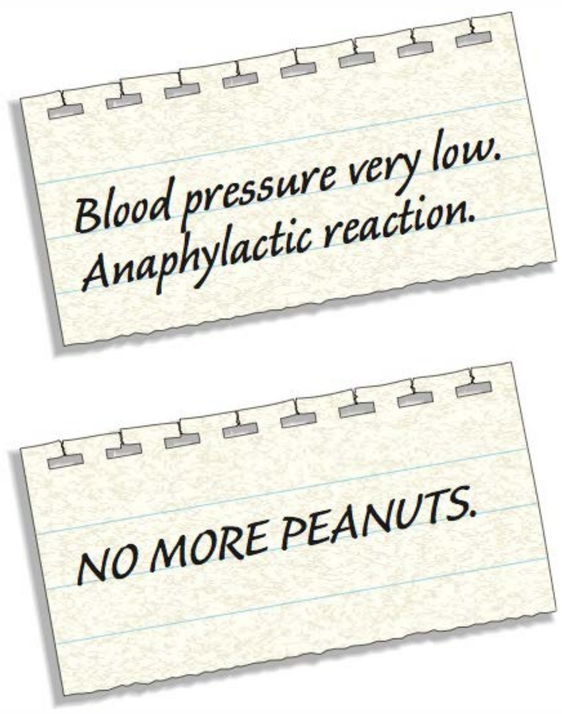
Fig. 49.3 IgE-mediated reactions to extrinsic antigens. All IgE-mediated responses involve mast-cell degranulation, but the symptoms experienced by the patient can be very different depending on whether the allergen is injected, inhaled, or eaten, and depending on the dose of the allergen.

Within minutes of the epinephrine injection, John's hoarseness improved, the wheezing diminished, and his breathing became less labored (Fig. 49.4). His blood pressure rose to 50/30 mmHg, the pulse decreased to 145 beats min^{-1} and his breathing to 61 min^{-1}. Thirty minutes later, the hoarseness and wheezing got worse again and his blood pressure dropped to 40/20 mmHg, his pulse increased to 170 beats min^{-1} and his respiratory rate to 70 min^{-1}.

John was given another intramuscular injection of epinephrine and was made to inhale nebulized albuterol (a β_2-adrenergic agent). This treatment was repeated once more after 30 minutes. One hour later, he was fully responsive, his blood pressure was 70/50 mmHg, his pulse was 116 beats min^{-1} and his respiratory rate had fallen to 46 min^{-1}. John was admitted to the hospital for further observation.

Treatment with Benadryl and methylprednisolone intravenously every 6 hours was continued for 24 hours, by which time the facial swelling had subsided and John's blood pressure, respiratory rate, and pulse were normal. He had stopped wheezing and when the doctor listened to his chest with a stethoscope it was clear.

He remained well and was discharged home with an Epi-Pen. His parents were instructed to avoid giving him foods containing peanuts in any form, and were asked to bring him to the Allergy Clinic for further tests.



Acute systemic anaphylaxis.

Anaphylaxis presents a medical emergency and is the most urgent of clinical immunologic events; it requires immediate therapy. It results from the generation and release of a variety of potent biologically active mediators and their concerted effects on a number of target organs. John showed classic rapid-onset symptoms of anaphylaxis, starting with vomiting and swelling of the

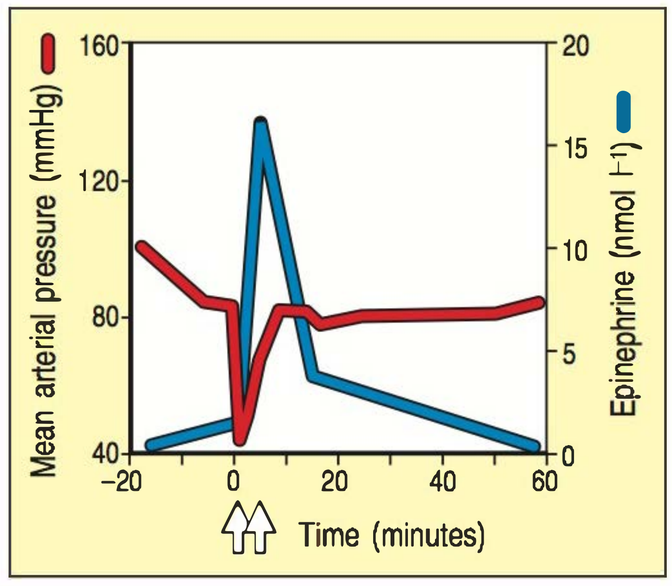


Fig. 49.4 Mean arterial pressure and epinephrine levels in a representative patient with insect-sting anaphylactic shock. Time 0 indicates the onset of the anaphylactic reaction as reported by the patient. The arrows indicate administration of antihistamines and epinephrine.

face and throat, and constriction of the bronchial smooth muscle, which led to his difficulty in breathing. This was soon followed by a catastrophic loss of blood pressure, due to leakage of fluid from the blood vessels. Anaphylaxis can also cause urticaria (hives), heart arrhythmias, and myocardial ischemia, and gastrointestinal symptoms such as nausea, vomiting, and diarrhea. All these signs and symptoms can occur singly or in combination.

Fatal allergic reactions to the venoms in bee and wasp stings have been recognized for at least 4500 years and account today for roughly 40 deaths each year in the United States. In 1902, Portier and Richet reported that a second injection of a protein from a sea anemone caused a fatal systemic reaction in dogs that had been injected previously with this protein. Because this form of immunity was fatal rather than protective, it was termed 'anaphylaxis' to distinguish it from the 'prophylaxis' (protection) generated by immunization.

Anaphylaxis requires a latent period for sensitization after the first introduction of antigen followed by reexposure to the sensitizing agent, which can be any foreign protein or a hapten. In the early part of the 20th century, the most frequent cause of systemic anaphylaxis was horse serum, which was used as a source of antibodies to treat infectious diseases.

In many cases, the presentation of food allergy occurs on the first known ingestion, suggesting that routes other than the oral one may be important in sensitization. For example, epidemiologic data suggest that sensitization to peanut protein may occur in children through the application of peanut oil to inflamed skin. A recent study demonstrated that the incidence of peanut allergy in children who avoided peanut ingestion correlated with the level of peanut consumption in their homes, which is consistent with the skin's being an important route of allergen sensitization. At present there is no cure for food allergy. Current therapy relies on allergen avoidance and the treatment of severe reactions with epinephrine.

Anaphylaxis is increasing in prevalence and is a frequent cause of visits to the emergency room, with 50–2,000 episodes per 100,000 persons, or a lifetime prevalence of 0.05–2.0%. The rate of fatal anaphylaxis from any cause is estimated at 0.4 cases per million individuals per year. Although in John's case the reaction was brought on by eating a food, an antigen administered by subcutaneous, intramuscular, or intravenous injection is more likely to induce a clinical anaphylactic reaction than one that enters by the oral or respiratory route.

Questions.

1 *Anaphylaxis results in the release of a variety of chemical mediators from mast cells, such as histamine and leukotrienes. Angioedema (localized swelling caused by an increase in vascular permeability and leakage of fluid into tissues) is one of the symptoms of anaphylaxis. With the above in mind, why did John get hoarse and why did he wheeze?*

2 *When his parents brought John back to the Allergy Clinic, a nurse performed several skin tests by pricking the epidermis of his forearm with a shallow plastic needle containing peanut antigens. John was also tested in a similar fashion with antigens from nuts as well as from eggs, milk, soy, and wheat. Within 5 minutes John developed a wheal, 10 mm × 12 mm*

in size, surrounded by a red flare, 25 mm × 30 mm (see Fig. 50.5), at the site of application of the peanut antigen. No reactions were noted to the other antigens. A radioallergosorbent test (RAST) was performed on a blood sample to examine for the presence of IgE antibodies against peanut antigens. It was positive. What would you advise John's parents to do?

3 Why was John treated first with epinephrine in the emergency room?

4 Why was John given a blood test for histamine and the enzyme tryptase?

5 Why was the skin testing for peanuts not done in the hospital immediately after John had recovered, instead being done at a later visit?

6 The incidence of peanut allergy is increasing. Why?

7 John's parents want to know whether there are therapies that might cure him of his peanut allergy. What do you tell them?

CASE 50 Allergic Asthma

Chronic allergic disease caused by an adaptive immune response to inhaled antigen.

Chronic allergic reactions are much more common than the acute systemic anaphylaxis reaction discussed in Case 49. Among these are allergic reactions to inhaled antigens (Fig. 50.1), which range in severity from a mild allergic rhinitis (hay fever) to potentially life-threatening allergic asthma, the disease discussed in this case. Once an individual has been sensitized, the allergic reaction becomes worse with each subsequent exposure to allergen, which not only produces allergic symptoms but also increases the levels of antibody and T cells reactive to the allergen.

Allergic asthma is an example of a type I hypersensitivity reaction. Type I reactions involve the activation of helper CD4 T_H2 cells, IgE antibody formation, mast-cell sensitization, and the recruitment of eosinophils. The allergen-specific IgE antibodies formed in sensitized individuals bind to and occupy high-affinity Fcε receptors (FcεRI) on the surfaces of tissue mast cells and basophils (Fig. 50.2). When the antigen is encountered again, it cross-links these bound IgE molecules, which triggers the immediate release of mast-cell granule contents, in particular histamine and various enzymes that increase blood flow and vascular permeability. This is the early phase of an immediate allergic reaction.

Within 12 hours of contact with antigen, a late-phase reaction occurs (Fig. 50.3). Arachidonic acid metabolism in the mast cell generates prostaglandins and leukotrienes, which further increase blood flow and vascular permeability. Cytokines such as interleukin-3 (IL-3), IL-4, IL-5, and tumor necrosis factor-α (TNF-α) are produced by both activated mast cells and helper T cells, and these further prolong the allergic reaction. The mediators and cytokines released by mast cells and helper T cells cause an influx of monocytes, more

This case was prepared by Raif Geha, MD, in collaboration with Lisa Bartnikas, MD.

Topics bearing on this case:

- Inflammatory reactions
- iNKT cells
- Differential activation of T_H1 and T_H2 cells
- IgE-mediated hypersensitivity
- Skin tests for hypersensitivity
- Radioimmunoassay
- Tests for immune function

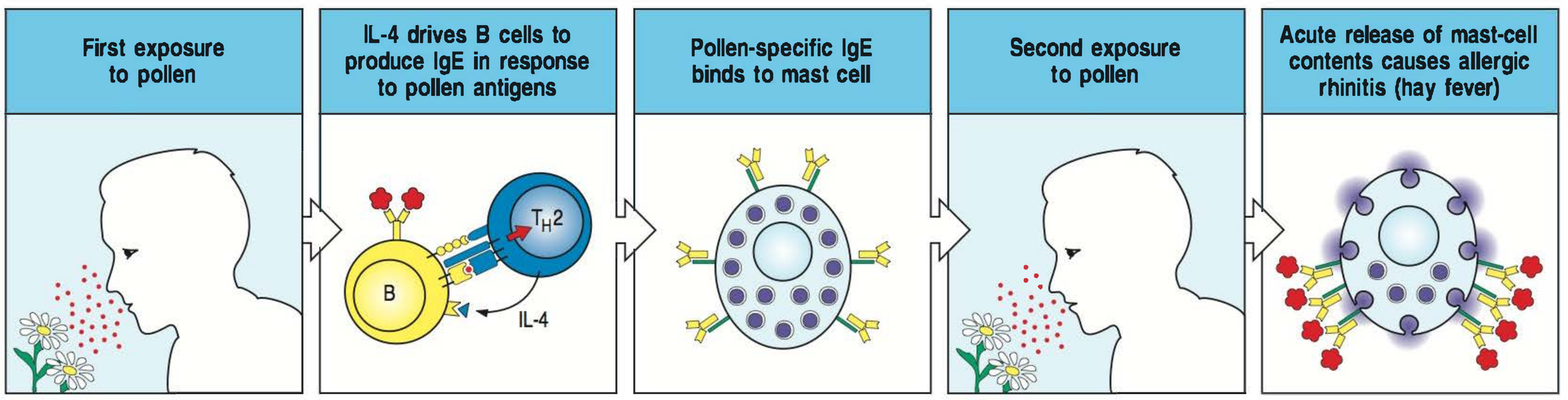


Fig. 50.1 Allergic reactions require previous exposure to the allergen. In this example, the first exposure to pollen induces the production of IgE anti-pollen antibodies, driven by the production of IL-4 by helper T cells (T_H2). The IgE binds to mast cells via FcεRI. Once enough IgE antibody is present on mast cells, exposure to the same pollen induces mast-cell activation and an acute allergic reaction, here allergic rhinitis (hay fever). Allergic reactions require an initial sensitization to the antigen (allergen), and several exposures may be needed before the allergic reaction is initiated.

T cells, and eosinophils into the site of allergen entry. The late-phase reaction is dominated by this cellular infiltrate. The cells of the infiltrate, particularly the eosinophils, make a variety of products that are thought to be responsible for much of the tissue damage and mucus production that is associated with chronic allergic reactions. Cytokine-producing NKT cells have also been implicated in allergic asthma.

Approximately 15% of the population suffers from IgE-mediated allergic diseases. Many common allergies are caused by inhaled particles containing foreign proteins (or allergens) and result in allergic rhinitis, asthma, and allergic conjunctivitis. In asthma, the allergic inflammatory response increases the hypersensitivity of the airway not only to allergen reexposure but also to nonspecific agents such as exercise, pollutants, and cold air.

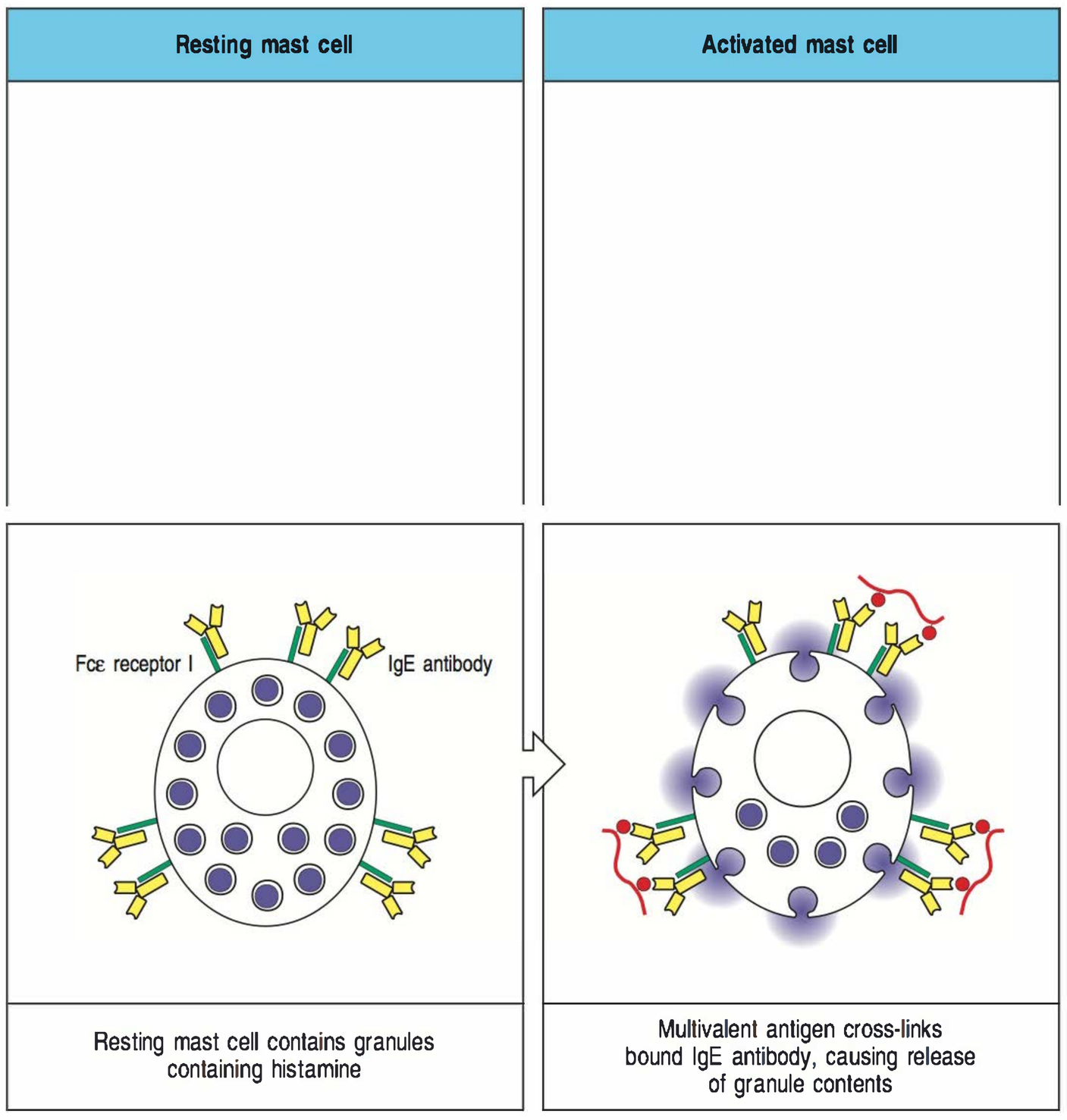


Fig. 50.2 Cross-linking of IgE antibody on mast-cell surfaces leads to a rapid release of inflammatory mediators by the mast cells. Mast cells are large cells found in connective tissue that can be distinguished by secretory granules containing many inflammatory mediators. They bind stably to monomeric IgE antibodies through the very high-affinity Fcε receptor (FcεRI). Antigen cross-linking of the bound IgE antibody molecules triggers rapid degranulation, releasing inflammatory mediators into the surrounding tissue. These mediators trigger local inflammation, which recruits cells and proteins required for host defense to sites of infection. It is also the basis of the acute allergic reaction causing allergic asthma, allergic rhinitis, and the life-threatening response known as systemic anaphylaxis (see Case 49). Photographs courtesy of A.M. Dvorak.

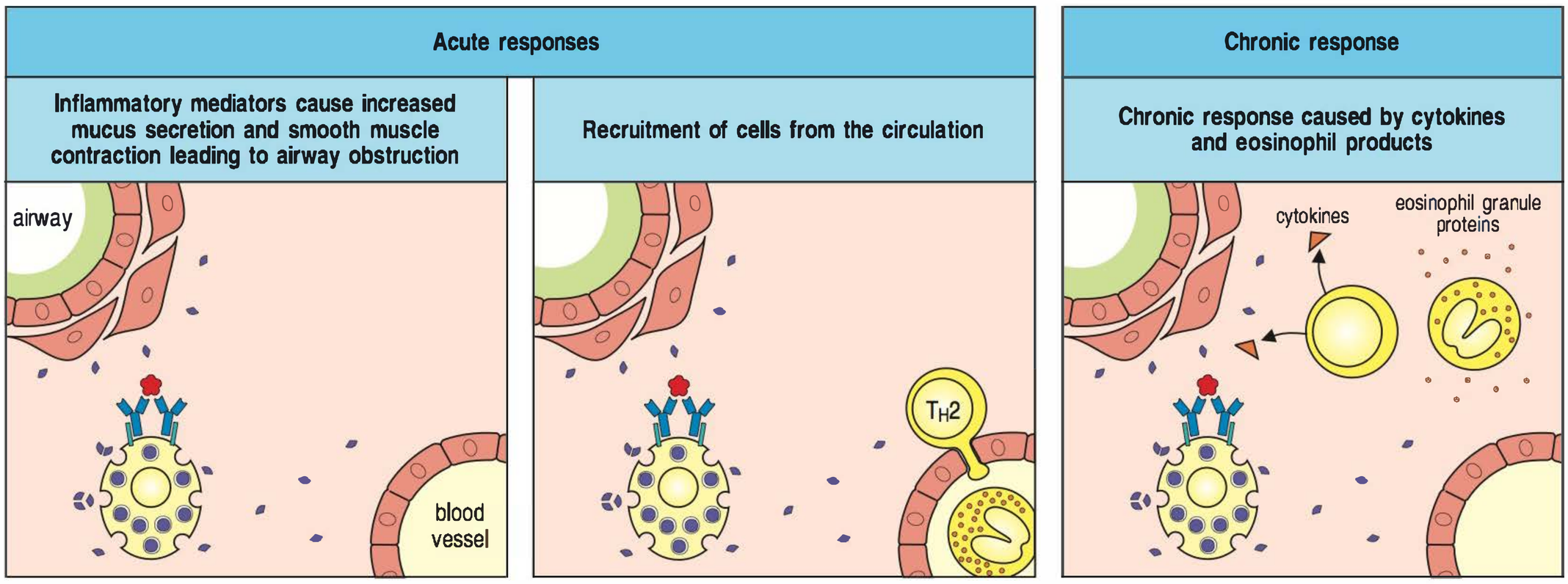


Fig. 50.3 The acute response in allergic asthma leads to T_H2-mediated chronic inflammation of the airways. In sensitized individuals, cross-linking of specific IgE on the surface of mast cells by inhaled allergen triggers them to secrete inflammatory mediators, causing bronchial smooth muscle contraction and an influx of inflammatory cells, including eosinophils and T_H2 lymphocytes. Activated mast cells and T_H2 cells secrete cytokines that also augment eosinophil activation, which causes further tissue injury and influx of inflammatory cells. The end result is chronic inflammation, which may then cause irreversible damage to the airways.

The case of Frank Morgan: a 14-year-old boy with chronic asthma and rhinitis.

Frank Morgan was referred by his pediatrician to the allergy clinic at 14 years of age because of persistent wheezing for 2 weeks. His symptoms had not responded to frequent inhalation treatment (every 2–3 hours) with a bronchodilator, the β_2-adrenergic agonist albuterol.

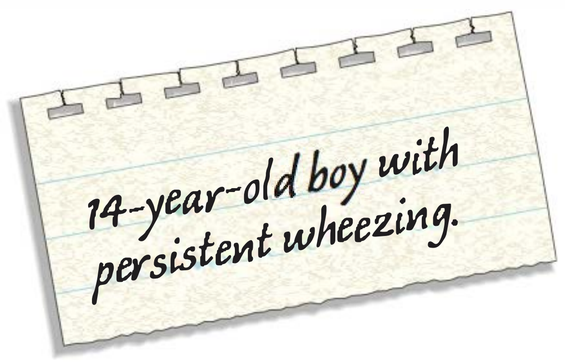
This was not the first time that Frank had experienced respiratory problems. His first attack of wheezing occurred when he was 3 years old, after a visit to his grandparents who had recently acquired a dog. He had similar attacks of varying severity on subsequent visits to his grandparents. Beginning at age 4 years, he had attacks of coughing and wheezing every spring (April and May) and toward the end of the summer (second half of August and September). A sweat test at age 5 years to rule out cystic fibrosis, a possible cause of chronic respiratory problems, was within the normal range.

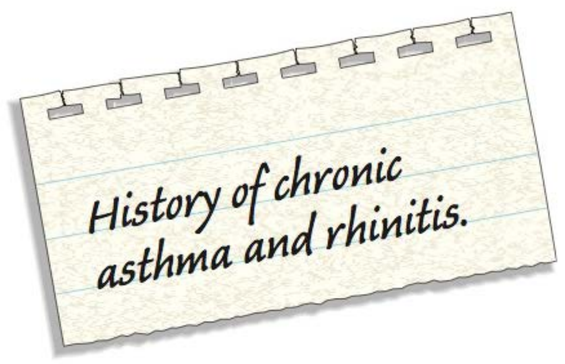
As Frank got older, gym classes, basketball, and soccer games, and just going outside during the cold winter months could bring on coughing and sometimes wheezing. He had been able to avoid wheezing induced by exercise by inhaling albuterol 15–20 minutes before exercise. Frank had frequently suffered from a night-time cough, and his colds had often been complicated by wheezing.

Frank's chest symptoms had been treated as needed with inhaled albuterol. During the previous 10 years, Frank had been admitted to hospital three times for treatment of his asthma with inhaled bronchodilators and intravenous steroids. He had also been to the Emergency Room many times with severe asthma attacks. He had maxillary sinusitis at least three times, and each episode was associated with green nasal discharge and exacerbation of his asthma.

Since he was 4 years old, Frank had also suffered from intermittent sneezing, nasal itching, and nasal congestion (rhinitis), which always worsened on exposure to cats and dogs and in the spring and late summer. The nasal symptoms had been treated as needed with oral antihistamines with moderate success. Frank had had eczema as a baby, but this cleared up by the time he was 5 years old.

Family history revealed that Frank's 10-year-old sister, his mother, and his maternal grandfather had asthma. Frank's mother, father, and paternal grandfather suffered from allergic rhinitis.





When he arrived at the allergy clinic, Frank was thin and unable to breathe easily. He had no fever. The nasal mucosa was severely congested, and wheezing could be heard over all the lung fields. Lung function tests were consistent with obstructive lung disease with a reduced peak expiratory flow rate (PEFR) of 180 liter min^{-1} (normal more than 350–400 liter min^{-1}), and forced expiratory volume in the first second of expiration (FEV_1) was reduced to 50% of that predicted for his sex, age, and height. A chest radiograph showed hyperinflation of the lungs and increased markings around the airways (Fig. 50.4).

A complete blood count was normal except for a high number of circulating eosinophils (1200 μl^{-1}; normal range less than 400 μl^{-1}). Serum IgE was high at 1750 ng dl^{-1} (normal less than 200 ng dl^{-1}). Radioallergosorbent assays (RAST) for antigen-specific IgE revealed IgE antibodies against dog and cat dander, dust mites, and tree, grass, and ragweed pollens in Frank's serum. Levels of immunoglobulins IgG, IgA, and IgM were normal. Histological examination of Frank's nasal fluid showed the presence of eosinophils.

Frank was promptly given albuterol nebulizer treatment in the clinic, after which he felt better, his PEFR rose to 400 liter min^{-1}, and his FEV_1 rose to 65% of predicted. He was sent home on a 1-week course of the oral corticosteroid prednisone. He was told to inhale albuterol every 4 hours for the next 2–3 days, and then to resume taking albuterol every 4–6 hours as needed for chest tightness or wheezing. He was also started on fluticasone propionate (Flovent), an inhaled corticosteroid, and montelukast (Singulair), a leukotriene receptor antagonist for long-term control of his asthma. To relieve his nasal congestion, Frank was given the steroid fluticasone furoate (Flonase) to inhale through the nose, and was advised to use an oral antihistamine as needed. He was asked to return to the clinic 2 weeks later for follow-up, and for immediate hypersensitivity skin tests to try to detect which antigens he was allergic to (Fig. 50.5).

On the next visit Frank had no symptoms except for a continually stuffy nose. His PEFR and FEV_1 were normal. Skin tests for type I hypersensitivity were positive for multiple tree and grass pollens, dust mites, and dog and cat dander. He was advised to avoid contact with cats and dogs. To reduce his exposure to dust mites the pillows and mattresses in his room were covered with zippered covers. Rugs, stuffed toys, and books were removed from his bedroom. He was also started on immunotherapy with injections of grass, tree, and ragweed pollens, cat, dog, and house dust mite antigens, to try to reduce his sensitivity to these antigens.

A year and a half later, Frank's asthma continues to be stable with occasional use of albuterol during infections of the upper respiratory tract and in the spring. His rhinitis and nasal congestion now require much less medication.

Fig. 50.4 Chest radiographs of a patient with asthma. Top: anteroposterior (A–P) view. Bottom: lateral view. The volume occupied by the lungs spans eight to nine rib spaces instead of the normal seven in the A–P view and indicates hyperinflation. The lateral view shows an increased A–P dimension, also reflecting hyperinflation. Hyperinflation indicates air trapping, which is a feature of the obstructive physiology seen in asthma. The bronchial markings are accentuated and can be seen to extend beyond one-third of the lung fields. This indicates inflammation of the airways.

Allergic asthma.

Like Frank, millions of adults and children suffer from allergic asthma. Asthma is the most common chronic inflammatory disorder of the airways and is characterized by reversible inflammation and obstruction of the small airways. Asthma has become an epidemic; the prevalence in the United States is increasing by 5% per year, with more than 500,000 new cases diagnosed annually. It is the most common cause of hospitalization and days lost from school in children. About 70% of patients with asthma have a family history of allergy. This genetic predisposition to the development of allergic diseases is called atopy. Wheezing and coughing are the main symptoms of asthma, and both are due to the forced expiration of air through airways that have become temporarily narrowed by the constriction of smooth muscle as a result of the

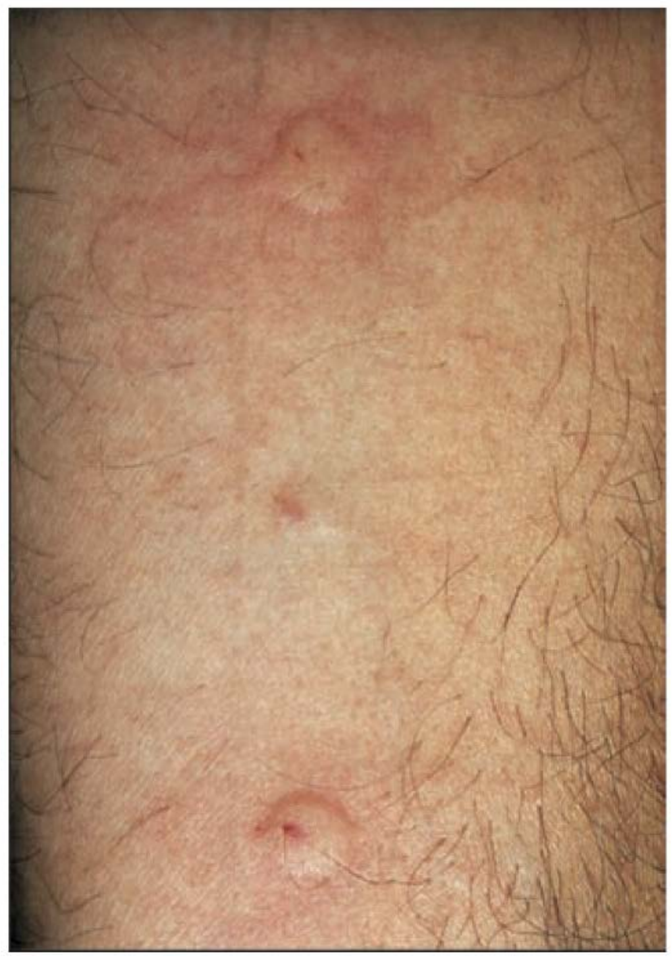


Fig. 50.5 An intradermal skin test. The photograph was taken 20 minutes after intradermal injections had been made with ragweed antigen (top), saline (middle), and histamine (bottom). A central wheal (raised swelling), reflecting increased vascular permeability, surrounded by a flare (red area), reflecting increased blood flow, is observed at the sites where the ragweed antigen and the positive histamine control were introduced. The small wheal at the site of saline injection is due to the volume of fluid injected into the dermis.

allergic reaction. As a consequence of the narrowed airways, air gets trapped in the lung, and the lung volume is increased during an attack of asthma (Fig. 50.6).

Once asthma is established, an asthma attack can be triggered not only by the allergen but by viral infection, cold air, exercise, or pollutants. This is due to a general hyperirritability or hyperresponsiveness of the airways, leading to constriction in response to nonspecific stimuli, thus reducing the air flow. The degree of hyperresponsiveness can be measured by determining the threshold dose of inhaled methacholine (a cholinergic agent) that results in a 20% reduction in airway flow. Airway irritability correlates positively with eosinophilia and serum IgE levels.

CD4 T cells are the central effector cells of airway inflammation in asthma. During asthma exacerbations, secretion of the T_H2-specific cytokines IL-4, IL-5, IL-9, and IL-13 is increased. Clinical improvement in asthma is associated with decreased T cells in the airways. Mast cells are also important effector cells in asthma and, after stimulation by allergen, release preformed and newly generated mediators, contributing to acute and chronic mucosal inflammation. Cysteinyl leukotrienes, a product of arachidonic metabolism, are also key inflammatory mediators in asthma (Fig. 50.7). Cysteinyl leukotriene receptors include at least three types of transmembrane receptors. Activation of the cysteinyl leukotriene receptor 1 ($CysLT_1$) leads to bronchial smooth muscle constriction and muscle-cell proliferation, plasma leakage,

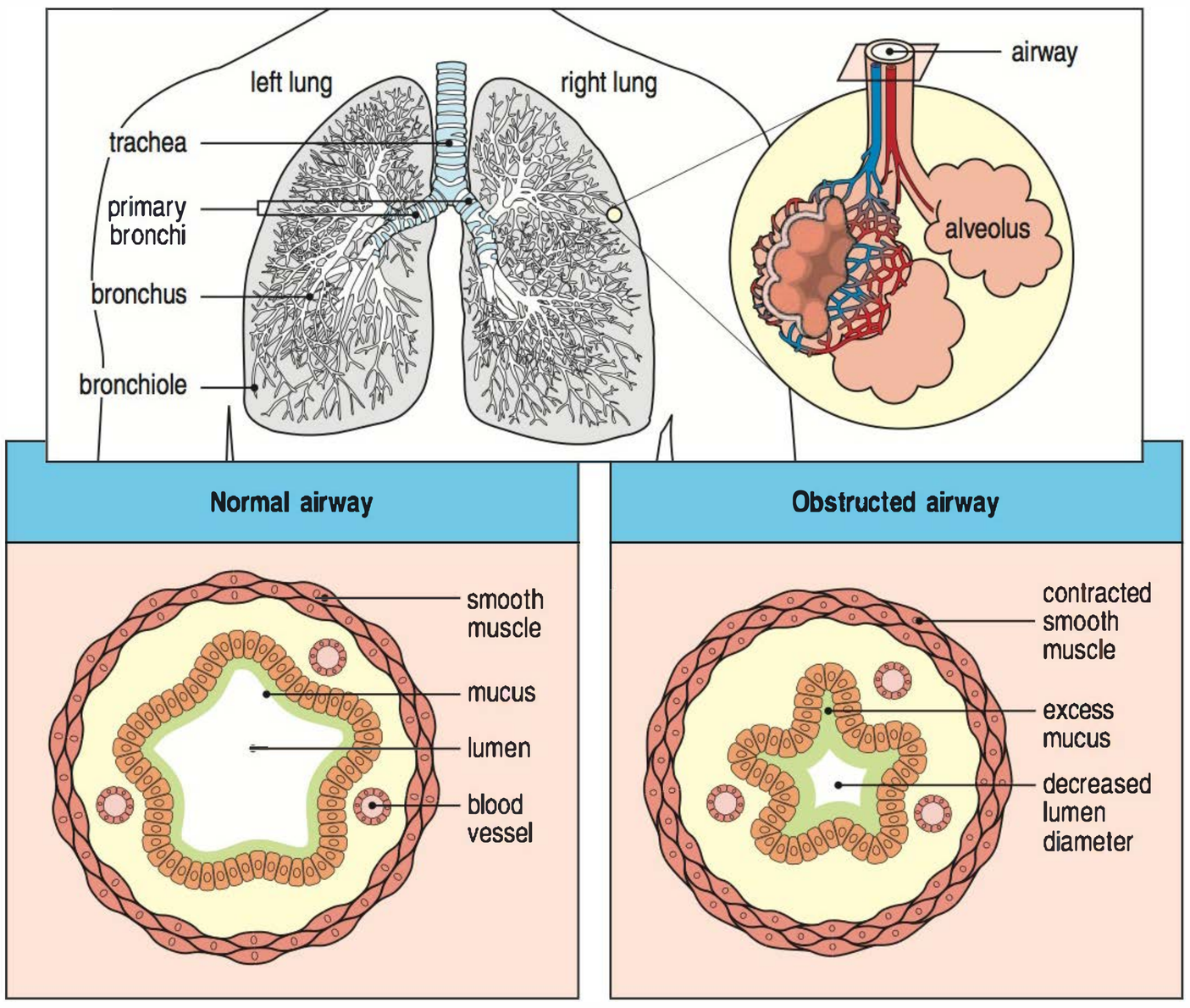
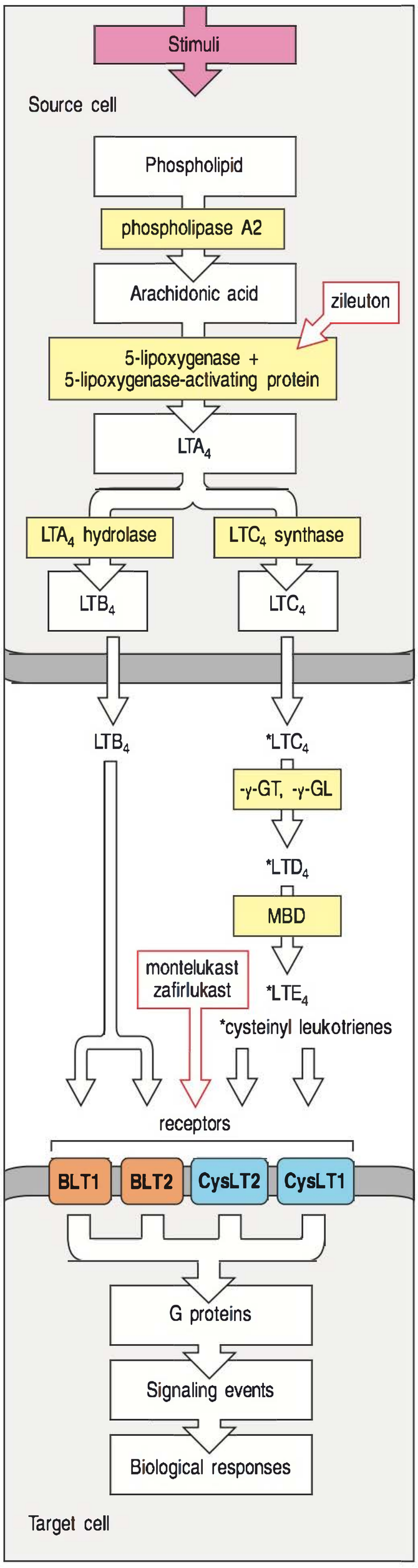


Fig. 50.6 Obstruction of the airways in chronic asthma. The top panels show the general anatomy of the lungs. Asthma is a chronic inflammatory disorder of the small airways—the bronchi and the bronchioles. In susceptible individuals, inflammation leads to recurrent wheezing, shortness of breath, chest tightness, and coughing. In between asthma attacks, patients are often asymptomatic, with normal physical exams and breathing tests. The bottom panels show schematic diagrams of sections through a normal airway (left) and an obstructed airway as a result of chronic asthma (right). During an asthma attack, there is infiltration of blood vessels of the small airways with immune cells (T_H2 lymphocytes and eosinophils), hypersecretion of mucus, and constriction and proliferation of bronchial smooth muscle. This leads to a decreased diameter of the airway lumen, resulting in wheezing and difficulty in breathing. In patients with severe asthma, there may be permanent airway remodeling.



hypersecretion of mucus, and eosinophil migration. The role of neutrophils in asthma is less clear. Elevated neutrophil numbers are more frequently seen in non-allergic asthma, steroid-unresponsive asthma, and in fatal asthma, suggesting that neutrophil-dominated asthma may represent a distinct asthma phenotype. Elevated neutrophil numbers in asthmatic lungs are associated with increased expression of IL-17.

The subset of T cells called invariant NKT cells (iNKT cells) is also elevated in asthmatic airways, suggesting that they may be important in human asthma. iNKT cells are a subpopulation of thymus-derived T cells that express markers of both T cells (such as the T-cell receptor:CD3 complex) and NK cells (such as NK1.1 and Ly-49). In humans, iNKT cells express an invariant antigen receptor with a variable region composed of $V_{\alpha}24$--$J_{\alpha}15$ paired with $V_{\beta}11$. Unlike conventional T cells, iNKT cells can recognize glycolipid antigens bound and presented by the major histocompatibility complex (MHC) class Ib molecule CD1d. On activation, iNKT cells rapidly produce large amounts of the T_H1-type cytokine IFN-γ, the T_H2-type cytokines IL-4 and IL-13, and TNF-α and IL-2. The trigger for their activation in people with asthma could be glycolipids derived from microbes colonizing asthmatic airways.

Although asthma is a reversible disease, severe uncontrolled asthma can lead to airway remodeling, and a severe attack can be fatal. The mortality from asthma has been rising alarmingly in recent years. Risk factors for fatal asthma include frequent use of β_2-agonist therapy, poor perception of asthma severity, membership in a minority group, low socioeconomic status, adolescence, and male gender.

Several classes of drugs are commonly used to treat asthma, including corticosteroids, leukotriene antagonists, anti-IgE antibodies, anticholinergics, and β_2-adrenergic agonists. Corticosteroids (oral prednisone and inhaled fluticasone) inhibit the transcription of allergic and pro-inflammatory cytokines and can also activate the transcription of anti-inflammatory cytokines. This leads to a decrease in the numbers of mast cells, eosinophils, and T lymphocytes in the bronchial mucosa. Leukotriene antagonists (zileuton, montelukast, and zafirlukast) inhibit the synthesis of leukotrienes (which are products of arachidonic acid metabolism) or their receptor binding (see Fig. 50.7). Leukotriene modifiers have both mild bronchodilator and anti-inflammatory properties. Anti-IgE therapy uses a humanized monoclonal antibody (omalizumab) directed against the IgE that forms complexes with free IgE and prevents its biding to the receptor FcεRI on the surfaces of mast cells and basophils. This results in a decrease in circulating free IgE and the downregulation of FcεRI expression on the cell surfaces. β_2-agonists (for example albuterol) bind to the β_2-adrenergic receptor, which is expressed on the surface of bronchial smooth muscle cells. β_2-agonists relax smooth muscle, thus rapidly relieving airway constriction, and are helpful in treating the immediate phase of the allergic reaction in the lungs. The treatment of allergic asthma also includes minimizing exposure to allergens and, in cases of severe or refractory environmental allergies, trying to desensitize the patient by immunotherapy.

Fig. 50.7 Leukotriene synthesis pathways and receptors. The biosynthetic pathway leading from arachidonic acid to the various leukotrienes is shown here, along with the sites of action of drugs used in asthma to block leukotriene synthesis and action (shown in red boxes). γ-GL, γ-glutamyl leukotrienase; γ-GT, γ-glutamyl transferase; MBD, membrane-bound dipeptidase. BLT1, BLT2, CysLT2, and CysLT1 are receptors.

Questions.

1 Explain the basis of Frank's chest tightness and the radiograph findings.

2 Explain the failure of Frank's asthma to improve despite the frequent use of bronchodilators, and his response to steroid therapy.

3 Eosinophilia is often detected in the blood and in the nasal and bronchial secretions of patients with allergic rhinitis and asthma. What is the basis for this finding?

4 What is the basis of the wheal-and-flare reaction that appeared 20 minutes after Frank had had a skin test for hypersensitivity to ragweed pollen?

5 Frank called 24 hours after his skin test to report that redness and swelling had recurred at several of the skin test sites. Explain this observation.

6 Frank developed wheezing on several occasions after taking the nonsteroidal anti-inflammatory drugs (NSAIDs) aspirin and ibuprofen (Motrin). Explain the basis for these symptoms.

7 How would the immunotherapy that Frank received help to alleviate his allergies?

8 Although atopic children are repeatedly immunized with protein antigens such as tetanus toxoid, they almost never develop allergic reactions to these antigens. Explain.

CASE 42 Myasthenia Gravis

The immune response turns against the host.

The specific adaptive immune response can, in rare instances, be mounted against self antigens and cause autoimmune disease. Injury to body tissues can result from antibodies directed against cell-surface or extracellular-matrix molecules, from antibodies bound to circulating molecules that deposit as immune complexes, or from clones of T cells that react with self antigens. A special class of autoimmune disease is caused by autoantibodies against cell-surface receptors (Fig. 42.1). Graves' disease and myasthenia gravis are two well-studied examples. Graves' disease is caused by autoantibodies against the receptor on thyroid cells for thyroid-stimulating hormone (TSH), secreted by the pituitary gland. In this disease, autoantibody binds to the TSH receptor; like TSH, it stimulates the thyroid gland to produce thyroid hormones. In myasthenia gravis, the opposite effect is observed: antibodies against the acetylcholine receptor at the neuromuscular junction impede the binding of acetylcholine and stimulate internalization of the receptor, thereby blocking the transmission of nerve impulses by acetylcholine (Fig. 42.2). In addition, the presence of autoantibodies at the neuromuscular junction initiates complement-mediated lysis of the muscle endplate and damages the muscle membrane.

Myasthenia gravis means severe (*gravis*) muscle (*my*) weakness (*asthenia*). This disease was first identified as an autoimmune disease when an immunologist immunized rabbits with purified acetylcholine receptors to obtain antibodies against this receptor. He noticed that the rabbits developed floppy ears, like the droopy eyelids (ptosis) that are the most characteristic symptom of myasthenia gravis in humans. Subsequently, patients with this disease were found to have antibodies against the acetycholine receptor. In addition, pregnant women with myasthenia gravis transfer the disease to their newborn infants. As IgG is the only maternal serum protein that crosses the placenta from mother to fetus, neonatal myasthenia gravis is clear evidence that myasthenia gravis is caused by an anti-IgG antibody. More recently, patients with myasthenia gravis have been identified who have autoantibodies against muscle-specific kinase (MUSK) rather than the acetylcholine receptor. MUSK is a tyrosine kinase receptor involved in clustering acetylcholine receptors; therefore, these autoantibodies also inhibit signaling through the neuromuscular junction.

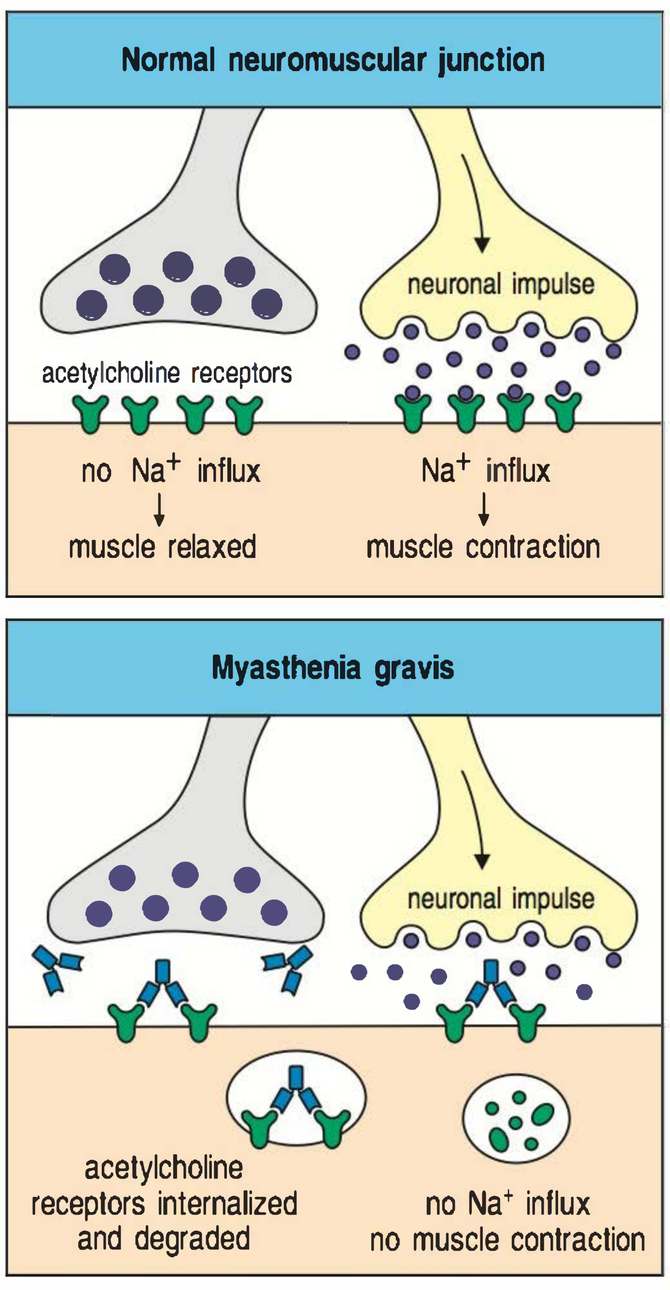
This case was prepared by Raif Geha, MD, in collaboration with Janet Chou, MD.

Topics bearing on this case:
Humoral autoimmunity
Transfer of maternal antibodies
Mechanisms for breaking tolerance

Fig. 42.1 Autoimmune diseases caused by antibody against surface or matrix antigens. These are known as type II autoimmune diseases. Damage by IgE-mediated responses (type I) does not occur in autoimmune disease. In most type II diseases, autoantibodies bind to the cell surface or extracellular matrix and target them for destruction by phagocytes (often with the help of complement) and/or natural killer cells. A special class of autoimmune diseases is caused by autoantibodies that bind cellular receptors and either stimulate or block their normal function. Immune-complex disease (type III) is discussed in Case 37. T cell-mediated disease (type IV) is discussed in Cases 36, 40, and 53.

Some common type II autoimmune diseases caused by antibody against surface or matrix antigens

Syndrome	Autoantigen	Consequence
Autoimmune hemolytic anemia (see Case 41)	Rh blood group antigens, I antigen	Destruction of red blood cells by complement and phagocytes, anemia
Autoimmune thrombocytopenic purpura	Platelet integrin GpIIb:IIIa	Abnormal bleeding
Goodpasture's syndrome	Noncollagenous domain of basement membrane collagen type IV	Glomerulonephritis Pulmonary hemorrhage
Pemphigus vulgaris (see Case 43)	Epidermal cadherin	Blistering of skin
Graves' disease	Thyroid-stimulating hormone receptor	Hyperthyroidism
Myasthenia gravis	Acetylcholine receptor	Progressive weakness
Insulin-resistant diabetes	Insulin receptor (antagonist)	Hyperglycemia, ketoacidosis
Hypoglycemia	Insulin receptor (agonist)	Hypoglycemia



The case of Mr Weld: from floppy ears to droopy eyelids.

Mr Weld, a 71-year-old retired engineer, had been in good health and active all his life. He developed double vision (diplopia). Initially, he did not want to seek medical attention because the double vision sometimes improved spontaneously. However, it gradually worsened over the course of 4 months and he finally scheduled an appointment with his physician.

On examination, the doctor noticed that Mr Weld had ptosis of both eyelids so that they covered the upper third of the irises of his eyes. When the doctor asked Mr Weld to look to the right and then to the left, he noticed limitations in the ocular movements of both eyes, as shown in Fig. 42.3.

The remainder of the neurological examination was normal. No other muscle weakness was found during the examination.

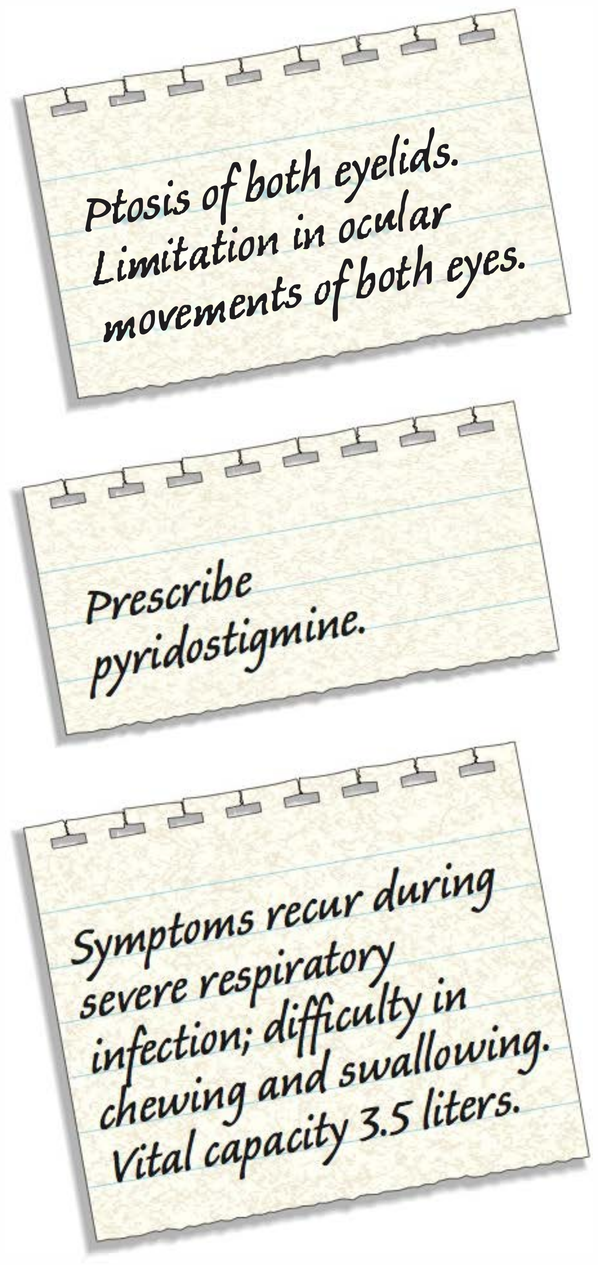
Fig. 42.2 Autoantibodies against the acetylcholine receptor weaken the reception of the signal from nerve ends that cause the muscle cell to contract. At the neuromuscular junction, acetylcholine is released from stimulated neurons and binds to acetylcholine receptors, triggering muscle contraction. The acetylcholine is destroyed rapidly by the enzyme acetylcholinesterase after release. In myasthenia gravis, autoantibodies against the acetylcholine receptor induce its endocytosis and degradation, and prevent muscles from responding to neuronal impulses.

A radiological examination of the chest was performed, and it was normal. There was no evidence in the radiograph of enlargement of the thymus gland. A blood sample was taken from Mr Weld, and his serum was tested for antibodies against the acetylcholine receptor. The serum contained 6.8 units of antibody against the acetylcholine receptor (normal less than 0.5 units). Mr Weld was told to take pyridostigmine, an inhibitor of cholinesterase. His double vision improved steadily but he developed diarrhea from the pyridostigmine, and this limited the amount he could take.

Three years later, Mr Weld developed a severe respiratory infection. Soon afterward, his ptosis became so severe that he had to lift his eyelids by taping them with adhesive tape. His diplopia recurred and his speech became indistinct. He developed difficulty in chewing and swallowing food. He could only tolerate a diet of soft food and it would take him several hours to finish a meal.

On examination the neurologist noted that Mr Weld now had weakness of the facial muscles and the tongue, and the abnormality in ocular movements again became apparent. Because of the diarrhea Mr Weld was only able to tolerate one-quarter of the prescribed dose of pyridostigmine. He also developed difficulty in breathing. His vital capacity (the amount of air he could exhale in one deep breath) was low, at 3.5 liters.

He was admitted to hospital and treated with azathioprine. Thereafter he showed steady improvement. His ptosis and diplopia improved remarkably and he was able to eat normally. His vital capacity returned to normal and was measured to be 5.1 liters.



Myasthenia gravis.

The defining characteristic of myasthenia gravis is a fluctuating weakness that worsens with activity and improves with rest. Normally, repetitive nerve stimulation during sustained physical activity results in the release of decreased amounts of acetylcholine with each successive stimulus; however, enough acetylcholine is released to achieve the desired muscle strength in healthy individuals. In contrast, patients with myasthenia gravis have fewer functional acetylcholine receptors as a result of the presence of anti-receptor autoantibodies. During repetitive nerve stimulation, the combination of fewer functional acetylcholine receptors with the physiologic decrease in neurotransmitter release results in muscular weakness.

Mr Weld experienced a common type of myasthenia gravis, called the oculobulbar form because it primarily affects the muscles of the eye. Older patients tend to have more generalized muscle weakness as well, and often have autoantibodies against muscle proteins in addition to anti-acetylcholine receptor antibodies. In very severe cases, difficulty in swallowing can cause the aspiration of food particles into the lung and impaired breathing, which may be fatal. Plasmapheresis (the filtration and removal of plasma from whole blood) can be used to remove the autoantibodies and treat a myasthenic crisis.

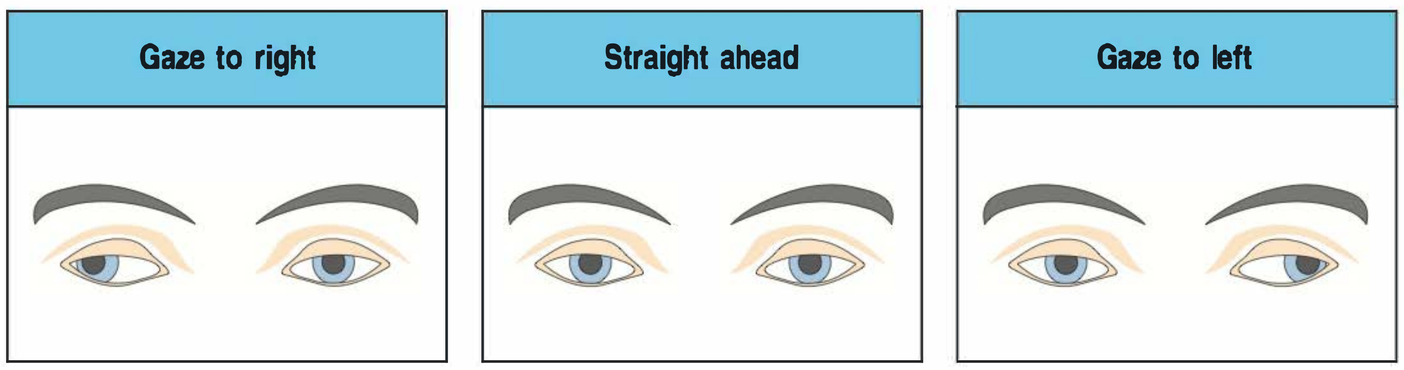


Fig. 42.3 Diagram of ocular movement limitation.

In younger people, the disease presents most often with weakness in the eye muscles. Chest radiographs of younger people with myasthenia gravis frequently reveal enlargement of the thymus gland; however, an association between myasthenia gravis and tumors of the thymus (thymomas) is more common in adults. Early removal of the thymus gland (thymectomy), particularly in those with thymomas, may lead to symptomatic improvement. Although the definitive mechanisms underlying the association between thymomas and myasthenia gravis are not yet identified, it has been hypothesized that neoplastic epithelial cells in the thymoma express selflike epitopes resembling proteins such as the acetylcholine receptor. In addition, thymomas have been found to have decreased expression of the autoimmune regulator gene (*AIRE*; see Case 17) and smaller numbers of regulatory T cells, indicating that an abnormal microenvironment within the thymomas results in impaired negative selection. However, as the occurrence of myasthenia gravis does not correlate with decreased expression of *AIRE* in the thymus, there are still unidentified factors that influence the development of myasthenia gravis in patients with thymomas.

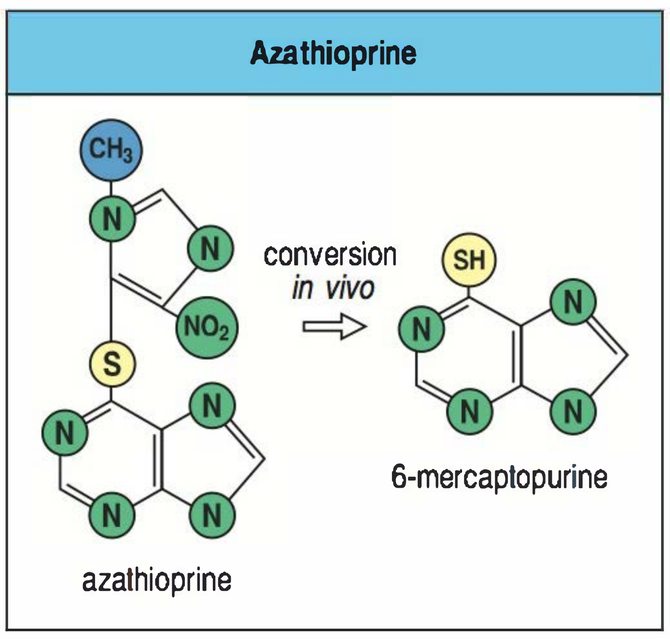


Fig. 42.4 The structure of azathioprine and its active product, mercaptopurine.

Questions.

1 *Newborn infants of mothers with myasthenia gravis exhibit symptoms of myasthenia gravis at birth. How long would the disease be likely to last in these infants?*

2 *Pyridostigmine is an ideal drug for the treatment of myasthenia gravis. It inhibits the enzyme cholinesterase, which normally cleaves and inactivates acetylcholine. In this way, pyridostigmine prolongs the biological half-life of acetylcholine. Unfortunately, it also causes diarrhea by increasing the amount of acetylcholine in the intestine. Acetylcholine binds to the muscarinic receptors in the intestine and increases intestinal motility. Because he could not tolerate full therapeutic doses of pyridostigmine and was getting worse, Mr Weld was given azathioprine (Fig. 42.4) and showed marked improvement. What did the azathioprine do? What would concern you about prolonged use of this drug?*

3 *Mr Weld had a severe relapse in his disease after a respiratory infection. Many autoimmune diseases seem to be triggered by infection, and relapses in autoimmune diseases frequently follow an infection. Can you explain how this might happen?*

CASE 37 Systemic Lupus Erythematosus

A disease caused by immune complexes.

Immune complexes are produced whenever there is an antibody response to a soluble antigen. As the immune response progresses, larger immune complexes form that trigger the activation of complement. These activated complement components then bind the triggering immune complexes. Large complexes are efficiently cleared by binding to complement receptor 1 (CR1) on erythrocytes, which convey the immune complexes to the liver and spleen. There, they are removed from the red-cell surface through interaction with a variety of complement and Fc receptors on Kupffer cells and other phagocytes (Fig. 37.1). When antigen is released repeatedly, there may be a sustained formation of small immune complexes; these complexes tend to be trapped in the small blood vessels of the renal glomeruli and synovial tissue of the joints.

The most common immune-complex diseases are listed in Fig. 37.2. In subacute bacterial endocarditis, bacteria reside for a protracted period on the heart valves. This infection and subsequent inflammation damage the valve. At the same time, the antibody response to the prolonged presence of the bacteria is intense, and immune complexes of IgG antibodies and bacterial antigens are formed. These complexes become trapped in the renal glomeruli and cause glomerulonephritis. The immunoglobulins in the immune complexes provoke the formation of anti-IgG IgM antibodies known as rheumatoid factor (see Case 36). In a similar fashion, viral hepatitis can become a chronic infection that provokes a marked IgG antibody response, with the consequent formation of virus-containing immune complexes and rheumatoid factor. The immune complexes can be entrapped in the renal glomeruli as well as in small blood vessels of the skin, nerves, and other tissues, where they cause inflammation of the blood vessels (vasculitis). The antibodies in the virus-containing immune complexes have the property of precipitating in the cold (less than 37°C) and are therefore termed cryoglobulins (see Case 38).

This case was prepared by Raif Geha, MD, in collaboration with Erin Janssen, MD.

Topics bearing on this case:

- Clearance of immune complexes by complement
- Immune-complex disease
- Coombs' tests

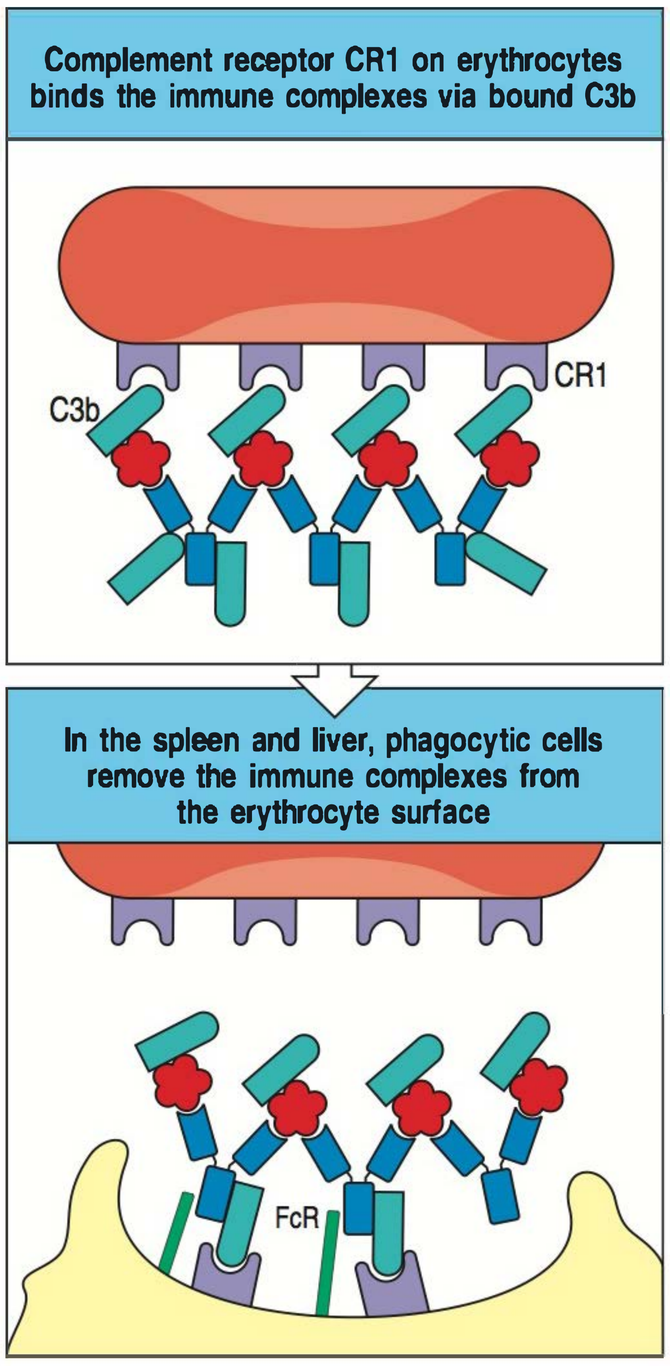
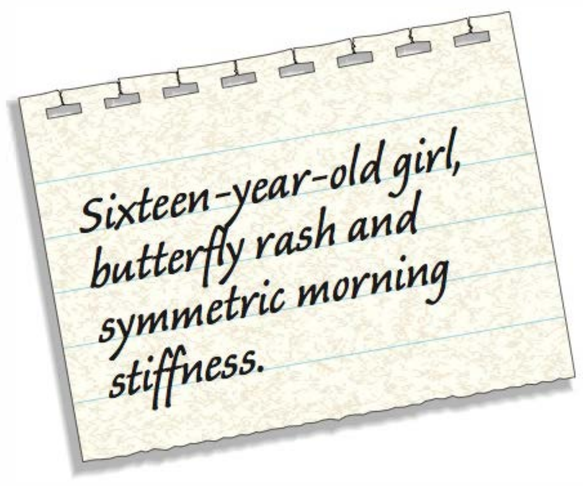


Fig. 37.1 Immune complexes are cleared from the circulation by binding to complement and Fc receptors. Immune complexes activate C3 in the serum, and bind activated complement components C3b, C4b, and C2a. C3b binds to complement receptors on erythrocytes, which transport the immune complexes to the spleen and liver, where complement receptors and Fc receptors on phagocytic cells bind to complement components and to the Fc portion of antibodies, and are thereby stimulated to engulf the complexes and degrade them.

The most prevalent immune-complex disease is systemic lupus erythematosus (SLE), which is characterized by the formation of antibodies against DNA. Every day, millions of nuclei are extruded from erythroblasts in the bone marrow as they mature into red blood cells (erythrocytes). This event, among others, provides a rich source of DNA in those individuals prone to making an immune response to DNA and developing SLE.

The case of Nicole Chawner: too much sun at the beach.



Nicole Chawner was a healthy 16-year-old until this summer. A few days after excessive exposure to the sun on the beach, Nicole developed a red rash on her cheeks. She saw her family doctor, who recognized that the butterfly rash on her cheeks and bridge of her nose was typical of systemic lupus erythematosus (SLE) (Fig. 37.3).

He referred Nicole to the Children's Hospital, where she was asked about any other problems she might have noticed. Nicole said that when she woke up in the morning her fingers and knees were stiff, although they got better as the day wore on. Nicole had also noticed some symmetric swelling in her fingers.

A blood sample was taken from Nicole to ascertain whether she had anti-nuclear antibodies (ANA). These were positive, at a titer of 1:1280. Because of this result, further tests were performed for antibodies characteristically found in SLE. An elevated level of antibodies against double-stranded DNA was also found. Her serum C3 level was 73 mg dl^{-1} (normal 100–200 mg dl^{-1}). Her platelet count was normal at 225,000 μl^{-1}, and her direct and indirect Coombs tests were negative, as was a test for anti-phospholipid antibodies. A urine sample was also found to be normal.

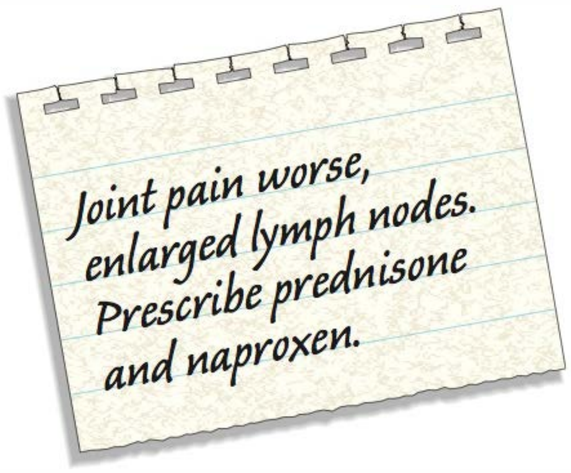
Nicole was advised to take an antimalarial agent, hydroxychloroquine sulfate (Plaquenil), and to avoid direct sunlight. She did well for a while but, after a month, the

Fig. 37.2 Three autoimmune diseases that result in damage by immune complexes.

Immune-complex disease		
Syndrome	**Autoantigen**	**Consequence**
Subacute bacterial endocarditis	Bacterial antigen	Glomerulonephritis
Mixed essential cryoglobulinemia (see Case 38)	Rheumatoid factor IgG complexes (with or without hepatitis C antigens)	Systemic vasculitis
Systemic lupus erythematosus	DNA, histones, ribosomes, snRNP, scRNP	Glomerulonephritis, vasculitis, arthritis

morning stiffness in her fingers and knees worsened. She developed a fever of 39°C each evening accompanied by shaking chills. Enlarged lymph nodes were felt behind her ears and in the back of her neck. She also lost 4.6 kg over the course of the next 2 months.

When she returned to the hospital for a check-up, it was noted that her butterfly rash had disappeared. She had diffuse swelling of the proximal joints in her fingers and toes. Blood was drawn at this time, and the level of anti-DNA antibodies was found to have increased. The serum C3 level was 46 mg dl^{-1}. Nicole was advised to take 10 mg of prednisone twice a day, as well as 250 mg of the nonsteroidal anti-inflammatory drug naproxen twice a day. This quickly controlled her symptoms, and she remained well. At her next visit, her serum C3 level was 120 mg dl^{-1}.



Systemic lupus erythematosus (SLE).

Systemic lupus erythematosus (SLE) is the most prevalent immune-complex disease in developed countries. For reasons that are not clear, it affects 10 times as many females as males. Patients with SLE usually have antibodies against multiple autoantigens. The most common autoantibody, which is found in the serum of 60% of all SLE patients, is against double-stranded DNA. Other commonly found antibodies are against small ribonucleoproteins. Autoantibodies against blood cells, such as platelets and red blood cells, as well as against the phospholipid complex that is formed by the activation of the proteins of the clotting system (antiphospholipid antibodies), are not infrequently seen. Most patients tend to have a range of these autoantibodies.

The immune complexes in SLE are small and tend to be trapped or formed inside tissues, primarily in the kidney and, to a lesser extent, in the synovial tissues of joints. For this reason, glomerulonephritis and arthritis are two of the most frequently encountered symptoms of SLE. These immune complexes fix complement efficiently, and tissue injury to the kidney or joints is mediated by activation of the complement system.

Cytokine signaling pathways have also been implicated in the pathogenesis of lupus. Type I interferons (IFN-α and IFN-β), which are important in suppressing viral replication, are secreted in response to the triggering of Toll-like receptors (TLRs). Immune complexes may trigger this response in SLE. Type I IFNs promote the activation of autoreactive T cells and augment class switching and antibody production in B cells. IRF5 is a transcription factor involved in interferon synthesis, and IRF5 haplotypes were one of the first genetic susceptibility factors identified in SLE. Further support for the link between IFNs and SLE is provided by the fact that a small percentage of patients on IFN-α treatment develop lupus. This drug-induced SLE occurs regardless of gender and tends to clear once the IFN-α is withdrawn.

The word 'lupus' is Latin for wolf, and this word is applied to a common symptom of SLE, the butterfly rash on the face. In the 19th century, the severe scarring rash on the face was named lupus because it was said to resemble the bite of a wolf. At that time, it was not possible to distinguish lupus erythematosus from lupus vulgaris, a scarring rash caused by tuberculosis. For unknown reasons, the rash is evoked by exposure to the sun (ultraviolet light). There is a seasonal variation to the onset of SLE, which is greatest in the Northern Hemisphere between March and September, when the greatest amount of ultraviolet light penetrates the atmosphere. Antimalarials such as hydroxychloroquine seem particularly helpful in the treatment of lupus skin disease.

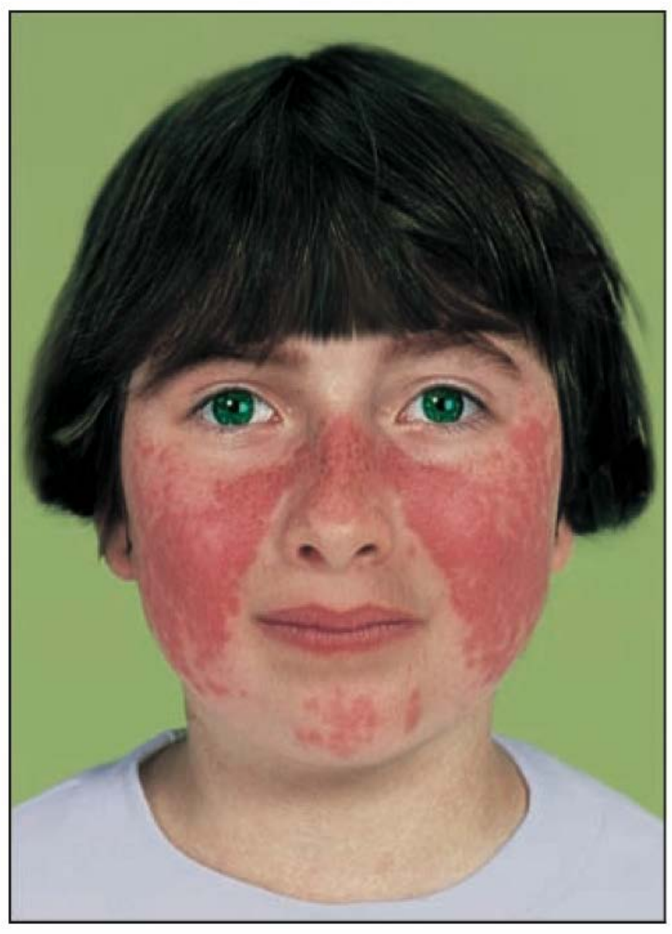


Fig. 37.3 The so-called butterfly rash typical of systemic lupus erythematosus. Photograph courtesy of M. Walport.

Questions.

1 Why do you think Nicole's serum C3 was measured, both on her first visit to the hospital and after therapy?

2 What are the direct and indirect Coombs tests, and what did they tell us in this case?

3 Why was Nicole told to avoid direct exposure to sunlight?

4 Repeated analysis of Nicole's urine was negative. What does this mean?

5 Nicole had a serum IgG level of 2020 mg dl^{-1}. This substantially elevated level of IgG is commonly found in patients with SLE. How could you explain this? And what would you expect to find if we took a biopsy of Nicole's swollen lymph nodes?

6 The antigen in the immune complexes formed in SLE is often a complex antigen, such as part of a nucleosome or a ribonucleoprotein particle, which contains several different molecules. Patients often produce autoantibodies against each of these different components. What is the reason for the production of this variety of autoantibodies, and what type of failure in tolerance could be responsible for autoantibody production?

CASE 40 Multiple Sclerosis

An autoimmune attack on the central nervous system.

As we saw in rheumatoid arthritis (Case 36), autoimmune disease can be caused by activated effector T cells specific for self peptides. When T cells recognize self-peptide:MHC complexes and become activated, they can cause local inflammation by activating macrophages, for example, with consequent tissue damage. Another example of a T cell-mediated autoimmune disease is the neurologic disease multiple sclerosis (MS). However, unlike the involvement of autoantibodies in some types of autoimmune diseases (see Case 42), it has been difficult to prove the involvement of T cells in MS, because T cells do not cross the placenta into the fetus and experimental T-cell transfer is not allowed in humans.

Seventy years ago, an experimental model of MS was established in mice, in which the injection of myelin in adjuvant caused the development of neurologic symptoms similar to those of MS. This disease is called experimental autoimmune encephalomyelitis (EAE). The antigens in myelin that can induce EAE are myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG). The disease can be transferred to syngeneic animals by cloned antigen-specific T-cell lines derived from animals with EAE (Fig. 40.1). When the recipient animals are immunized with MBP, for example, they develop active disease. T cells specific for MBP, PLP, and MOG have been found in the blood and cerebrospinal fluid (CSF) of patients with MS.

This case was prepared by Raif Geha, MD, in collaboration with Andrew Shulman, MD, PhD.

Topics bearing on this case:

- Inflammatory reactions
- Interactions of co-stimulatory molecules with their receptors
- The development of tolerance to self antigens
- Activation of self-reactive T cells
- Immunologically privileged sites
- Experimental autoimmune encephalomyelitis
- Induction of oral tolerance

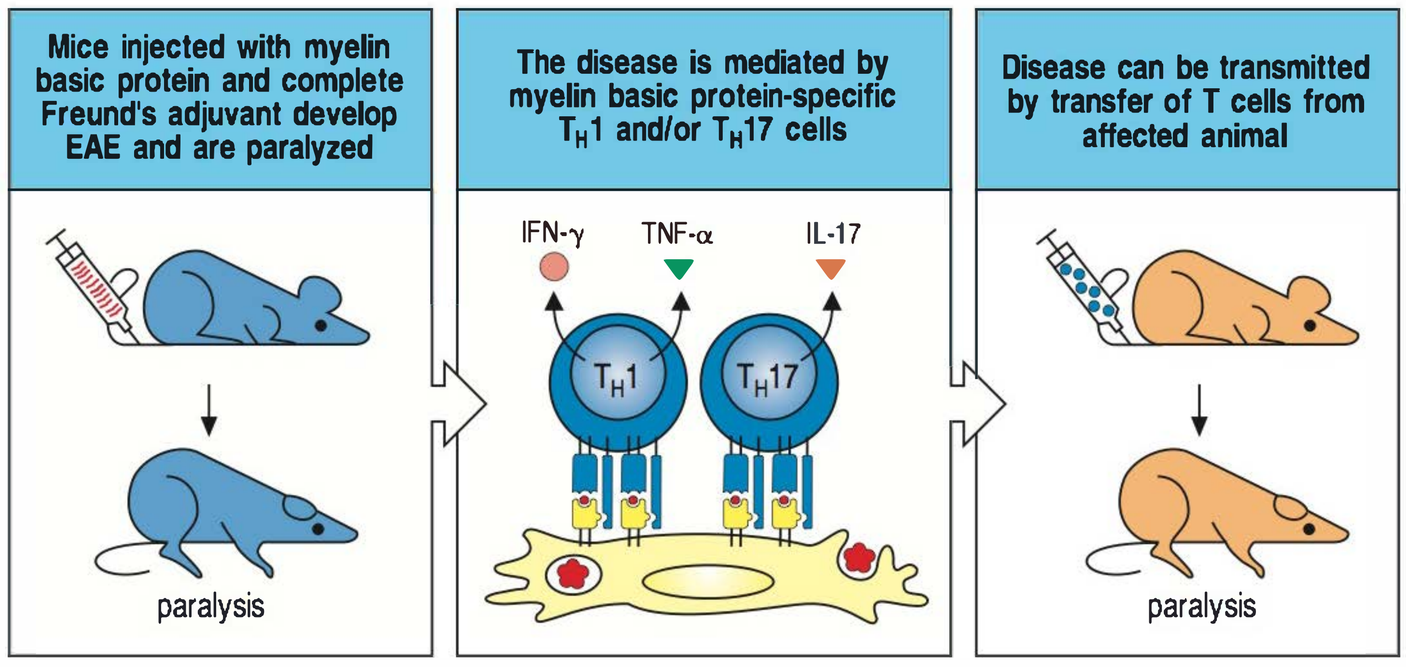
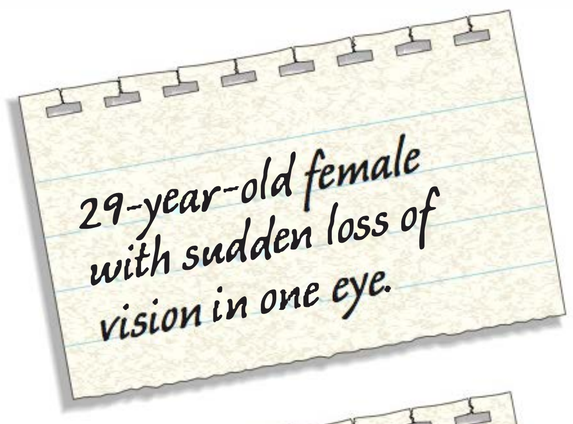


Fig. 40.1 T cells specific for myelin basic protein mediate inflammation of the brain in experimental autoimmune encephalomyelitis (EAE). This disease is produced in experimental animals by injecting them with isolated spinal cord homogenized in complete Freund's adjuvant. EAE is due to an inflammatory reaction in the brain that causes a progressive paralysis affecting first the tail and hindlimbs before progressing to forelimb paralysis and eventual death. One of the autoantigens identified in the spinal cord homogenate is myelin basic protein (MBP). Immunization with MBP alone in complete Freund's adjuvant can also cause these disease symptoms. Inflammation of the brain and paralysis are mediated by T_H1 and/or T_H17 cells specific for MBP. Cloned MBP-specific T_H1 and/or T_H17 cells can transfer symptoms of EAE to naive recipients provided that the recipients carry the correct MHC allele. In this system it has therefore proved possible to identify the peptide:MHC complex recognized by the TH clones that transfer disease.



The case of Vivie Warren: an oboist who has difficulty reading a musical score.

Mrs Vivie Warren, a 29-year-old professional oboe player, was in good health until one morning she noticed a loss of vision in her left eye. Her physician referred her to a neurologist, who found that her eye movement was normal and not accompanied by any pain. The visual acuity in Vivie's left eye was 20/100 and in her right eye 20/200. Her retina was normal, and a detailed neurologic examination also proved normal. The neurologist diagnosed optic neuritis (inflammation of the optic nerve). Her family history was informative, however, in that her mother had severe MS and was permanently disabled, and a magnetic resonance imaging (MRI) brain scan was ordered.

Vivie was given a 5-day course of intravenous corticosteroids, and her vision returned to normal over the next 3 weeks. The MRI scan revealed multiple lesions in the white matter of the brain under the cortex and around the ventricles (Fig. 40.2). Intravenous injection of gadolinium, a contrast agent that leaks from blood vessels in recently inflamed tissue, showed that some of the brain lesions were probably of recent origin (Fig. 40.3). The neurologist told Vivie that she had a high probability of developing MS and advised her to return for frequent neurologic examination.

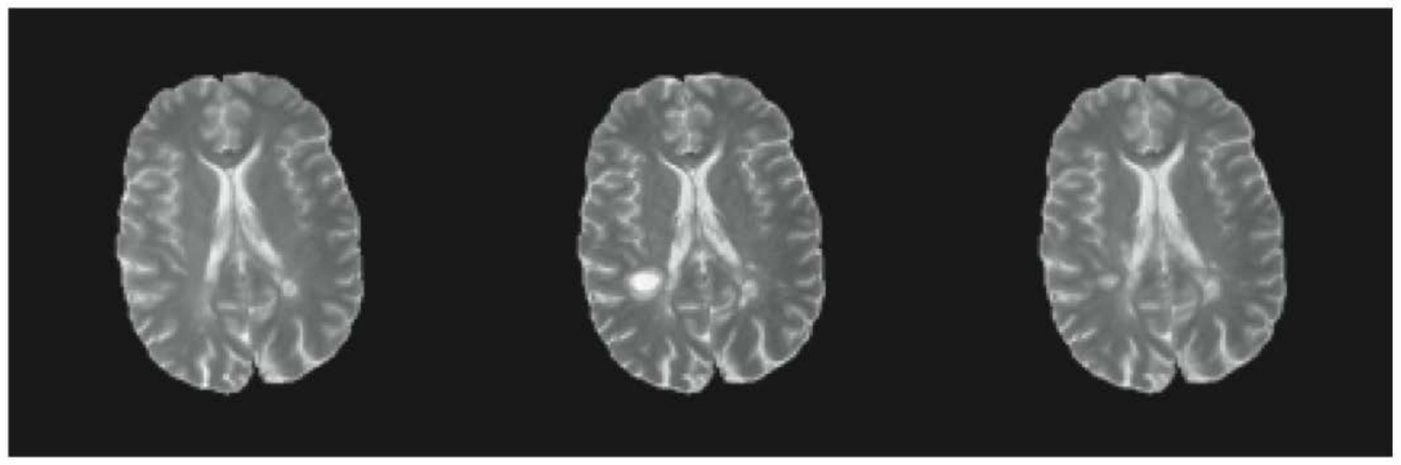


Fig. 40.2 Three-dimensional magnetic resonance images of the brain at three different time points in the course of MS. Left, early; center, during acute exacerbation; right, after therapy. The technique used causes fluid to appear white. The lateral ventricles in the middle of the brain scan and the sulci of the cerebral cortex around the edge appear white as a result of normal cerebrospinal fluid. The white spots, which are due to edema fluid and decreased myelin, are MS lesions.

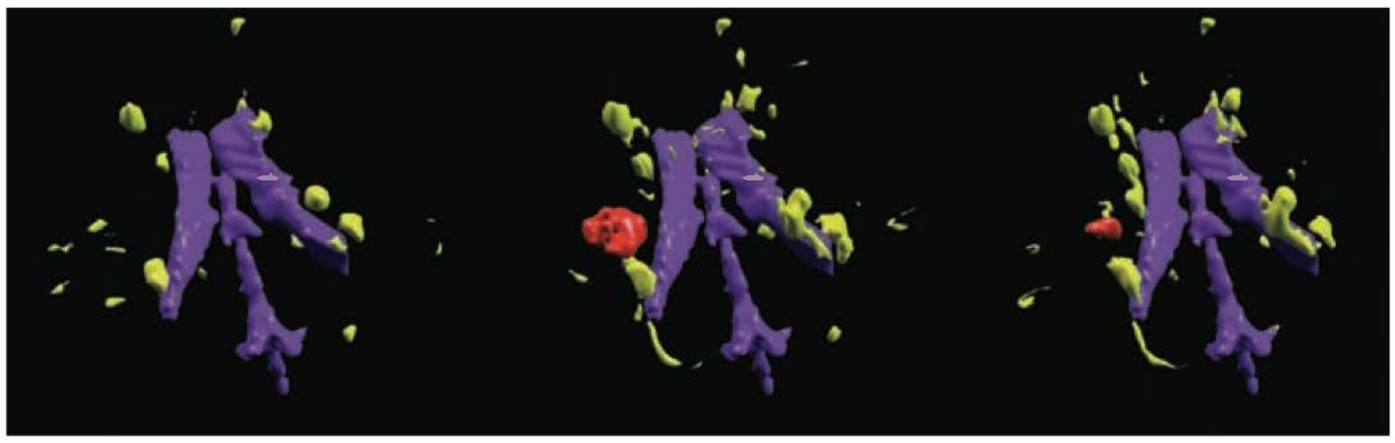


Fig. 40.3 Three computer-generated images of MRI scans of the brain shown in Fig. 40.2 at the same time points. Several levels of MRI scans have been integrated by computer. The lateral and middle ventricles appear in purple. Old MS lesions appear yellow-green. A new, gadolinium-enhanced, lesion appears orange. On the right, this lesion has diminished in size after immunosuppressive therapy.

Vivie remained well for a further 3 years and then developed weakness of the muscles on the left side of her face that were innervated by the seventh cranial nerve. A repeat MRI scan with gadolinium enhancement showed new lesions in the left middle cerebellar peduncle and in the pons. CSF was obtained by lumbar puncture. It contained 28 mg dl^{-1} protein (normal) and 8 lymphocytes ml^{-1} (normal 0–3 ml^{-1}). At this point a firm diagnosis of MS was made. Despite the normal level of protein in the CSF, the IgG content was raised. On electrophoresis, discrete bands of IgG were observed, indicating clonal expansion of restricted B-cell populations in the central nervous system (CNS). Another 5-day course of corticosteroids was administered intravenously, and Vivie's symptoms improved. Weekly intramuscular injections of interferon (IFN)-β were started to prevent progression of the disease.

Vivie did well for 3 more years, after which she developed a weakness in her left leg and left hand. Her speech became slurred. She developed nystagmus (rapid uncontrolled horizontal jerking eye movements when attempting to fix the gaze on something) and ataxia (wide-based staggering gait). Vivie was given another course of corticosteroids, after which her symptoms improved, but 8 months later they recurred. The injections of IFN-β were stopped and she was put on high doses of cyclophosphamide and corticosteroids at monthly intervals. After 3 months of this therapy, the cyclophosphamide and corticosteroid injections were gradually reduced to every 12 weeks. Her neurological examination became normal and no new lesions were observed on gadolinium-enhanced MRI.

Multiple sclerosis.

Multiple sclerosis (MS) was first described by the great French neurologist Jean-Martin Charcot in the 1860s. It was noted at autopsy that patients who died of this disease had multiple hard (sclerotic) plaques scattered throughout the white matter of the CNS. The disease is 10 times more frequent in women than in men and is associated with HLA-DR2. Those affected have a variety of nervous symptoms, such as urinary incontinence, blindness, ataxia, muscle weakness, and paralysis of limbs. The plaques characteristic of the disease show dissolution of myelin along with infiltrates of lymphocytes and macrophages, particularly along blood vessels. The inflammatory exudate causes increased vascular permeability.

The CNS is a relatively immunologically privileged site from which antigens do not normally reach the lymphoid tissues, and so there is no negative selection of T cells with the potential to react against CNS antigens. In MS, an unknown injurious event is presumed to provoke the release of CNS antigens and their presentation to lymphocytes in the peripheral lymphoid organs. This results in the expansion of clones of autoreactive T cells and their differentiation into T_H1 cells, which home to the CNS and initiate inflammation. These T_H1 cells can be readily identified in the CNS of patients with MS.

Lymphocytes and other blood cells do not normally cross the blood–brain barrier. If tissue becomes inflamed, however, activated CD4 T cells autoreactive for a brain antigen and expressing α_4:β_1 integrin, which binds vascular cell adhesion molecules (VCAM) on the surface of activated venule endothelium, can migrate out of the blood into the brain. There they reencounter their specific autoantigen presented by MHC class II molecules on microglial cells and produce pro-inflammatory cytokines such as IFN-γ (Fig. 40.4). Microglia are phagocytic macrophage-like cells of the innate immune system resident in the CNS and, like macrophages, can act as antigen-presenting cells. Inflammation causes increased vascular permeability, and the site becomes heavily infiltrated by activated macrophages and T_H1 cells, which produce pro-inflammatory cytokines that exacerbate the inflammation, resulting in the further recruitment of T cells, B cells, macrophages, and dendritic cells to the site of the lesion. Autoreactive B cells produce autoantibodies against myelin antigens with help from T cells. Activated mast cells release histamine, contributing to the inflammation. In some way that is not yet fully understood, these combined activities lead to demyelination and interference with neuronal function.

Mice deficient in IFN-γ are not protected from the development of EAE, suggesting that additional T_H cells may drive CNS inflammation. T_H17 cells are a recently identified helper T-cell population that can be induced from memory T cells by a combination of cytokines including IL-6, IL-21, and transforming growth factor-β (TGF-β) and are sustained by IL-23. Although T_H17 cells do not seem to mediate inflammation in all contexts, studies in mouse EAE and human MS reveal a pathologic role for T_H17 cells and their associated cytokine, IL-17. Adoptive transfer of MOG-specific T_H1 and T_H17 cells differentiated *in vitro* induces EAE with a distinct histologic appearance, indicating that several effector T-cell types drive autoimmunity in MS. Autoreactive T_H1 cells with specificity for MS-associated myelin antigens can be found in healthy patients without MS. Investigators searching for the suppressive mechanisms that inhibit potentially autoreactive T cells in healthy individuals have focused on regulatory T cells (T_{reg} cells), a subset of naturally occurring CD4 $CD25^+$ T cells that promote peripheral tolerance and inhibit autoimmunity in multiple organs. Although patients with MS have normal numbers of T_{reg} cells, the cells have a decreased ability to suppress autoreactive T cells *in vitro*. Future research will seek to further investigate the regulation of T_H-cell subtypes and the interplay of autoreactive and suppressive T cells in MS.

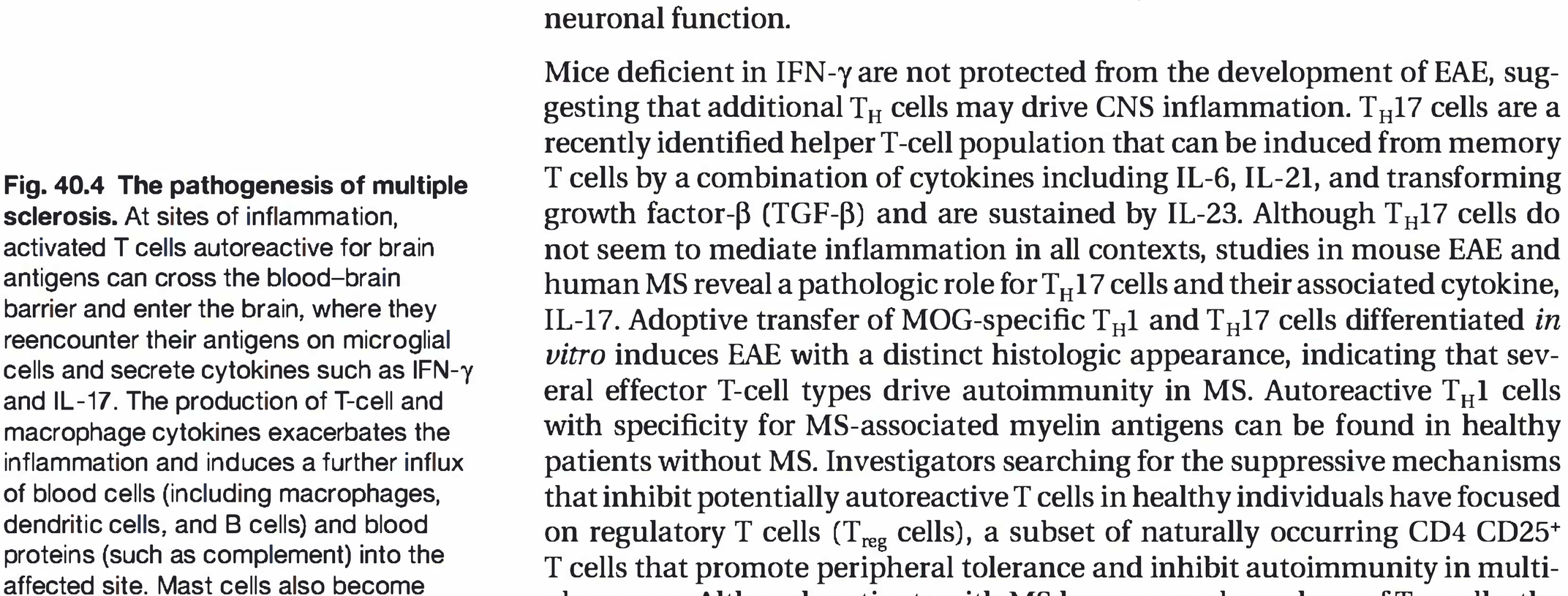


Fig. 40.4 The pathogenesis of multiple sclerosis. At sites of inflammation, activated T cells autoreactive for brain antigens can cross the blood–brain barrier and enter the brain, where they reencounter their antigens on microglial cells and secrete cytokines such as IFN-γ and IL-17. The production of T-cell and macrophage cytokines exacerbates the inflammation and induces a further influx of blood cells (including macrophages, dendritic cells, and B cells) and blood proteins (such as complement) into the affected site. Mast cells also become activated. The individual roles of these components in demyelination and loss of neuronal function are still not well understood.

In mice, feeding with MBP before immunization with MBP in adjuvant protects against the development of EAE. MBP-specific T cells can be identified in the brain of protected mice, but they secrete TGF-β rather than IFN-γ and fail to initiate an inflammatory reaction. Furthermore, adoptive transfer of T cells from mice fed MBP can confer protection against EAE. Therapeutic attempts to treat patients with MS by oral MBP have not been successful, however. This suggests that therapeutic interventions that induce oral tolerance may not be effective in already established disease.

Questions.

1 *Oligoclonal immunoglobulins were found in Mrs Warren's central nervous system. How do you explain this?*

2 *Mrs Warren was treated with corticosteroids, cyclophosphamide, and IFN-β. What was the aim of this therapy?*

3 *An attempt has been made to treat MS patients with IFN-γ. Can you predict what the outcome was and why?*

4 *What is the rationale behind feeding MBP to mice to prevent EAE?*

5 *Can you predict whether EAE can be induced in CD28 knockout mice?*

CASE 10 Acquired Immune Deficiency Syndrome (AIDS)

Infection can suppress adaptive immunity.

Certain infectious microorganisms can suppress or subvert the immune system. For example, in lepromatous leprosy, *Mycobacterium leprae* induces T cells to produce lymphokines that stimulate a humoral response but suppress the development of a successful inflammatory response to contain the leprosy bacillus. The leprosy bacillus multiplies and there is a persistent depression of cell-mediated immune responses to a wide range of antigens (see Case 48). Another example of immunosuppression is provided by bacterial superantigens, such as toxic shock syndrome toxin-1. Superantigens bind and stimulate large numbers of T cells by binding to certain V_β chains of the T-cell receptor, inducing massive production of cytokines by the responding T cells (see Case 47). This, in turn, causes a temporary suppression of adaptive immunity.

At the beginning of the 20th century, when tuberculosis was the leading cause of death and fully half the population was tuberculin-positive, it was well known that an intercurrent measles infection would cause a well-contained tuberculosis infection to run rampant and result in death. The mechanism responsible is now known to be the suppression of IL-2 synthesis after binding of measles virus to CD46 on macrophages.

Some of the microorganisms that suppress immunity act by infecting lymphocytes. Infectious mononucleosis or glandular fever is caused by a virus (Epstein–Barr virus) that infects B lymphocytes. The infection activates cytotoxic CD8 T cells, which destroy the B cells in which the Epstein–Barr virus is replicating. In the third week of infection, at the height of activation of CD8 T cells, all adaptive immunity is suppressed. The cytokines responsible for the immunosuppression are not well defined but probably include IL-10 and TGF-β (see Case 45).

Topics bearing on this case:
Failure of cell-mediated immunity
Infection with the human immunodeficiency virus (HIV)
Control of HIV infection
Drug therapy for HIV infection
ELISA test

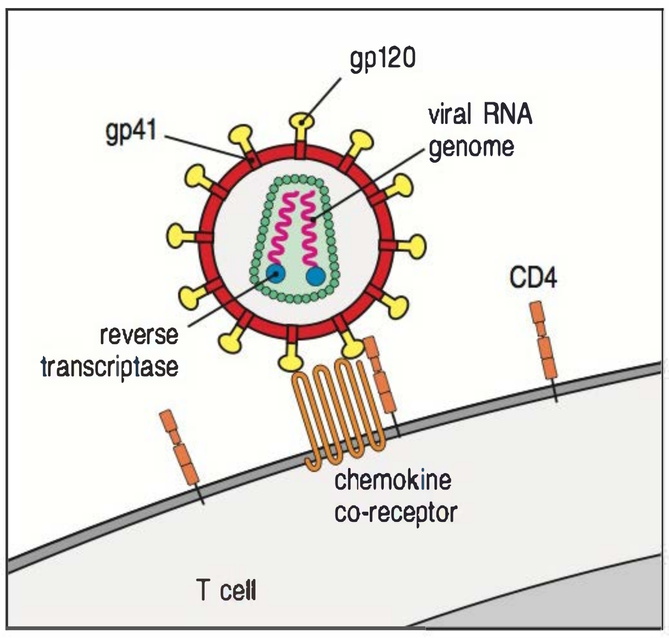
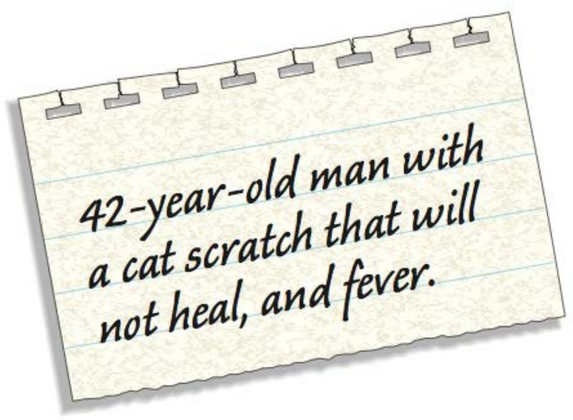
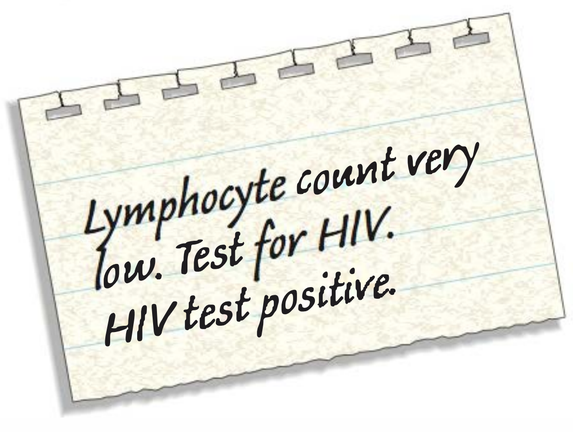


Fig. 10.1 HIV binds to CD4 T cells through its coat glycoprotein gp120. The gp120 molecule on the surface of the virus binds CD4 on T cells and macrophages; the viral protein gp41 then mediates fusion of the enveloped virus with the target cells, allowing the viral genome to enter the cell. The chemokine receptors CCR5 and CXCR4 act as co-receptors of HIV.

The human immunodeficiency virus (HIV) presents a chilling example of the consequences of infection and destruction of immune cells by a micro-organism. CD4 molecules on the T-cell surface act as the receptors for HIV (Fig. 10.1). CD4 is also expressed on the surface of cells of the macrophage lineage and they, too, can be infected by this virus. The chemokine receptors CCR5 and CXCR4 act as obligatory co-receptors for HIV. As we shall see, the primary infection with HIV may go unnoticed, and the virus may replicate in the host for many years before symptoms of immunodeficiency are seen. During this period of clinical latency, the level of virus in the blood and the number of circulating CD4 cells remain fairly steady, but in fact both virus particles and CD4 cells are being rapidly destroyed and replenished, as rounds of virus replication take place in newly infected cells. When the rate at which CD4 cells are being destroyed exceeds the capacity of the host to replenish them, their number decreases to a point at which cell-mediated immunity falters. As we have seen in other cases, such as severe combined immunodeficiency (see Case 5), the failure of cell-mediated immunity renders the host susceptible to fatal opportunistic infections.

The case of Martin Thomas: a police officer whose past comes back to haunt him.





Martin Thomas is a 42-year-old African-American police officer who has always been in good health. He has been married for 10 years and has one child, an 8-year-old daughter. Six months ago he went to the emergency room at the local hospital complaining of a fever and a swollen right hand. He was admitted to hospital for the hand infection, which was assumed to be the result of a cat scratch. His blood lymphocyte count was found to be very low, so a blood sample was sent to be tested for antibodies against the human immunodeficiency virus (HIV). Both an ELISA (enzyme-linked immunosorbent assay) (Fig. 10.2) and a Western blot (Fig. 10.3) revealed the presence of anti-HIV antibodies. Officer Thomas was referred to Dr Wright, an AIDS specialist, at the Massachusetts General Hospital.

Martin Thomas told Dr Wright that he had had several homosexual encounters before his marriage 10 years ago. He had always been in good health until 6 months before the present consultation, when he began to have drenching night sweats several times a week. Over this period his body weight had gone down from 94.5 kg to 90 kg. He could not remember having any infections other than the one in his hand, nor any rashes, gastrointestinal problems, cough, shortness of breath, or any other symptoms. His mother had been 84 years old when she died of a heart attack, and his father had died at age 87 from cirrhosis of the liver, cause unknown. His wife and child were both in good health and his wife had recently tested negative for anti-HIV antibodies. Mr Thomas told Dr Wright that he did not smoke or use intravenous drugs. He drank large amounts of beer at weekends. A cat and a dog were the only pets in the house.

On physical examination his blood pressure was 130/90, his pulse rate 92, and temperature 37.5°C (all normal). Nothing abnormal was found during the physical examination. His white blood cell count was 5800 μl^{-1} (normal), his hematocrit was 31.3, and his platelet count was 278,000 μl^{-1} (both normal). His CD4 T-cell count was very low at 170 μl^{-1} (normal 500–1500 μl^{-1}) and his load of HIV-1 RNA was 67,000 copies ml^{-1}.

Mr Thomas was prescribed trimethoprim sulfamethoxazole for prophylaxis against *Pneumocystis jirovecii* pneumonia (see Case 5). He was also given a combination antiretroviral therapy consisting of zidovudine (Retrovir, AZT), lamivudine (Epivir, 3TC), and efavirenz (Sustiva). He was counseled about safe sex with his wife.

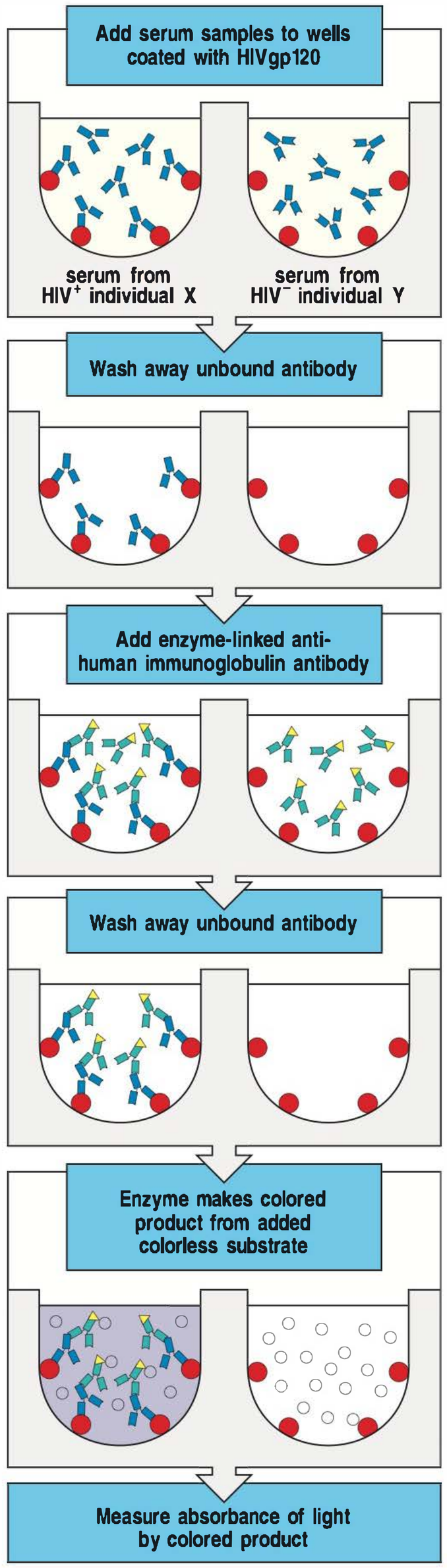


Fig. 10.2 Use of the enzyme-linked immunosorbent assay (ELISA) to detect the presence of antibodies against the HIV coat protein gp120. Purified recombinant gp120 is coated onto the surface of plastic wells to which the protein binds nonspecifically; residual sticky sites on the plastic are blocked by adding irrelevant proteins (not shown). Serum samples from the individuals being tested are then added to the wells under conditions where nonspecific binding is prevented, so that only binding to gp120 causes antibodies to be retained on the surface. Unbound antibody is removed from all wells by washing, and anti-human immunoglobulin that has been chemically linked to an enzyme is added, again under conditions that favor specific binding alone. After further washing, the colorless substrate of the enzyme is added, and colored material is deposited in the wells in which the enzyme-linked anti-human immunoglobulin is found. This assay allows arrays of wells known as microtiter plates to be read in fiberoptic multichannel spectrometers, greatly speeding the assay.

After 5 weeks of this therapy his HIV-1 viral load declined to 400 copies of RNA ml^{-1} and after 8 weeks to <50 copies of RNA ml^{-1}, in other words to undetectable levels. In the meantime his CD4 T-cell count rose to 416 μl^{-1}. The prophylaxis for *Pneumocystis* was discontinued. Mr Thomas remains well and active and works full time.

Acquired immune deficiency syndrome (AIDS).

AIDS is caused by the human immunodeficiency virus (HIV), of which there are two known types, HIV-1 and HIV-2. HIV-2 was largely confined to West Africa but now seems to be spreading into Southeast Asia. HIV infections in North and South America and in Europe are exclusively from HIV-1. HIV can be transmitted by homosexual and heterosexual intercourse, by infusion of contaminated blood or blood products, or by contaminated needles, which are the major source of infection among drug addicts. The infection can also be passed from mother to child during pregnancy, during delivery or, more uncommonly, by breastfeeding. In the past, between 25% and 35% of infants born to HIV-positive mothers were infected, but the rate of vertical transmission in industrialized countries has more recently dropped to 3–10% by giving HIV-positive pregnant women antiretroviral drugs such as zidovudine.

Contact with the virus does not necessarily result in infection. The standard indicator of infection is the presence of antibodies against the virus coat protein gp120. The initial infection, as in Mr Thomas's case, may pass unnoticed and without symptoms. More often, a mild viral illness within 6 weeks of infection is sustained, with fever, swollen lymph nodes, and a rash. It subsides at about the time that seroconversion (the appearance of anti-HIV antibodies) occurs, and although virus and antibody persist, the patient feels well. A period of clinical latency lasting years, and perhaps even decades, may ensue during which the infected person feels perfectly well. Then they begin to experience low-grade fever and night sweats, excessive fatigue, and perhaps candidiasis (thrush) in the mouth. Lymph nodes in the neck or axillae (armpits) or groin may swell. Weight loss may become very marked. These are the prodromal symptoms of impending AIDS. (A prodrome is a concatenation of signs and symptoms that predict the onset of a syndrome.) The number of CD4 T cells in the blood may have been normal up to this time but, with the onset of the prodrome, the CD4 T-cell count begins to fall (Fig. 10.4). When the number of CD4 T cells decreases to the range of 200–400 cells μl^{-1}, the final phase of the illness, which is called AIDS, starts. At this time serious, eventually fatal, opportunistic infections as well as certain unusual malignancies occur (Fig. 10.5).

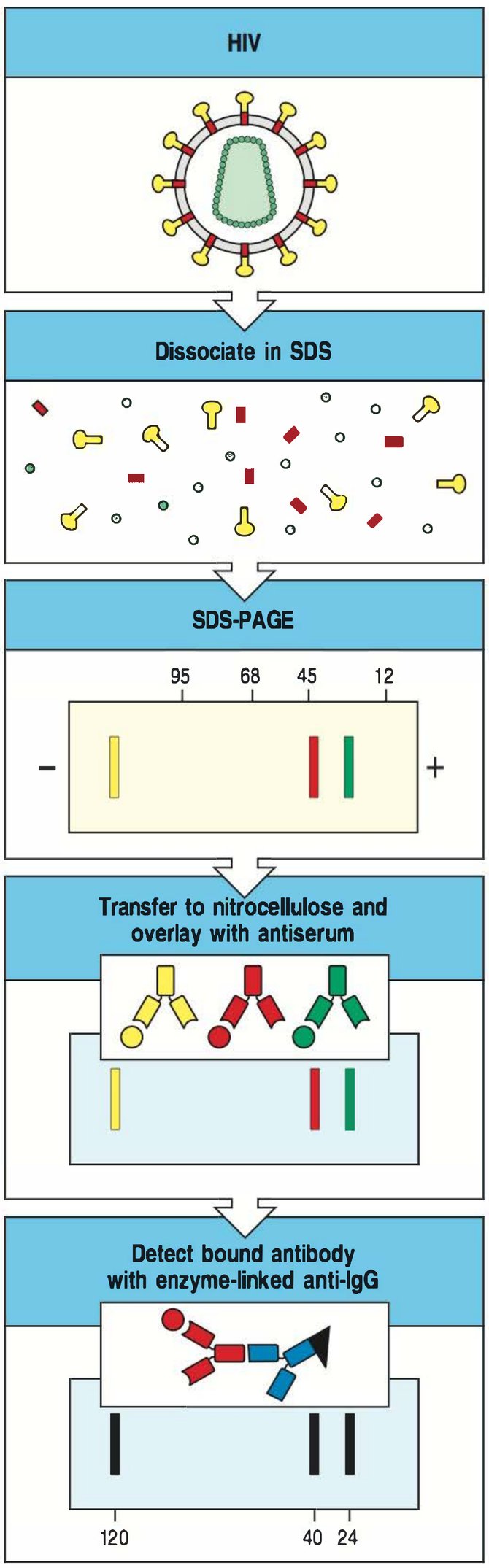


Fig. 10.3 Western blotting is used to identify antibodies against the human immunodeficiency virus (HIV) in serum from infected individuals. The virus is dissociated into its constituent proteins by treatment with the detergent SDS, and its proteins are separated by SDS-PAGE. The separated proteins are transferred to a nitrocellulose sheet and reacted with the test serum. Anti-HIV antibodies in the serum bind to the various HIV proteins and are detected by using enzyme-linked anti-human immunoglobulin, which deposits colored material from a colorless substrate. This general methodology will detect any combination of antibody and antigen and is used widely, although the denaturing effect of SDS means that the technique works most reliably with antibodies that recognize the antigen when it is denatured.

At any time after the infection, HIV may infect megakaryocytes, which have some surface CD4. Because megakaryocytes are the bone marrow progenitors of blood platelets, extensive infection of megakaryocytes causes the platelet count to fall (thrombocytopenia) and bleeding to occur. HIV may also infect the glial cells of the brain. Glial cells are of the monocyte–macrophage lineage and have some CD4 on their surface. The infection of glial cells may cause dementia and other neurological symptoms.

Questions.

1 *When Mr Thomas was first seen by Dr Wright, a chest X-ray revealed some enlarged hilar lymph nodes. If a lymph-node biopsy had been obtained, in what way would its histopathology have differed from that of lymph nodes from patients with severe combined immunodeficiency (SCID; see Case 5) or X-linked agammaglobulinemia (XLA; see Case 1)?*

2 *The course of an HIV infection in adults is very different from that in an infant infected in utero or intrapartum (during birth). What are the major differences between pediatric and adult AIDS and how do you account for them?*

3 *What are the mechanisms of resistance to the progression of HIV infection?*

4 *A few individuals, mostly hemophiliacs, are known to have been infected with HIV as long as 20 years ago and yet they remain asymptomatic. What factors may contribute to long-term survival with this infection?*

5 *Dr Wright told Mr Thomas to take zidovudine, lamivudine, and efavirenz. What are these drugs?*

6 *What do HIV protease inhibitors do? Does Mr Thomas need one of these drugs?*

7 *What is the mechanism of CD4 T-cell depletion in HIV infection?*

8 *What is the most important known determinant of the progression of HIV infection?*

9 *Which cytokine released during an HIV infection causes weight loss?*

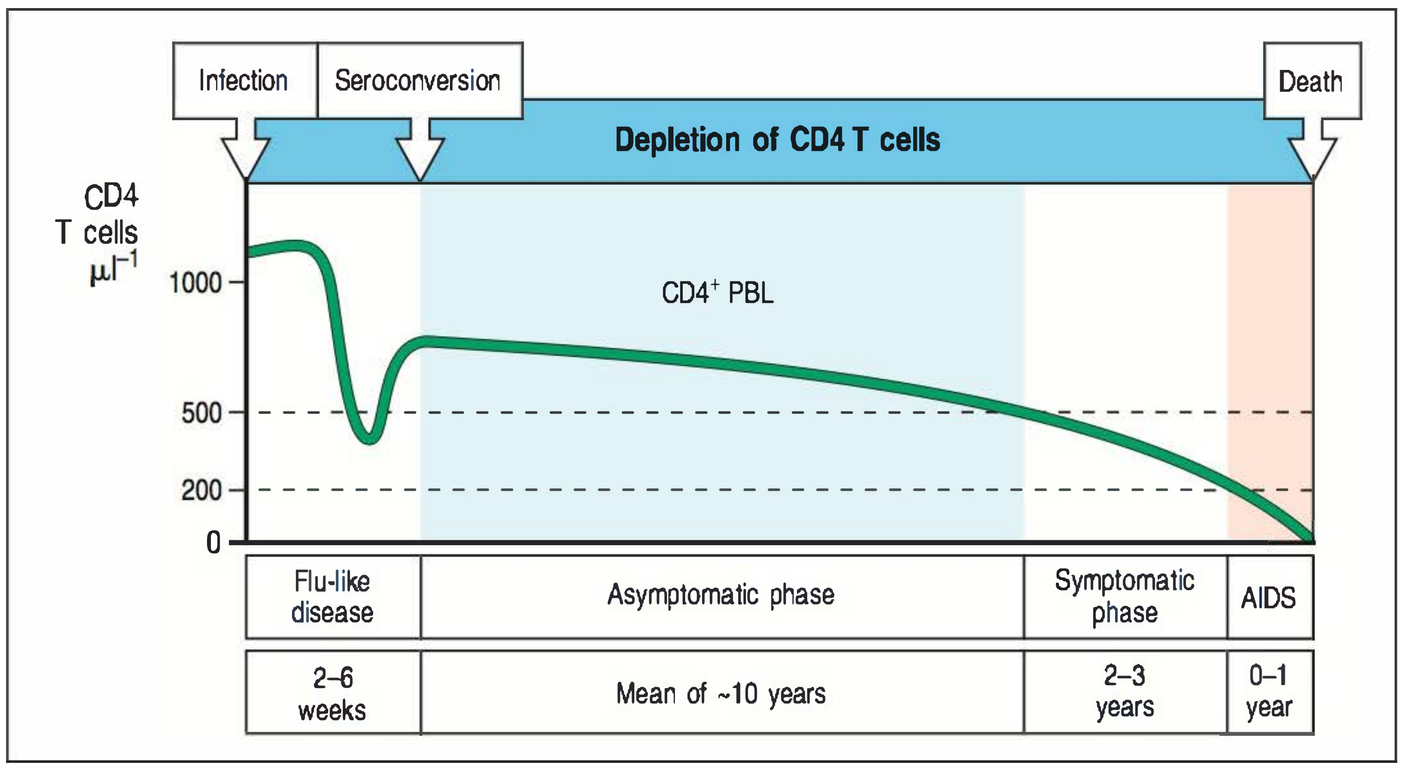


Fig. 10.4 The typical course of infection with HIV. The first few weeks are typified by an acute influenza-like viral illness, with high titers of virus in the blood. An adaptive immune response follows, which controls the acute illness and largely restores CD4 T cell levels but does not eradicate the virus. Opportunistic infections and other symptoms become more frequent as the CD4 T-cell count falls, starting at around 500 cells ml^{-1}. The disease then enters the symptomatic phase.

Infections	
Parasites	*Toxoplasma* spp. *Cryptosporidium* spp. *Leishmania* spp. *Microsporidium* spp.
Bacteria	*Mycobacterium tuberculosis* *Mycobacterium avium intracellulare* *Salmonella* spp.
Fungi	*Pneumocystis jirovecii* *Cryptococcus neoformans* *Candida* spp. *Histoplasma capsulatum* *Coccidioides immitis*
Viruses	Herpes simplex Cytomegalovirus Herpes zoster

Malignancies
Kaposi's sarcoma (invasive) Non-Hodgkin's lymphoma, including EBV-positive Burkitt's lymphoma Primary lymphoma of the brain

Fig. 10.5 A variety of opportunistic pathogens and cancers can kill AIDS patients. Infections are the major cause of death in AIDS; of these, respiratory infection with *Pneumocystis jirovecii* is the most prominent. Most of these pathogens require effective macrophage activation by CD4 T cells or effective cytotoxic T cells for host defense. Opportunistic pathogens are present in the normal environment but cause severe disease primarily in immunocompromised hosts, such as AIDS patients and cancer patients. AIDS patients are also susceptible to several rare cancers, such as Kaposi sarcoma and lymphomas, suggesting that immune surveillance by T cells may normally prevent such tumors. EBV, Epstein–Barr virus.

Answers

Case 1

Answer 1

In every somatic cell of a female, one of the two X chromosomes is inactivated. Which of the X chromosomes is inactivated is a random process, so each is normally active in 50% of the cells on average. However, if the normal X chromosome is inactivated in a pre-B cell of Bill's mother or grandmother, that cell has no normal *BTK* gene product and cannot mature. All of their B cells therefore have the normal X chromosome active (Fig. A1.1). This makes it seem that in the B cells of Bill's mother and grandmother the inactivation of the X chromosome has been nonrandom. If we have a marker that allows us to distinguish between the two X chromosomes of Bill's aunts we can determine whether their B cells exhibit random or nonrandom X inactivation. In fact it turned out that one of Bill's aunts was a carrier and the other was not.

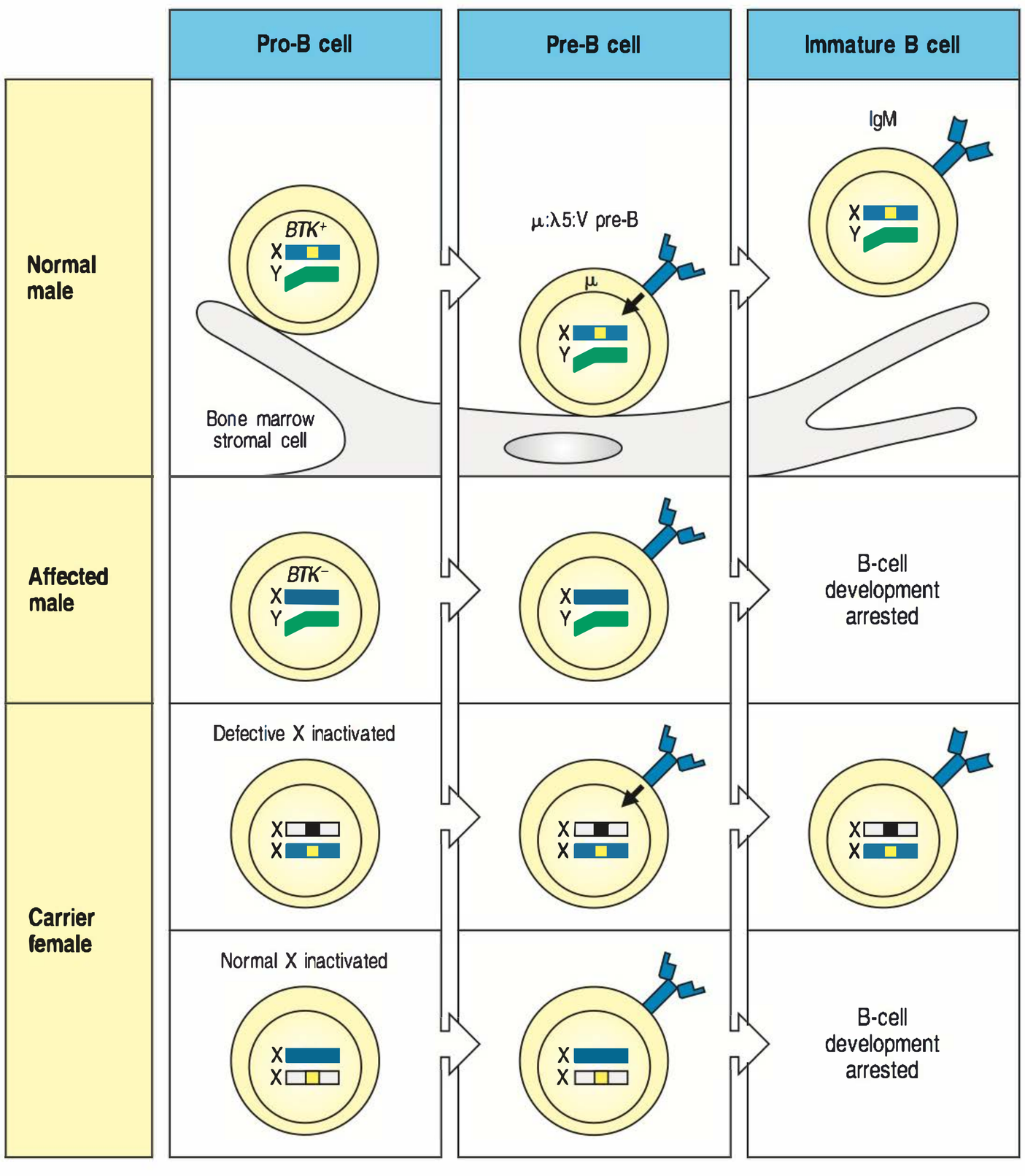


Fig. A1.1 Arrested B-cell development in X-linked agammaglobulinemia is responsible for apparently nonrandom X inactivation in the B cells of female carriers. In normal individuals, B-cell development proceeds through a stage in which the pre-B-cell receptor consisting of μ:λ5:Vpre-B tranduces a signal via Bruton's tryosine kinase (BTK), triggering further B-cell development. In males with XLA, no signal can be transduced and, although the pre-B-cell receptor is expressed, the B cells develop no further. In females, one of the two X chromosomes in each cell is permanently inactivated early in development. Because the choice of which chromosome to inactivate is random, half of the pre-B cells in a carrier female express a normal *BTK*, and half express the defective gene (*BTK*$^-$). None of the B cells that express *BTK*$^-$ from the defective chromosome can develop into mature B cells. Therefore, in the carrier, mature B cells always have the nondefective X chromosome active. This is in sharp contrast to all other cell types, which express the nondefective chromosome in only half of the population. Apparently nonrandom X chromosome inactivation in a particular cell lineage is a clear indication that the product of the X-linked gene is required for the development of cells of that lineage. It is also sometimes possible to identify the stage at which the gene product is required, by detecting the point in development at which X-chromosome inactivation develops bias. Using this kind of analysis, one can identify carriers of traits like XLA without needing to know the nature of the gene.

Answer 2
Maternal IgG crossed the placenta into Bill's circulation during fetal life. He was protected passively by the maternal IgG for 10 months.

Answer 3
Live polio vaccines are made from viruses with a disabling mutation in the gene that allows the virus to enter the motor nerve cells and cause paralysis. When a normal infant is given live attenuated poliovirus orally, the poliovirus establishes a harmless infection in the gut. Within 2 weeks the infant makes IgG and IgA antibodies that neutralize the poliovirus and prevent the infection from spreading, so that as the infected gut cells die the infection is terminated. When male infants with X-linked agammaglobulinemia are given this same vaccine, they are incapable of making any antibodies and the infection can persist. They continue to excrete the poliovirus from their gut. After a time there may be a mutation in some of the viruses that causes them to reacquire the ability to enter nerve cells (neurotropism). These so-called revertant viruses disseminate through the bloodstream and infect the neurons in the spinal cord, thus causing paralytic poliomyelitis. Another example of a virus that may disseminate from the gastrointestinal tract in males with X-linked agammaglobulinemia is the echoviruses. These are cleared readily by normal individuals; however, without the benefit of either IgA or IgG antibodies against the infecting serotype, the virus can disseminate to the central nervous system and cause meningoencephalitis.

Answer 4
Certain plant lectins, such as phytohemagglutinin and concanavalin A, cause virtually all T cells to divide and are therefore known as nonspecific mitogens. Antigens to which the host has previously been exposed also cause T cells to divide *in vitro*. After 72 hours exposure either to nonspecific mitogens or to specific antigens, ^{3}H-thymidine (tritiated thymidine) was added to Bill's T-cell cultures. Tritiated thymidine becomes incorporated into the DNA of dividing cells. The stimulation indices (the number of tritium counts in the stimulated cultures divided by the number of counts in similar cultures not exposed to mitogen or antigen) were normal for both mitogens and antigens. Bill's T cells responded to tetanus and diphtheria toxoids because he had been immunized with these inactivated bacterial toxins before the diagnosis was established.

Answer 5
Hereditary deficiency of complement component C3. The fixation of C3 to the bacterial surface, either by the classical or by the alternative pathways of complement activation, leads to its cleavage into a succession of fragments, two of which (C3b and iC3b) bind to complement receptors on the surface of phagocytic cells and enhance phagocytosis. iC3b binds the most potent complement receptor (CR3), and is the most important opsonizing agent for the ingestion and phagocytosis of encapsulated bacteria (see Case 32).

Answer 6
Tonsils are 80–90% B cells.

Answer 7
The rate at which IgG is catabolized depends on its concentration. In normal individuals, IgG has a half-life of approximately 21 days. In males with X-linked agammaglobulinemia, because the concentration is lower, IgG has a half-life of approximately 28 days. Overall, Bill's optimal level of 600 mg dl^{-1} decreases to about 450 mg dl^{-1} after a week because of catabolism, as well as minor losses in saliva, tears, the gut, and other secretions.

Bill weighs 75 kg.

The vascular volume is 8% of body weight or 0.08 liters kg^{-1}.

Bill's blood volume is therefore 75 × 0.08 = 6 liters.

Bill's hematocrit (the portion of the blood composed of cells) is 45%.

Therefore his plasma is 55% of his blood volume or 6 × 0.55 = 3.3 liters (3300 ml).

Bill injects himself with 10 g of gamma globulin or 10,000 mg. He thereby raises his IgG level by 10,000/3300 or roughly 3 mg ml^{-1}, or 300 mg dl^{-1}.

Half of the IgG equilibrates into the extravascular pool, so that Bill has really raised his plasma level by only 150 mg dl^{-1}.

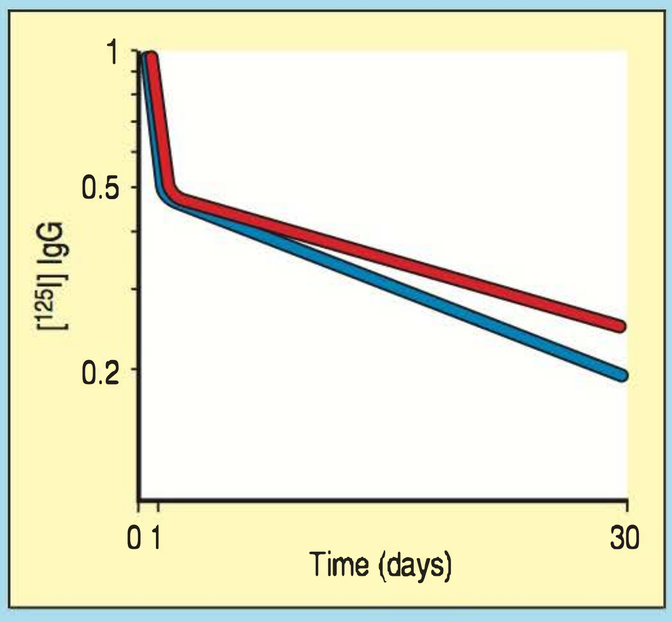


Fig. A1.7 IgG was radiolabeled with ^{125}I and then injected intravenously. After 10 minutes to allow for complete mixing of the radioactive dose in the blood, a plasma sample was obtained and the radioactivity was assayed. The amount of radioactivity at that time point was considered to be 100%. Plasma samples were obtained subsequently at frequent intervals and the percentage of residual radioactivity was determined and plotted on semi-logarithmic graph paper. The blue line shows the rate of disappearance of IgG in a normal person and the red line the rate in a male with X-linked agammaglobulinemia. From inspection of the curve it can be determined how long it takes for the radioactivity to decrease by 50%, say from 50% to 25%.

IgG, like all plasma proteins, distributes into the extravascular space: half the body IgG is in the blood and the other half is in the extravascular space. A dose of gamma globulin administered intravenously would equilibrate with the extravascular fluid in 24 hours. The dose of 10 g is arrived at as shown in Fig. A1.7.

Answer 8

Signaling via the pre-B-cell receptor is critical for B-cell development. Therefore, a deficiency of any one of the nonredundant components of pre-B-cell receptor signaling can cause failure of B-cell development. Defects transmitted as an autosomal recessive trait have been described in the μ heavy chain, the surrogate light chain λ5, the signaling components Igα and Igβ, and the B-cell linker protein BLINK. Another form of agammaglobulinemia is associated with a defect in the protein leucine-rich repeat containing 8 (LRRC8), namely a translocation that affects one *LRRC8* allele and results in a truncated LRRC8 protein. The role of LRRC8 in B-cell development is unknown.

Case 2

Answer 1

There is no DNA switch region 5′ to the Cδ gene. A single transcript of VDJCμCδ is alternatively spliced to yield either the μ or the δ heavy chain (see Fig. 2.4). In contrast, there are DNA switch regions 5′ to all the other heavy-chain C genes, and isotype switching must occur before functional transcripts of these genes

are made. Isotype switching requires the enzyme activation-induced cytidine deaminase, which is expressed in B lymphocytes in response to signals from T cells.

Answer 2

These experiments tell us that the activation of macrophages against this opportunistic microorganism requires binding of CD40 on the macrophage surface to the CD40L on the surface of activated T cells.

Answer 3

Patients with CD40L deficiency have impaired antibody responses to T-cell dependent antigens, but can make IgM antibodies to antigens that can stimulate a B-cell response without T-cell help. The blood group antigens are sugars that are also found on bacteria in the gut and can activate B cells in the absence of T-cell help. Tetanus toxoid, typhoid O and H antigens, and streptolysin, in contrast, are proteins, which cannot elicit a B-cell response in the absence of T cells. Without CD40L, Dennis's T cells cannot activate his B cells to respond to these protein antigens. He would also have been unable to make antibodies against *Streptococcus pyogenes*, because the antigenic component of the bacterial capsule is a protein.

Answer 4

The polysaccharide capsules of these pyogenic bacteria are resistant to destruction by phagocytes unless they are opsonized. IgM largely promotes the phagocytosis of bacteria by activating complement, leading to the deposition of C3 fragments on the bacterial surface. The C3 is recognized by complement receptors on the phagocytic cells. IgG, however, is more efficient than IgM in promoting the phagocytosis of most bacteria. In addition, there is a range of Fc receptors for IgG isotypes on phagocytes, and IgG1, IgG2, and IgG3 antibodies all promote complement activation on bacterial surfaces. This means that bacteria coated with IgG stimulate phagocytosis through two different classes of receptor, Fc and C3. This results in much more efficient phagocytosis than stimulation through a single class of receptor.

Answer 5

Both neonates and people taking immunosuppressive drugs such as cyclosporin A exhibit increased susceptibility to both pyogenic and opportunistic infections.

A second reason for the increased susceptibility of neonates to some pyogenic infections is the immaturity of many of their B cells. Neonates are normally protected by preexisting maternal IgG until their lymphocytes mature.

Cyclosporin A also inhibits transcription of the *IL2* gene, thereby preventing the expansion of T-cell clones activated by antigen. This means that all T cell-mediated immune responses, including cytotoxic T-cell responses, are suppressed by cyclosporin A.

Case 3

Answer 1

Although the most notable features of hyper IgM syndrome caused by CD40L deficiency (Case 2) are elevated IgM and a lack of other immunoglobulin isotypes, the defect is actually of T cells, not B cells. Lack of expression of CD40L by T cells does not only affect B-cell function: it also results in a failure to activate monocytes/macrophages and dendritic cells via CD40. Interleukin-12 is not synthesized. In the absence of CD40 signaling, pulmonary macrophages

are inefficient in killing *Pneumocystis jirovecii*, leading to pneumonia, and liver macrophages may be similarly deficient in killing *Cryptosporidium*, leading to chronic inflammation of the bile ducts (cholangitis). Lack of activation of dendritic cells via CD40 may impair their ability to elicit robust T-cell responses. This may contribute to the susceptibility to opportunistic infections in CD40L deficiency. In AID deficiency, the defect is solely in the B cells, and only affects antibody production, resulting in a phenotype similar to other B-cell deficiencies such as X-linked agammaglobulinemia (Case 1). There is susceptibility to pyogenic bacteria in particular, leading to frequent bacterial infections of the ears, lungs, and sinuses, but no increased risk of opportunistic infections.

Answer 2

Because the syndrome in this patient resembles that seen in CD40 ligand deficiency, we might hypothesize that she has a defect in CD40, the receptor for CD40 ligand. In fact, several cases of hyper IgM syndrome secondary to defects in CD40 have now been reported. As might be expected, these patients are clinically indistinguishable from those with CD40 ligand deficiency, except in the pattern of inheritance.

Answer 3

In this case it is likely that the defect is in another of the proteins required in the B cell for class switching. One of the genes involved in class switching is that for uracil-DNA glycosylase (*UNG*), which removes uracil from uridine. Defects in *UNG* have been described in a few patients with hyper IgM syndrome.

Answer 4

B cells from patients deficient in CD40L undergo normal class-switch recombination in response to CD40 ligation and cytokine action, whereas B cells from AID patients do not. Measurement of IgE synthesis after stimulation of cultured blood lymphocytes with anti-CD40 antibody plus IL-4 is an excellent indicator of class switching, because blood lymphocytes normally contain very few or no B cells that have already switched to IgE. B cells from patients deficient in CD40L make normal amounts of IgE in response to anti-CD40 plus IL-4. In contrast, B cells from patients with AID deficiency, or CD40 deficiency or defects in genes downstream of CD40, fail to secrete IgE in response to anti-CD40 and IL-4.

Answer 5

Enlargement of lymph nodes in children is usually due to expansion in the number and size of germinal centers. The interaction of CD40 with its ligand is required for germinal center formation, and patients with CD40L deficiency or CD40 deficiency have no germinal centers. In contrast, AID is not required for germinal center formation. Mitotic stimuli result in normal cell proliferative responses in these patients, and infections result in enlarged lymph nodes, as observed in Daisy.

Case 4

Answer 1

TACI, like other members of the TNFR family, such as TNFR-I and Fas, might require ligand-induced trimerization for signaling. The TRAFs that associate with TACI cytoplasmically have been shown to have a higher affinity for trimeric receptors. Therefore, in the case of mutations in the cytoplasmic domain of TACI, the recruitment of mutant and normal TACI subunits into a trimeric complex will compromise the binding of downstream signaling molecules and thus interfere with signaling. In this case, the mutant TACI will be acting as a dominant negative.

Answer 2
There is considerable variation in the severity of clinical symptoms in both related and unrelated individuals with the same *TACI* mutations. As seen in this case, in the same family, the *TACI* mutation could be associated with CVID and IgA deficiency. This suggests that the penetrance of the *TACI* mutation is determined by the particular genotype of the individual. In addition, environmental modifiers could also be involved. Indeed, some family members of patients with CVID carry the same *TACI* mutation but have few or no symptoms. Furthermore, some of the common mutations in *TACI* found in CVID have been identified in a small percentage of 'normal' subjects on blood screening.

Answer 3
A deficiency in CD19, which is a component of the B-cell co-receptor complex, mutations in the co-stimulatory protein ICOS, and possibly a mutation in BAFF-R have been found.

Case 5

Answer 1
His mother had nonrandom inactivation of the X chromosome in her T cells, thereby demonstrating that she carried an X-linked gene required for the normal maturation of T cells.

Answer 2
From what we know of their functions, defects in the receptors for IL-2, IL-4, IL-9, IL-15, and IL-21 do not seem relevant to the early block in T-cell development seen in X-linked SCID, because all of them activate mature lymphocytes or other effector cells. However, the receptor for IL-7 is thought to be important for pre-T-cell growth in humans and mice, and also for pre-B-cell growth in mice (which are deficient in both B and T cells when they lack the γ_c chain). Mice with a defect in the IL-7 receptor alone suffer from blocks in T- and B-cell development that resemble those seen in mice lacking the γ_c chain. A loss of IL-7 receptor function is therefore likely to be the most important loss of function responsible for X-linked SCID.

Answer 3
To prevent graft-versus-host disease. The mother's bone marrow donation also contained mature T cells capable of reacting with the paternal HLA antigens inherited by Martin from his father. Recognition of the paternal HLA antigens in Martin by the T-cell antigen receptors of the maternal T cells would incite graft-versus-host disease. The CD34 antigen is expressed by hematopoietic stem cells and hematopoietic progenitors; therefore, positive selection of these cells depletes the mature T lymphocytes. Alternatively, several strategies can be used to deplete T lymphocytes contained in the bone marrow, including *in vitro* treatment of the bone marrow with monoclonal antibodies directed against T lymphocytes in the presence of complement (to lyse T lymphocytes).

Answer 4
The *P. jirovecii* organisms are present in lung fluid and in the pulmonary macrophages. They do not incite an inflammatory response until the infant has T cells bearing a T-cell antigen receptor for *P. jirovecii* antigens. After a successful transplant, the infant is rendered chimeric. The transplanted T cells recognize *P. jirovecii* antigens and incite an inflammatory response, which makes the pneumonia more severe.

Answer 5
They may develop disseminated BCG infection and even die because of this complication. This ordinarily harmless attenuated bacillus is a pathogen in individuals with compromised cell-mediated immunity.

Answer 6
They developed a progressive vaccinia infection, which spread contiguously in the skin from the site of inoculation—so-called vaccinia gangrenosa. It proved invariably fatal in these infants. No live vaccines of any kind should be given to children or adults with significant T- or B-cell immunodeficiency.

Answer 7
Martin was treated with bone marrow transplantation without chemotherapy: he could not reject maternal cells because he lacked mature T lymphocytes. This strategy is often used in patients with SCID. However, with this strategy, autologous B lymphocytes persist, if present. B lymphocytes from patients with X-linked SCID or with JAK3 deficiency are impaired in their response to IL-4 and IL-21, two cytokines that are important in the maturation of B-cell responses. In particular, IL-4 is important for class-switch recombination, whereas IL-21 is produced by follicular helper T cells and promotes germinal center B-cell reaction and terminal differentiation of B lymphocytes.

Answer 8
Gene therapy. The gene encoding γ_c is introduced by retroviral transfer into bone marrow stem cells of patients with X-linked SCID, and the cells are injected into the blood. This therapy has resulted in full immune reconstitution in patients with X-linked SCID. Unfortunately, some of these patients developed leukemia, prompting the development of novel and safer vectors that are currently being tested in clinical trials.

Case 6

Answer 1
The thymus gland can shrink (involute) as the result of many kinds of stress, particularly infection.

Answer 2
The T cells of a patient with SCID are incapable of responding to a mitogenic stimulus. Because Roberta's cells could not respond to John's it was not necessary to add mitomycin to the mixed lymphocyte reaction.

Answer 3
The metabolic abnormalities of ADA deficiency are present already in prenatal life, and continue after birth until detoxification is achieved with treatment. Accumulation of toxic metabolites of adenosine is particularly detrimental to the thymus, and may cause irreversible damage, so that full restoration of T-cell generation may not be achieved, even after detoxification.

Case 7

Answer 1
The few T cells produced and activated must have had a T_H2 phenotype and secreted large amounts of interleukin-4 (IL-4) and interleukin-5 (IL-5). IL-4 is required for switching to IgE synthesis and IL-5 for the recruitment

of eosinophils. The few B cells in the patient (which were below the limit of detection) must have been induced to switch immunoglobulin class to IgE.

Answer 2
The few clones of T cells that were able to mature were activated, as shown by their surface expression of CD45R0 and MHC class II molecules. The activated clones would have expanded within the lymph node.

Answer 3
Neither of Ricardo's parents had Omenn syndrome, but Ricardo had an affected brother and an affected sister. This indicates Mendelian autosomal recessive inheritance of the defect. If the defect had been X-linked recessive, a female would not have been affected; if it were dominant, one of the parents would have had to have been affected.

Answer 4
The T cells that are present are activated, as shown by their expression of CD45R0 and MHC class II molecules, and express homing receptors for the skin. In the skin, the activated T cells secrete chemokines that attract other inflammatory cells, such as monocytes and eosinophils, into the skin. The perivascular inflammation in the skin causes the blood vessels to dilate, and this appears as a bright red rash.

Case 8

Answer 1
The maturation of CD4 T cells in the thymus depends on the interaction of thymocytes with MHC class II molecules on thymic epithelial cells. When the MHC class II genes are deleted genetically in mice, the mice also exhibit a deficiency of CD4 T lymphocytes.

Answer 2
The polyclonal expansion of B lymphocytes and their maturation to immunoglobulin-secreting plasma cells requires helper cytokines, such as IL-4, from CD4 T cells. Helen's hypogammaglobulinemia is thus a consequence of her deficiency of CD4 T lymphocytes.

Answer 3
Helen's T cells, although decreased in number, are normal and are not affected by the defect. They are capable of normal responses to nonspecific mitogens and to an allogeneic stimulus in which the antigen is presented by the MHC molecules on the surface of the (nondefective) allogeneic cells and thus does not require to be processed and presented by the defective cells. However, the failure of her lymphocytes to respond to tetanus toxoid *in vitro* resulted from the fact that, in this situation, there were no cells that could present antigen on MHC class II molecules to the CD4 T cells.

Answer 4
Yes. Helen's T cells would be capable of recognizing the foreign MHC molecules on the grafted skin cells and would reject the graft.

Case 9

Answer 1
Bone marrow transplantation is not the treatment of choice in patients with

DiGeorge syndrome or with *FOXN1* deficiency, because in these patients the defect lies in the thymic epithelium, not in cells of hematopoietic origin. However, bone marrow transplantation has been successfully performed in patients with DiGeorge syndrome who have received a transplant from HLA-identical siblings. In this case, the bone marrow is not manipulated. The mature T lymphocytes contained in the graft expand in the recipient and provide immune reconstitution, but no new T cells are generated in the thymus.

Answer 2

The idea that T cells develop across the HLA barrier in patients with DiGeorge syndrome treated with unrelated thymic transplantation is indeed against the dogma that positive selection requires recognition of self-HLA molecules. Although this remains puzzling, one possibility is that dendritic cells from the host home to the thymus and mediate the positive selection of newly generated thymocytes.

Answer 3

In most patients with DiGeorge syndrome, some thymic (and parathyroid) tissue is present, even if it cannot be seen. These tissues undergo expansion after birth, leading to progressive correction of the hypocalcemia and some significant T-cell development.

Answer 4

The thymus has a crucial role in the establishment of tolerance. In particular, expression of the transcription factor Aire by medullary thymic epithelial cells induces the expression of peripheral tissue antigens in the thymus. These antigens can be presented to newly generated self-reactive thymocytes that are deleted. Severe defects of the thymic stroma (as in DiGeorge syndrome) may lead to a reduced expression of Aire and of peripheral tissue antigens, hence allowing the survival of self-reactive T cells. Furthermore, the thymus is also the tissue in which natural regulatory T (nT_{reg}) cells are generated. These cells suppress autoimmune manifestations in the periphery. The thymic defect in DiGeorge syndrome may also lead to a reduction of nT_{reg} cells and hence contribute to autoimmunity.

Case 10

Answer 1

The lymph node would exhibit marked, if not exuberant, follicular hyperplasia, indicating an ongoing B-cell response to the virus. In SCID and XLA the lymph nodes are very small. In SCID the lymph node would contain no or very, very few lymphoid cells. In XLA the lymph node would have no follicles, no germinal centers, and no B cells or plasma cells. T cells would be present but not in an organized array (Fig. A10.1).

Answer 2

In infants, the HIV infection typically runs a more rapid course, and infants often die before they reach 1 year old. Mr Thomas, however, had been infected for many years before symptoms of immunodeficiency started to appear. This difference is probably due to the fact that infants are immunologically immature and naive, whereas an infected adult has a functionally mature immune system and decades of acquired adaptive immunity to many different antigens. This has consequences for both the response to HIV itself and the susceptibility to other infections. We have seen that T cells in newborn infants are not fully 'turned on.' For example, in hyper IgM the CD40 ligand is not readily expressed when T cells of newborns are activated (see Case 2). Their T cells do not synthesize interferon-γ in normal amounts, nor are their cytotoxic

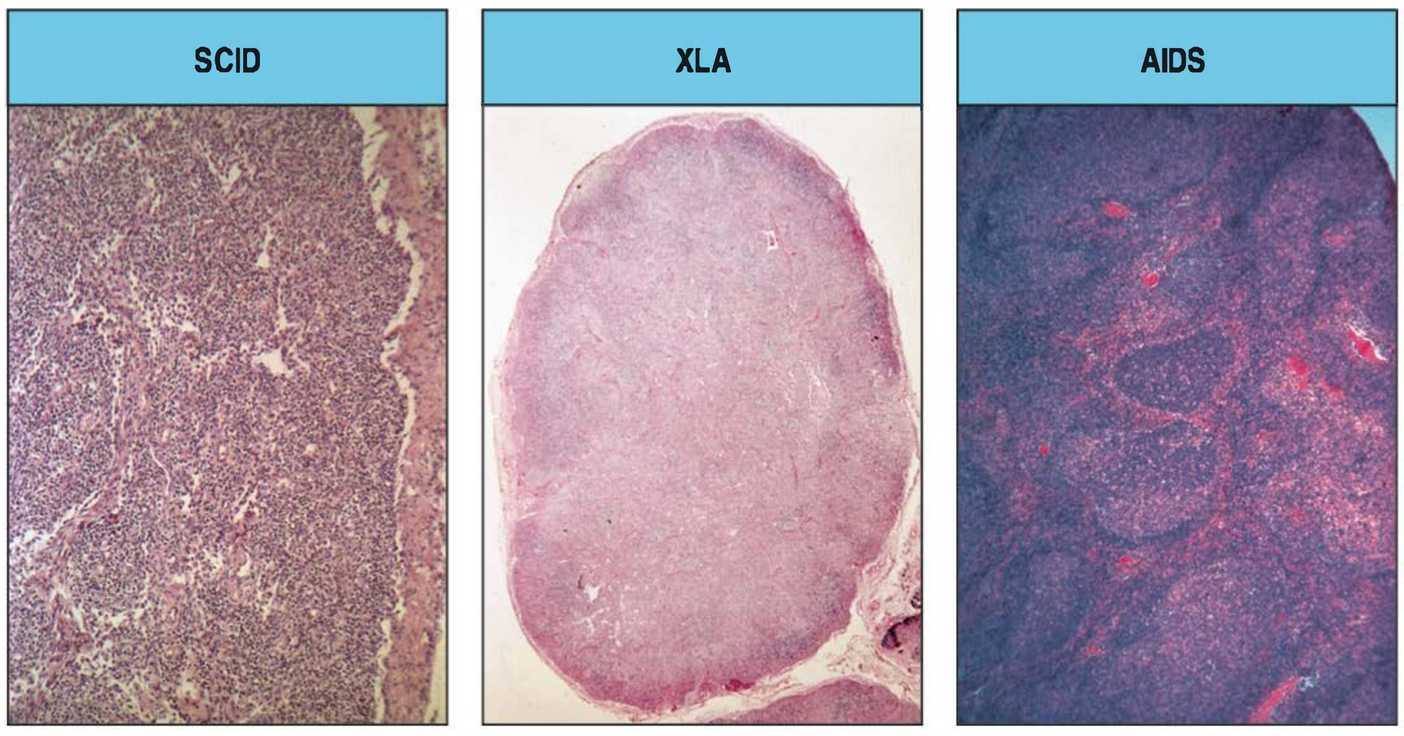


Fig. A10.1 Lymph-node sections from patients with severe combined immunodeficiency (SCID), X-linked agammaglobulinemia (XLA), and AIDS.

T lymphocytes readily activated. This functional immaturity is probably why young infants have difficulty confining and walling off infections, particularly those that require adaptive immunity mediated by T cells. Tuberculosis offers a clear example of this. It is a fast-spreading, highly lethal infection in young infants, whereas in older children and immunologically normal adults, the infection is usually confined to the lung, or more rarely to other organs.

HIV infection in infants occurs before they have had an opportunity to develop any adaptive immunity to common infections, and this means that they are prone to infections not seen in adult AIDS. An adult will already have antibodies to the common pyogenic bacteria and will not be particularly susceptible to pyogenic infections, whereas these are frequently observed in affected infants. Adults will also have been exposed to common viruses, such as Epstein–Barr virus (EBV). This is normally encountered early in life and contained as a latent infection. Virtually all adults have been infected with EBV by the end of the second or third decade of life, and primary EBV infection is therefore not a threat to HIV-infected adults. For an HIV-infected infant, however, a first encounter with this virus causes bizarre manifestations such as parotitis (inflammation of the parotid gland, like mumps) and a form of pneumonia characterized by pulmonary lymphoid hyperplasia.

Answer 3

The answer to this question is not known precisely. The immune response to the virus is illustrated in Fig. A10.3. Antibody against HIV seems to have a minor role in resisting the progress of infection. HIV-specific cytotoxic CD8

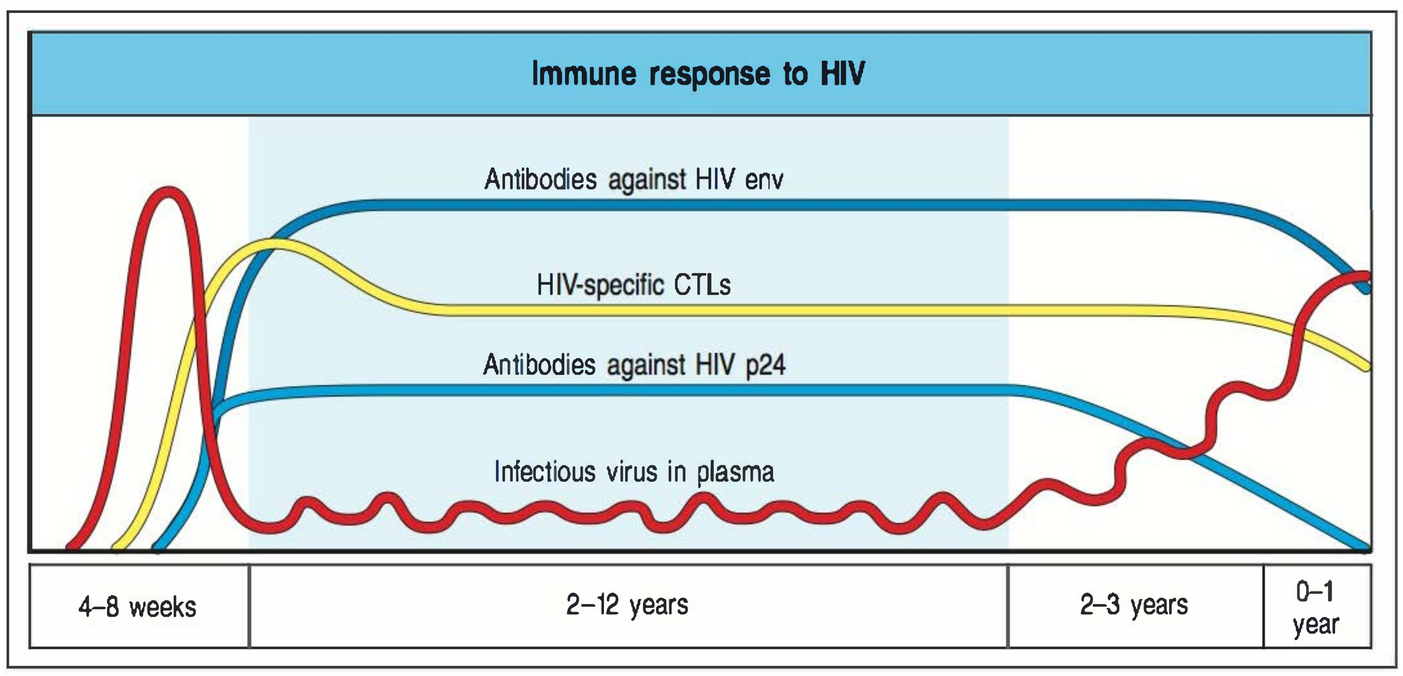


Fig. A10.3 The immune response to HIV. Infectious virus is present at relatively low levels in the peripheral blood of infected individuals during a prolonged asymptomatic phase but is replicated persistently in lymphoid tissues. During this period, CD4 T-cell counts gradually decline, although antibodies and CD8 cytotoxic T cells directed against the virus remain at high levels. Two different antibody responses are shown in the figure, one to the envelope protein of HIV, env, and one to the core protein p24. Eventually, the levels of antibody and HIV-specific cytotoxic T lymphocytes (CTLs) also fall, and more infectious HIV progressively appears in the peripheral blood.

cells arise as virus levels decline from the peak associated with primary infection, and seem to have a more important role in containing the infection. Rare individuals with mutations in the co-receptors for HIV (CCR5 and CXCR4) are resistant to HIV infection.

Answer 4

The virus burden in these individuals is very low; in some cases, the only detectable viruses carry mutations in genes such as *nef* or *tat*, which are vital to HIV replication in the infected host. However, viruses able to replicate in culture can be isolated from most of these so-called 'long-term non-progressors.' These patients seem to be able to contain replication-competent virus, most probably by continuing to maintain a successful cytotoxic CD8 T-cell response.

Answer 5

They inhibit the reverse transcriptase of HIV. Zidovudine (AZT; 3′-azido,2′,3′-dideoxythymidine) is a nucleoside analog that is phosphorylated inside the cell and used as a substrate by the reverse transcriptase of HIV (Fig. A10.5). HIV reverse transcriptase synthesizes a DNA complement of the viral RNA, at the start of a new round of virus replication in a newly infected cell. The incorporation of zidovudine blocks further extension of this DNA strand and thereby stops replication of the virus. Two other nucleoside analogs, ddI (dideoxyinosine) and ddC (dideoxycytosine) inhibit HIV replication by a similar mechanism and are also used to treat HIV infection. Lamivudine (also known as 3TC) is an enantiomer of a dideoxy analog of cytidine. Efavirenz (also called Sustiva) is a nonnucleoside inhibitor of HIV reverse transcriptase. Unfortunately, mutation allows the virus to acquire resistance to all these drugs. Replication of HIV (and other known retroviruses) is error-prone, and the virus mutates as it replicates in the infected host. Resistance to zidovudine requires multiple mutations but can arise in only a few months. Combining HIV protease inhibitors with zidovudine or other nucleoside analogs has markedly improved the survival of HIV-infected patients.

Answer 6

HIV produces an aspartyl protease, structurally related to pepsin and renin, which has two aspartic-acid residues in the active site. This protease is required to splice the HIV Gag proteins after they are synthesized and before the packaging of the virus into its coat and its budding from the cell surface. Inhibitors of this HIV protease have been designed and are very effective in halting HIV replication. If the HIV in Mr Thomas were to become resistant to the reverse transcriptase inhibitors, adding a protease inhibitor to his drug regimen might keep the virus in check for some time longer.

Answer 7

The precise answer to this question is unknown. It is clear that the cells producing the virus are killed, either by cytotoxic T lymphocytes or by direct cytotoxic effects of the virus. It is also possible that the death of uninfected 'bystander' cells contributes to CD4 T-cell depletion. HIV is known to have a cytotoxic effect on CD4 T cells in culture. The viral capsular gp120 binds and cross-links the CD4 molecule, which depresses T-cell function and may induce apoptosis, even when the CD4 cells are not themselves infected by HIV. If, as seems likely, the early killing of HIV-infected CD4 cells by cytotoxic T cells serves to contain the virus and prevent greater CD4 T-cell depletion in the next round of HIV infection and replication, a declining ability to mount cytotoxic responses, especially to new viral variants, could be very important. Patients infected with HIV show impairment of T-cell function, especially memory cell responses, even during the asymptomatic phase. They are hypergammaglobulinemic, and humoral responses seem favored at the expense of

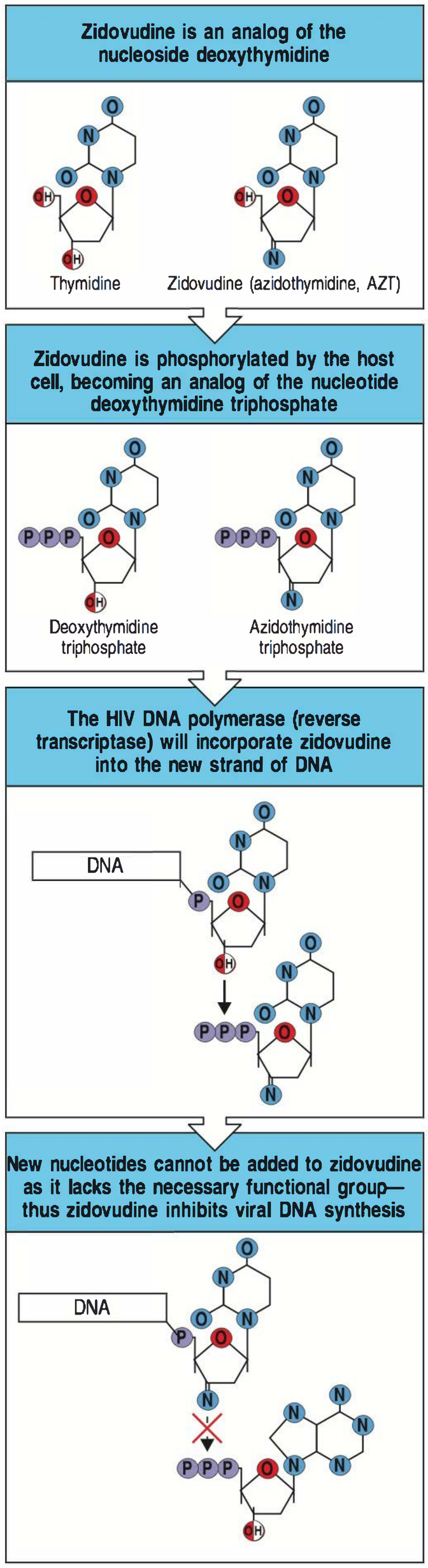


Fig. A10.5 The mechanism of action of the antiretroviral drug zidovudine. Zidovudine is azidothymidine (AZT), an analog of the nucleotide deoxythymidine.

cell-mediated immunity. This immunoregulatory bias impedes inflammatory responses, and possibly also cytotoxic responses to HIV-infected cells.

Answer 8
The CD4 T-cell count is, statistically speaking, the best indicator of the time-course of progression to AIDS. Other factors such as lifestyle and the incidence of intercurrent infections do not seem statistically significant.

Answer 9
Tumor necrosis factor-α (TNF-α). It causes loss of appetite (anorexia) and increased expenditure of body heat (thermogenesis).

Case 11

Answer 1
The engrafted T cells seem to recognize allogeneic antigens on the recipient's hematopoietic cells and thus will attack the leukemic cells. One such antigen, HB-1, which is a B-cell lineage marker, is expressed by acute lymphoblastic leukemia cells, which are B-lineage cells, and by B lymphocytes transformed by Epstein–Barr virus (EBV).

Answer 2
IFN-γ induces the expression of MHC molecules on cells; this makes GVHD worse, because it provides more targets for the donor T cells.

Answer 3
In vivo T-cell depletion can also be achieved with the intravenous injection of monoclonal antibodies, such as anti-CD3. This approach is also used to treat initial graft rejection in recipients of solid organ transplantation, but it is associated with a higher risk of lymphoproliferative disease induced by the Epstein–Barr virus. Monoclonal antibodies targeting activated, but not resting, T lymphocytes have been also used in the treatment of GVHD, and include anti-CD25 and anti-CD40L monoclonal antibodies.

Answer 4
One reason could be that the skin and intestine express a higher level of MHC molecules than other tissues. The intestinal tract is also likely to be damaged by the preparative cytotoxic treatments given to destroy the recipient's bone marrow. The damage induces the production of cytokines; as well as inducing MHC molecules, these can also drive GVHD and make the tissue susceptible to immunological attack.

Case 12

Answer 1
The intrathymic maturation of CD8 T cells depends on the expression of MHC class I molecules on thymic epithelial cells. Conversely, maturation as CD4 T cells requires interactions with the MHC class II molecules also present on thymic epithelium. Because Tatiana and Alexander do not lack MHC class II molecules, their CD4 T cells are normal and they have normal humoral immunity.

Answer 2
The maturation of CD8 T cells bearing γ:δ chains occurs after these cells emigrate from the thymus and is independent of MHC class I expression, whereas

the maturation of α:β CD8 T cells occurs in the thymus and is dependent on the expression of MHC class I molecules.

Answer 3
No. Delayed-type hypersensitivity reactions are provoked by antigen-specific CD4 T cells (see Case 8).

Answer 4
The factors that help B cells to mature and secrete immunoglobulins are derived from activated CD4 T cells. These cells were normal in Tatiana and Alexander. Factors that suppress B-cell responses are secreted by CD8 T cells, of which the children had very few. They were therefore not very efficient at terminating B cell-mediated humoral immune reactions and tended to over-produce antibody. In Case 8, we saw the opposite phenomenon in patients with MHC class II deficiency, who have a deficiency of CD4 T cells and very low levels of serum immunoglobulins.

Answer 5
The patients with TAP1 deficiency clinically resembled those with TAP2 deficiency.

Case 13

Answer 1
In retrospect, the family history and the patient's past medical history provided clues that he might be affected by XLP. The patient himself had low IgG and recurrent otitis. His uncle and grandfather were affected by some of the manifestations of XLP, including recurrent lymphoma and aplastic anemia.

Answer 2
In some patients, family history as well as a classic clinical presentation (fulminant infectious mononucleosis) provide a strong basis for a clinical diagnosis. Often, however, there is no clear family history and the severe lymphoproliferation cannot easily be distinguished from other forms of malignant lymphohistiocytosis with hemophagocytosis. The molecular defect responsible for most cases of XLP has been identified as a mutation or deletion of the gene encoding SAP (*SH2D1A*). In difficult cases, a molecular diagnosis is desirable, and PCR analysis of the four exons encoding SAP can be performed. Flow cytometry and Western blotting can be used to identify patients in whom *SH2D1A* mutations result in a lack of SAP protein expression. If family history, clinical data, and laboratory data are consistent with a diagnosis of XLP but no defects in SAP can be identified, mutation analysis should be targeted to the *BIRC4* (*XIAP*) gene, using PCR.

Answer 3
In a girl, mutation of an X-chromosome gene such as *SH2D1A* would be very unlikely to account for her problem. An autosomal disorder is more probable. If the parents were related, suspicion of an autosomal defect would be heightened. Autosomal recessive defects that may cause an XLP-like phenotype include ITK deficiency but also, and more commonly, defects in the cytolytic machinery that result in hemophagocytic lymphohistiocytosis (see Case 14).

Answer 4
Destruction of Alexander's bone marrow by radiation or chemotherapy (with busulfan and cytoxan) followed by administration of HLA-matched marrow from a normal sibling or unrelated donor would replace all lymphoid

precursors with normal SAP-expressing cells of donor origin. This approach has been used successfully in the treatment of XLP.

Case 14

Answer 1

FHL is characterized by impaired cytotoxic activity of CD8 T cells and NK cells, which are therefore unable to kill virus-infected cells. However, the ability of CD8 T cells to recognize virus-derived peptides in association with HLA class I molecules on the surface of infected cells is intact. Therefore, antigen-specific CD8 T cells continue to respond to the viral infection by becoming activated and proliferating. As part of this response, they secrete large amounts of IFN-γ, which drives the production of the pro-inflammatory cytokines IL-6 and TNF-α by macrophages.

Answer 2

The liver and spleen enlargement that Jude developed, which is typical of the accelerated phase of HLH, is the result of the marked expansion of CD8 T cells and the accumulation of activated macrophages.

Answer 3

The bone marrow is a target organ in HLH. Activated macrophages often engulf red cells, myeloid cells, lymphoid cells, and platelets, leading to bone marrow hypoplasia.

Case 15

Answer 1

The nitro blue tetrazolium (NBT) test measures the capacity of the lysosomes in a phagocyte to produce superoxide and other oxygen free radicals. The test is done by adding yellow NBT dye to phagocytes that are then stimulated with a cell activator, typically phorbol myristate acetate (PMA). Activation of the NADPH oxidase enzyme complex in the lysosome by PMA leads to the production of the reactive intermediates of oxygen that modify NBT into formazan, which has a deep blue color. Chediak–Higashi syndrome (CHS) affects the normal formation and traffic of vesicles in the cell, but does not affect function of the NADPH oxidase. Thus, Shweta's neutrophils could readily reduce NBT to a blue color.

Answer 2

An easy and non-invasive test with which to confirm CHS is microscopic observation of a hair shaft. The hair of patients with CHS has abnormal pigmentation: when observed under the microscope, the hair shaft is seen as speckled with clumps of pigment instead of the normal homogeneous distribution (see Fig. 15.5). When the hair bulb of CHS patients is examined under electron microscopy, melanocytes showing enlarged melanosomes with variable amounts of melanin pigment are seen.

Answer 3

The accelerated phase of CHS is thought to be due to impaired lymphocyte cytotoxicity, although the precise pathogenic mechanism is so far unclear. A similar process is observed in other diseases with impaired cytotoxicity such as familial hemophagocytic lymphohistiocytosis (FHL)(see Case 14). Many cases of FHL are due to mutations in *perforin 1*, a gene that encodes a lytic

enzyme that is contained in the granules of cytotoxic lymphocytes and is essential for cytotoxicity. Evidence suggests that the accelerated phase of CHS may typically occur after infection with Epstein–Barr virus. In this setting, the immune system is unsuccessful in killing the virus-infected cells; in attempting to control the infection, lymphocytes proliferate without restraint.

Answer 4

Bone marrow transplantation is only able to correct defects that are due to cells of hematopoietic origin. Oculocutaneous albinism and the neurological defects seen in patients with CHS are not due to dysfunction of hematopoietic cells, so these abnormalities cannot be corrected by a bone marrow transplant. At one time, it was believed that the neurological disease was due to persistent lymphocyte infiltration of the CNS, but later studies have shown intrinsic defects in the neurons and glia of patients with CHS, which have disproved that hypothesis. The oculocutaneous albinism is due to a defect in the melanocytes that produce pigment in the skin, eyes, and hair.

Case 16

Answer 1

Signals that direct the maturation of hematopoietic stem cells into the various lineages are transmitted by their contact with stromal cells in the bone marrow. Presumably, this interaction, like T-cell–B-cell interaction, requires cytoskeletal reorientation, and thus will be impaired in cells containing an active affected X chromosome. The stem cells bearing an active normal X chromosome thus have a survival advantage. An alternative hypothesis, based on studies in mice, is that fetal liver hematopoietic stem cells that express WASP have a significant advantage over WASP-negative cells in reaching the bone marrow.

Answer 2

You might try to give antibody against the B-cell cell-surface protein CD40 along with the immunogen. Ligation of CD40 by the CD40 ligand borne by activated T cells is a signal for a resting B cell to start dividing and to undergo isotype switching. The antibody should act like the CD40 ligand and induce isotype switching in B cells (see Case 2).

Answer 3

Measurement of mean platelet volume (MPV) may help diagnose XLT. WAS and XLT are the only conditions that are typically associated with low MPV. In contrast, the MPV is normal or even elevated in patients with chronic ITP.

Case 17

Answer 1

The loss of adrenal cortical hormones as a result of the autoimmune destruction of his adrenal cortex caused Robert's pituitary gland to secrete greatly increased amounts of ACTH. ACTH is composed of 39 amino acids, the amino-terminal 14 amino acids of which can be cleaved off by trypsin-like enzymes. This 14-residue peptide is called melanocortin and stimulates melanocytes in the skin to produce the brown pigment melanin. The receptor for melanocortin also binds intact ACTH, albeit at a lower affinity. The increased amounts of ACTH, and probably of melanocortin, were what led to the increased pigmentation of Robert's scrotum and the tissue around his nipples.

Answer 2
They had twice the normal number of CD4 and CD8 effector/memory cells in their lymph nodes. This apparently resulted from a lack of negative selection of autoreactive cells in the thymus.

Answer 3
This experiment implies, but does not prove, that *Aire* acts in the thymus, and that the expression of *Aire* in peripheral organs is less important. The lymphocytes transferred from the normal mice had left the thymus with self-reactive clones deleted, whereas this had not occurred in the *Aire*-deficient lymphocytes. To show definitively that the thymus is responsible for the disease, the investigators transplanted either knockout or control thymuses into *nude* mice (which do not have a thymus). Only those mice that received a knockout thymus developed autoimmune disease.

Answer 4
Autoantibodies against ovarian cells are common in females with APECED and may cause primary ovarian failure.

Case 18

Answer 1
In Foxp3-deficient mice, infusion of relatively small numbers of T_{reg} cells controls the disease symptoms. So it is likely that, in humans also, a small number of natural T_{reg} cells is sufficient for immune regulation, and Billy can make sufficient Treg $_{cells}$ that derive from his sister's bone marrow stem cells.

Answer 2
The 'conditioning' leading up to a bone marrow transplant comprises treatment with cytotoxic drugs (see Cases 8 and 27) that kill all rapidly dividing cells, including the CD4 effector T cells responsible for the uncontrolled inflammatory response in IPEX. As these cells are destroyed, the autoimmune response that they have produced will be dampened. Immunosuppressant drugs have the same effect in reducing the activation and proliferation of T cells, and this is why they are used to treat IPEX and other autoimmune disorders.

Answer 3
Because IPEX patients are unable to downregulate the immune activation triggered by infections, their disease frequently flares up on exposure to pathogens and even after vaccination. IVIG is useful in forestalling infections or ameliorating their impact when they occur. Vaccination is contraindicated in IPEX patients because of the risk of disease flare-up.

Answer 4
Venous infusion of immunocompetent purified naive CD4 T cells into mice with severe immunodeficiency (for example, *scid* mice or Rag-deficient mice (see Case 7)) results in colitis. Infusion of CD4 CD25 T_{reg} cells into these recipients can both prevent and reverse the colitis. This suggests that CD4 CD25 T_{reg} cells may have therapeutic potential in human autoimmune diseases.

Answer 5
Patients with IL-2Rα deficiency present a clinical picture with similarities to IPEX. IL-2 signaling is essential for the maintenance of T_{reg} cells. Therefore, T_{reg} activity is deficient in the absence of IL-2, IL-2Rα (CD25), or the transcription factor STAT5, which is important for transducing the IL-2 signal. This may explain the similarity of the symptoms of IL-2Rα deficiency to those of IPEX.

Case 19

Answer 1
Fas and FasL are homotrimeric signaling complexes (see Fig. 19.2). If one element of the trimer is mutant, the trimer is rendered ineffective and cannot deliver the signal to downstream elements of the pathway that ultimately cause cell death. This type of effect is called a dominant-negative effect.

Answer 2
Some family members, even though they show no clinical evidence of ALPS, will show impaired lymphocyte apoptosis *in vitro*. It is clear that environmental and/or other genetic factors have a role in the full expression of the ALPS phenotypes as in other genetically inherited diseases. This is called variable expressivity.

Answer 3
Vaccinia (the virus used for smallpox vaccination) expresses a protein, Crm A, that inhibits caspases. Herpes simplex has two genes, *Us5* and *Us3*, which encode proteins that also inhibit caspases. In contrast, Epstein–Barr virus, which causes acute infectious mononucleosis (see Case 45), produces a protein that resembles Bcl-2, which prevents apoptosis and renders infected cells resistant to killing by cytotoxic T cells (Fig. A19.3).

Answer 4
Yes, it would be. The lethality in mice of knocking out caspase-8 points to the importance of this enzyme in fetal tissue remodeling, but there are known differences between species. Moreover, a point mutation in human caspase-8, at the site where it interacts with the Fas complex, might interfere with its function in Fas-induced apoptosis but not in other, Fas-independent, processes. Thus a missense mutation in caspase-8 could conceivably cause ALPS.

Case 20

Answer 1
Bone marrow transplantation was attempted in a 7-year-old female patient with clinically diagnosed HIES in 2000. Despite good evidence of engraftment (full donor chimerism), within 4 years she again developed recurrent staphylococcal skin abscesses and her serum IgE levels rose to pre-transplant levels.

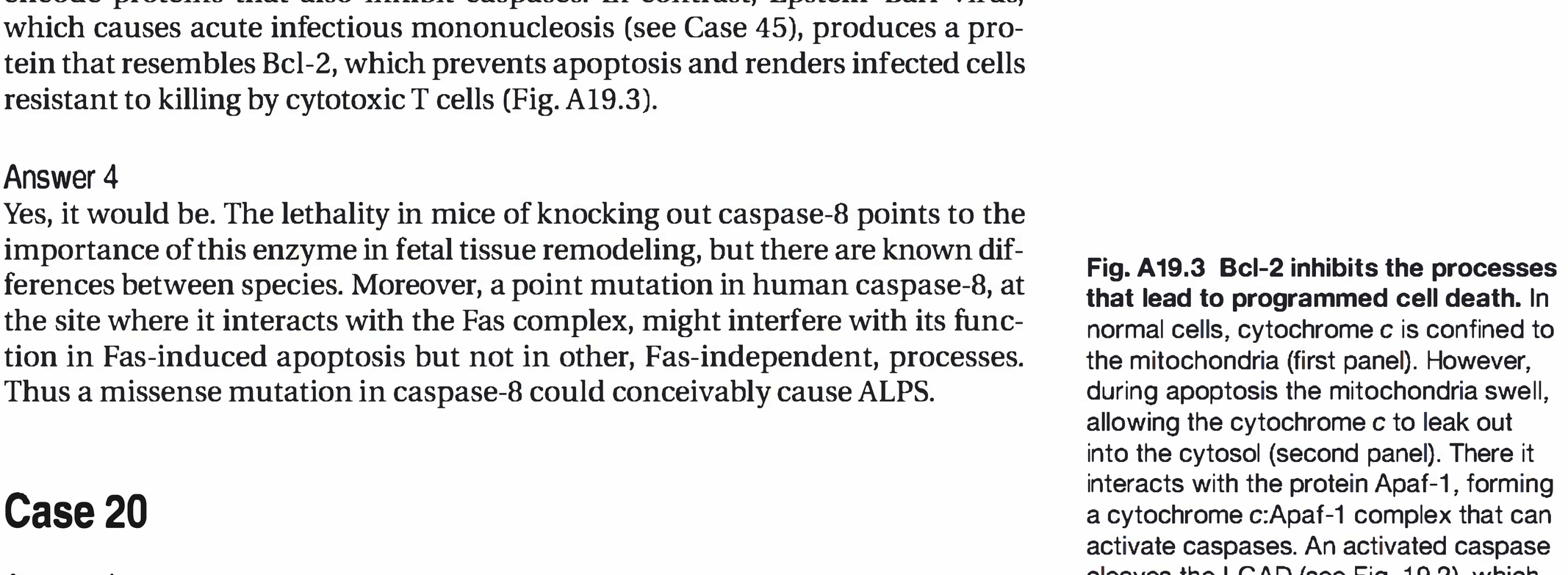


Fig. A19.3 Bcl-2 inhibits the processes that lead to programmed cell death. In normal cells, cytochrome *c* is confined to the mitochondria (first panel). However, during apoptosis the mitochondria swell, allowing the cytochrome *c* to leak out into the cytosol (second panel). There it interacts with the protein Apaf-1, forming a cytochrome *c*:Apaf-1 complex that can activate caspases. An activated caspase cleaves the I-CAD (see Fig. 19.2), which leads to DNA fragmentation (third panel). Bcl-2 interacts with the mitochondrial outer membrane and blocks the mitochondrial swelling that leads to cytochrome *c* release (last panel).

Although the experience with bone marrow transplant in HIES is limited, it seems that defects apart from those in the hematopoietic-cell precursors are important in mediating the immunologic abnormalities.

Answer 2

STAT3 forms homodimers, which translocate to the nucleus to induce the expression of RORγT. A fourfold decrease in RORγT expression is consistent with the fact that STAT3 activity depends on intact function of both molecules in the dimer. A single mutant allele of *STAT3* (heterozygous mutation) would render 75% of the STAT3 homodimers nonfunctional, which is consistent with a dominant-negative effect. Having only 25% of the functional STAT3 homodimers would lead to a roughly equivalent decrease in RORγT expression.

Answer 3

The elevation of IgE levels in HIES is likely to reflect IgE production by activated B cells and plasma cells in which T_H2-dependent class switching to IgE has already occurred. IgE synthesis by these cells is not subject to inhibition by IFN-γ, which only counteracts T_H2 cytokine induction of IgE switching in naive B cells.

Answer 4

IL-21 normally inhibits IgE synthesis in mice, and mice with a knockout of the IL-21 receptor (IL-21R) have elevated serum IgE. Because IL-21R signals via STAT3, decreased IL-21R signaling would be expected to result in increased IgE levels. In humans, however, IL-21 has been reported to increase IgE synthesis, so at present the cause of the elevation in IgE in patients with autosomal dominant HIES is not clear.

Case 21

Answer 1

Loss-of-function mutations in the genes encoding Artemis, DNA ligase IV, Cernunnos, or DNA-PKcs can lead to a radiosensitive T^- B^- NK^+ SCID phenotype. All of these proteins are essential for DNA repair in V(D)J recombination and general DNA repair via NHEJ.

Answer 2

Differences in modifier genes can affect the clinical presentation of a monogenic disease. In the mouse model of ataxia telangiectasia, hypermorphic mutations in the *RAD50* gene, which encodes a component of the MRN complex, can partly compensate for ATM deficiency.

Answer 3

Because there are ATM-independent mechanisms of DNA repair, even complete ATM deficiency results in the faulty repair of only a small fraction of double-strand DNA breaks, and the repair of single-strand DNA breaks does not require ATM. Therefore, the gradual clinical presentation in patients with ataxia telangiectasia is a result of the slow accumulation of unrepaired double-strand DNA breaks.

Answer 4

No. ATM is expressed at very low levels in peripheral blood lymphocytes, and Western blotting is not always reliable, especially if the sample size is less than 10 ml. It is therefore best if the diagnosis of ataxia telangiectasia is made by using a combination of clinical, laboratory, and genetic testing.

Case 22

Answer 1

Chronic neutropenia and hypogammaglobulinemia are also typical of CD40 ligand or CD40 deficiency (see Case 2). However, these conditions have X-linked and autosomal recessive inheritance respectively, whereas WHIM syndrome is typically autosomal dominant. Furthermore, the bone marrow of patients with CD40 ligand or CD40 deficiency shows an arrest in myeloid differentiation, whereas an accumulation of mature neutrophils is seen in patients with WHIM syndrome. Neutropenia may also be seen in other conditions with hypogammaglobulinemia. Autoimmune neutropenia may be observed in patients with common variable immunodeficiency; severe neutropenia may be transiently seen in patients with agammaglobulinemia (see Case 1) during acute infections.

Answer 2

There are two possible explanations for this. Sue's WHIM syndrome could be caused by a de novo mutation that arose in the paternal or maternal germline. Alternatively, it is possible that the disease was not clinically evident in one of the two parents, in spite of the presence of the mutation. Forms of WHIM syndrome that show only some of the symptoms of the disease have been reported.

Answer 3

CXCR4 mutations not only cause retention of neutrophils in the bone marrow but also interfere with the trafficking of other leukocytes. In particular, impaired migration of effector T cells, NK cells, and antigen-presenting cells (dendritic cells) might contribute to the increased susceptibility of patients with WHIM syndrome to viral cutaneous infections.

Answer 4

The receptor function of CXCR4 can be antagonized by competitive ligands, such as the drug AMD-3100 (plerixafor). Because CXCR4 action is important in retaining hematopoietic stem cells in the bone marrow, AMD-3100 is sometimes used to help mobilize stem cells from the bone marrow to the periphery to facilitate the collection of hematopoietic stem cells from blood (for example, for transplantation).

Case 23

Answer 1

One important defense against mycobacteria is the activation of macrophages to synthesize cyokines such as IL-18 and TNF-α after recognition of microorganisms via Toll-like receptors (TLRs). These cytokines amplify immune responses, activate macrophages' intracellular killing activity and induce nitric oxide, which is important in the destruction of the intracellular mycobacteria. In patients with *NEMO* mutations, these cytokines are not made in such quantity because TLRs signal via the activation of NFκB by the IKK complex, which contains NEMO. TNF-α and IL-18 themselves also signal through the NFκB pathway, so their effect is severely reduced. This in turn reduces the synthesis of the important cytokine interferon-γ (IFN-γ) by T cells, which is normally induced by IL-12 acting synergistically with IL-18. Thus, NEMO deficiency also results in decreased IFN-γ production. Because IFN-γ also activates macrophages' ability to kill intracellular bacteria, a lack of NEMO leads to an inability of macrophages to kill mycobacteria that have been taken up by phagocytosis and have become resident in their endosomes. The persistently infected macrophages continue to activate T cells, leading to granuloma

formation. Patients with NEMO mutations thus have some similarities to children with mutations in IL-12, the IL-12 receptor, and the IFN-γ receptor in terms of their susceptibility to mycobacteria (see Case 24).

Answer 2

The killer activity of natural killer (NK) cells is deficient in most patients with *NEMO* mutations. Patients deficient in NK cells are known to suffer from recurrent CMV and herpesvirus infections. This suggests that activation of NK cells via their invariant activating receptors in response to viruses is dependent on intact IKK and NFκB activation.

Answer 3

Class switching can be induced by various members of the TNF family (to which CD40 ligand belongs), which are expressed on the surface of activated dendritic cells and engage receptors on B cells. This engagement activates NFκB in a NEMO-independent fashion by selective activation of IKKα dimers by the kinase NIK, which results in the processing of the auto-inhibited NFκB subunit p100 into its active form, p52. This small amount of B-cell activation might also explain the presence of lymph nodes in patients with *NEMO* mutations.

Answer 4

IKK is the major means of phosphorylating IκB, but other kinases can also perform this function, thereby circumventing the block to some extent. When IκB cannot be phosphorylated at all because of a mutation at the phosphorylation site, the pathway is completely blocked and a more severe immunodeficiency results.

Answer 5

It is still possible that the patients have NEMO deficiency despite a normal coding sequence. Mutations in the 5′ untranslated region of a gene may result in severely reduced mRNA levels and protein levels with normal coding sequence. NEMO deficiency due to this type of mutation has been observed, with a fivefold reduction of NEMO protein levels, impaired TLR function, and impaired antibody responses to several vaccines.

Case 24

Answer 1

Mycobacteria, particularly atypical mycobacteria, are ubiquitous in the environment, and any infection is normally contained byT-cell action. AIDS, however, is characterized by a marked reduction in the number of CD4 T_H1 cells. Hence the production of IFN-γ is compromised, and patients with AIDS have difficulty in activating their macrophages and clearing mycobacteria.

Answer 2

The delayed-type hypersensitivity reaction is provoked by a few T cells that are specific for tuberculin. After binding antigen, these T cells secrete chemokines that nonspecifically recruit macrophages and other inflammatory cells. The secretion and action of these chemokines are not dependent on IFN-γ. It is therefore not surprising that Clarissa and her cousins developed positive tuberculin skin tests.

Answer 3

Granulomas form where there is local persistence of antigen, antigen-specific T cells, and activated macrophages. These children could not activate their

macrophages, so it is not surprising that they did not form granulomas. Granulomas can be beneficial in that they wall off and prevent the spread of microorganisms. These children could not do that and we find the mycobacteria spreading via the bloodstream—a highly unusual finding in mycobacterial disease. However, granuloma formation is preserved in patients with autosomal recessive partial IFN-γR1 deficiency and in patients with IL-12Rβ1 deficiency.

Answer 4

Infection with pyogenic bacteria such as pneumococci is controlled and terminated by antibody and complement. In fact Clarissa had a very high level of IgG (1750 mg dl^{-1}), probably as a result of increased IL-6 production induced by the chronic mycobacterial infection. Most viral infections, such as chickenpox, are terminated by cytotoxic CD8 cells. Activation of these cells is not dependent on IFN-γ. However, there is evidence from mice that a lack of the IFN-γ receptor increases susceptibility to certain viruses, including vaccinia virus and lymphocytic choriomeningitis virus. Salmonellae take up residence as an intracellular infection of macrophages and become inaccessible to antibody and complement; they can be destroyed in this site only when macrophages are activated by IFN-γ.

Case 25

Answer 1

If the neutropenia is due to increased peripheral destruction of neutrophils, the ability to produce neutrophils in the bone marrow is likely to be normal. In such cases, a bone marrow aspirate will demonstrate the presence of myeloid cells, including neutrophils, in all stages of differentiation. In contrast, neutropenia due to a decreased production of neutrophils is associated with a reduction of myeloid cells in the bone marrow. This reduction may involve cells at all stages of differentiation (as seen in patients with leukemia, whose bone marrow is occupied by cancer cells), or it may affect more mature myeloid cells only, with a normal presence of more immature and progenitor cells, as is typically the case in patients with SCN.

Answer 2

Neutrophils have a very rapid turnover (their half-life being about 8 hours). Therefore, almost all of the neutrophils infused intravenously will die within a day. Furthermore, such transfusions are associated with a significant risk of inflammatory reactions. These observations significantly limit the therapeutic use of transfusions of granulocytes generally. Nonetheless, this approach could be considered in patients with severe numerical or functional defects of neutrophils (SCN and chronic granulomatous disease, respectively) who have life-threatening infections that fail to respond to conventional treatment.

Answer 3

The binding of cytokines to their specific receptors elicits intracellular signaling and may promote cellular activation, leading to proliferation and/or differentiation. Negative regulation of cytokine-mediated signaling may involve internalization of the cytokine receptor, or modification of its intracytoplasmic tail (such as ubiquitination) followed by proteasome-mediated degradation. The intracytoplasmic tail of the G-CSF receptor (G-CSFR) includes a ubiquitination site. Somatic mutations of the G-CSFR that result in truncation of the intracytoplasmic tail impede G-CSFR ubiquitination and cause increased G-CSF-mediated signaling and cellular hyperactivation, thus contributing to leukemic transformation.

Case 26

Answer 1

There has been random inactivation of the X chromosomes in Randy's mother's neutrophils. Therefore 50% of her neutrophils have a normal X chromosome and 50% have an X chromosome bearing the CGD defect. The cells bearing the CGD-defective X chromosome have not been selected against. That half does not reduce NBT, whereas the half bearing the normal X chromosome does.

Answer 2

Streptococcus pneumoniae, the pneumococcus, does not produce catalase, an enzyme that converts hydrogen peroxide (H_2O_2) to water and oxygen, and is thus far less resistant to intracellular killing than are microorganisms that are catalase producers.

Answer 3

Because of persistent antigenic stimulation, Randy is making more immunoglobulins (antibodies) than a normal person. In fact, all chronic infections, such as malaria, result in hypergammaglobulinemia.

Answer 4

Rac2 is important for both the function of the membrane cytochrome b_{558} complex and for the chemotaxis of many cells including neutrophils. These two defects coexist both in humans and in the mouse model of Rac2 deficiency, but not in defects that affect other genes that encode other subunits of the cytochrome b_{558} complex.

Case 27

Answer 1

It is autosomal recessive. The parents are both healthy; they have had an affected male and an affected female child. These facts lead to the conclusion that the leukocyte adhesion deficiency (LAD) is inherited as an autosomal recessive trait. In fact, the gene encoding CD18 has been mapped to the long arm of chromosome 21 at position 21q22.

Answer 2

Monoclonal antibodies against CD18 can induce a mild phenotype of LAD. Such monoclonal antibodies have been used to prevent graft rejection in recipients of kidney grafts and, when administered before bone marrow transplantation, can prevent graft-versus-host disease.

Answer 3

T cells express the β_1 integrin VLA-4 (Fig. A27.3), and its interaction with VCAM-1 on endothelia seems to be sufficient to enable T cells to home and function normally. VLA-4 is not expressed on neutrophils and macrophages, which are much more dependent on β_2 integrins for their adhesion to other cells. B cells also home normally in LAD, and this is probably due to the integrin α_4:β_7, which is not defective in this disease.

Answer 4

Luisa's brother had delayed separation of the umbilical cord and Luisa developed an infection at the site of umbilical cord separation. The role of neutrophils and macrophages in wound healing is not well understood.

Nevertheless, it is apparent from cases of LAD that the movement of white blood cells into wounds is vital to normal tissue repair.

Answer 5
Fucose is a defining determinant of the sialyl-Lewis^x element, which is the ligand whereby white blood cells bind to selectins on endothelial cells (see Fig. CS6-0301/27.1). In patients with LAD type 2, impaired transport of fucose into the Golgi apparatus prevents the fucosylation of newly synthesized glycoproteins, such as sialyl-Lewis^x. Infants with LAD type 2 have very high white blood cell counts because their leukocytes cannot roll on the endothelium to begin the process of leukocyte emigration from the bloodstream.

Answer 6
After the transplant, her leukocytes were found to express CD18.

Answer 7
This was done to destroy her abnormal cells and create 'space' for the transplanted cells. Such preparation can be avoided in the case of severe combined immunodeficiency because the lymphoid compartment is already devoid of T cells and thus space is available for the transplanted cells without any treatment.

Answer 8
PHA is a so-called nonspecific T-cell mitogen. The mitogenic response requires cell–cell interactions that depend on the interaction of LFA-1 with ICAM-1, as well as that of VLA-4 with VCAM-1. In LAD, one of these interactions is missing.

Answer 9
In LAD, there is a defect in the mobility of the leukocytes so that their emigration from the circulation to sites of infection is impeded, whereas in chronic granulomatous disease (CGD), the microbicidal capacity of phagocytes (neutrophils and macrophages) is impaired. The microbicidal capacity of phagocytes in LAD is normal. Children with LAD therefore never develop the inflammatory lesions characteristic of CGD, which are caused by activated phagocytes. In contrast, the leukocytes in CGD have normal mobility and can reach sites of infection in a normal manner but cannot efficiently destroy the infecting bacteria when they reach them. Hence the development of abscesses and granulomas. Unlike individuals with CGD, children with LAD are no more susceptible to pneumococcal infections than they are to other bacterial infections.

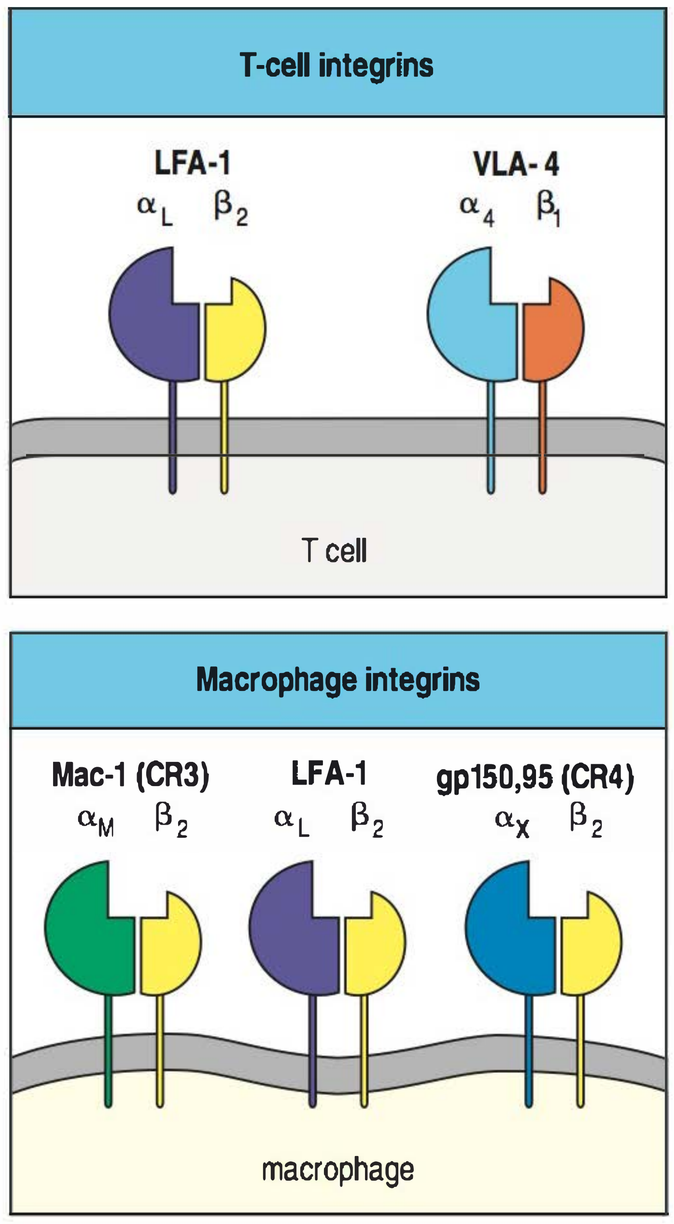


Fig. A27.3 T cells and phagocytes express different integrins. Integrins are heterodimeric proteins containing a β chain, which defines the class of integrin, and an α chain, which defines the different integrins within a class. The α chain is larger than the β chain and contains binding sites for divalent cations that may be important in signaling. Upper panel: LFA-1, a β_2 integrin, and VLA-4, a β_1 integrin, are expressed on T cells and are important in the migration and activation of these cells. Lower panel: macrophages and neutrophils express all three members of the β_2 integrin family: LFA-1, Mac-1 (also known as CR3), and gp150,95 (also known as CR4). Like LFA-1, Mac-1/CR3 binds the immunoglobulin-superfamily molecules ICAM-1, ICAM-2, and ICAM-3, but in addition it is a complement receptor (for the fragment iC3b). gp150,95/CR4 also binds complement and stimulates phagocytosis.

Case 28

Answer 1
The reasons for the central nervous system (CNS)-specific phenotype of patients with TLR-3 signaling defects remain poorly defined. However, the CNS-restricted phenotype suggests that TLR-3 signaling is redundant for the systemic control of viral infections. Indeed, leukocytes may respond to the TLR-3 agonist poly(I:C) in a TLR-3-independent manner, which indicates that there is redundancy in the mechanisms of recognition and response to double-stranded RNA (dsRNA) viral intermediates within the immune system.

Answer 2
Identification of the molecular basis of HSE could have important therapeutic implications. In particular, administration of type 1 interferons might be

beneficial in patients with mutations of *TLR3*, *UNC93B*, and *TRAF3*, who are otherwise unable to produce these interferons in response to HSV-1 infection in the CNS.

Case 29

Answer 1

Several immunodeficiencies result in susceptibility to pneumococcal infection. These include defects of innate immunity, including congenital asplenia (see Case 30) and defects within the complement pathways (see Cases 31–33). Other defects in the NFκB activation pathway that lies downstream of TLRs and other cell-surface receptors include NEMO deficiency (see Case 23) and mutations in IκB that prevent its degradation and release of NFκB. In addition, defects of adaptive immunity that result in impaired antibody production, such as X-linked agammaglobulinemia (see Case 1) and common variable immunodeficiency (see Case 4), result in increased susceptibility to Gram-positive bacteria, such as pneumococci and staphylococci.

Answer 2

These infections are usually associated with fever. IRAK4 deficiency, however, leads to an early block in the TLR/IL-1R signaling pathways and barely detectable or no TLR-induced production of pro-inflammatory cytokines. The virtual absence of pro-inflammatory cytokines and the inability of IRAK4-deficient patients to respond to what little IL-1 might be produced results in an impaired febrile response (see Case 34). Thus, a history of little or no fever associated with recurrent pyogenic infections supports a possible diagnosis of IRAK4 deficiency.

Answer 3

So far, there are too few IRAK4-deficient patients for us to be sure. More cases of IRAK4 deficiency need to be identified and followed throughout their lives before conclusions can be drawn. The 18 patients identified so far all show an increased susceptibility to invasive infections with pyogenic bacteria in childhood. As they mature into adolescence, however, susceptibility to such infections becomes variable, and many no longer show significantly increased susceptibility.

Answer 4

The antibody response to polysaccharides seems to require initial signaling via TLR-2 (which recognizes lipoteichoic acid of Gram-positive bacterial cell walls and lipoproteins of Gram-negative bacteria) and TLR-4 (which recognizes LPS) in dendritic cells. This may be because stimulation of dendritic cells via these TLRs is essential for their ability to induce the differentiation of T cells (especially T_H1) that help B cells to make the antipolysaccharide antibodies. The response to Pneumovax, for example, seems to depend on the presence of such TLR ligands in the vaccine. It has been shown that depletion of endotoxin from the vaccine renders it unable to elicit an antibody response in mice.

Answer 5

The answer to this important question is not clear. The production of type I interferons in response to the ligation of TLRs 7, 8, or 9 is markedly diminished in IRAK4-deficient patients, and interferon synthesis is variably affected in response to TLR-3 ligation by long double-stranded RNA. The apparent integrity of the antiviral defenses in these patients is therefore surprising.

One explanation could be that humans can make relatively intact adaptive immune response to viruses as a result of responses by cytotoxic T cells, which kill the infected cells, and through antiviral antibodies produced by B cells. There is probably redundancy between the adaptive and innate immune responses, with (innate system) NK cells cytotoxic for virus-infected cells being activated by T-cell-derived IL-2 and IFN-γ. Other intracellular antiviral immune responses are produced via activation of the RNA-dependent protein kinase pathway and via activation of the cytoplasmic protein RIG1 by double-stranded RNA, which leads to the synthesis of type I interferons.

Answer 6

Experience in managing these patients is limited. However, they are maintained in good health into their adolescence by a combination of prophylactic antibiotics and regular infusions of intravenous immunoglobulin.

Case 30

Answer 1

First you find out that Nicholas has had all his routine immunizations. He received DPT (diphtheria, pertussis, and tetanus antigens) and oral live poliovirus vaccine at ages 3, 4, and 5 months, and a booster of both before entering kindergarten. He was also given MMR (mumps, measles, and rubella live vaccines) at 9 months of age. At the same time, he was given Hib vaccine (the conjugated capsular polysaccharide of *Haemophilus influenzae*, type b; Fig. A30.1). His growth and development have been normal. He suffered a middle ear infection (otitis media) at age 24 months. Other than that he has had no other illnesses, except for a common cold each winter. You feel comfortable that he is protected against infection with *H. influenzae* from the Hib vaccine. However, your concern about the possibility of pneumococcal infection leads you to advise the surgeon to immunize Nicholas against pneumococcal capsular poysaccharides by giving him conjugated pneumococcal polysaccharide vaccine. You also advise prophylactic antibiotics, to be taken at a low dose daily but at higher doses when Nicholas has any dental work done, or any invasive surgical procedure.

Answer 2

The typhoid vaccine was given subcutaneously and a response was mounted in a regional lymph node. The sheep red blood cells were given intravenously, and, in the absence of a spleen, failed to enter any peripheral lymphoid tissue where an immune response to them could occur.

Answer 3

The defect is inherited as an autosomal recessive. The parents are normal but each carries this recessive gene. Furthermore, they are consanguineous, a setting in which autosomal recessive disease is encountered more frequently

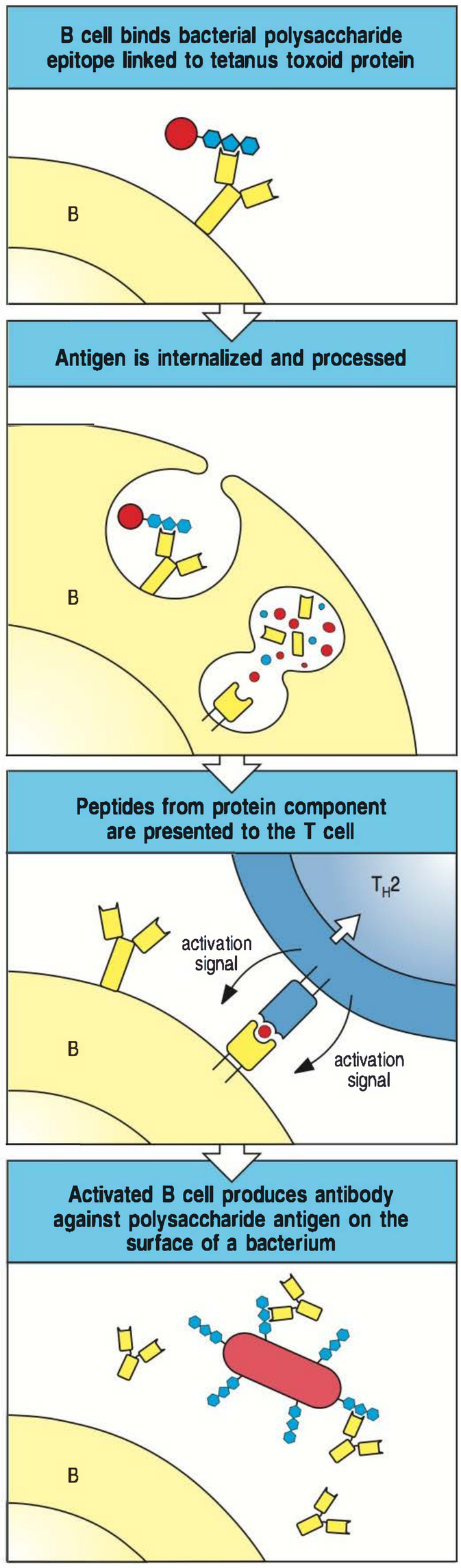


Fig. A30.1 *Haemophilus influenzae* b vaccine is a conjugate of bacterial polysaccharide with the tetanus toxoid protein, which enhances the immune response by allowing a polysaccharide-specific B cell to recruit T-cell help. The B cell recognizes and binds the polysaccharide, internalizes and degrades the toxoid protein to which it is attached, and displays the peptides derived from it on surface MHC class II molecules. Helper T cells generated in response to the protein moiety of the toxoid recognize the complex on the B-cell surface and activate the B cell to produce antibody against the polysaccharide. This antibody can then protect against infection with *H. influenz*ae, type b.

than when parents are unrelated. Chance would predict that one in four (that is, two) of their eight children would be affected. Each pregnancy provides a one in four chance that the fetus will inherit the abnormal gene from both parents. As it turned out, this happened in five of Mrs Vanderveer's eight pregnancies. Because Betsy married a normal man, all her children are heterozygous for the defect, like their maternal grandparents, and have normal spleens.

Case 31

Answer 1

Histamine release on complement activation is caused by C3a (the small cleavage fragment of C3), and the main chemokine is C5a (the small cleavage fragment of C5). These are both generated by the C3/C5 convertase, which in the classical pathway is formed from C4b and C2a. In HAE, C4b and C2a are both generated free in plasma. C4b is rapidly inactivated if it does not bind immediately to a cell surface; for that reason, and because the concentrations of C4b and C2a are relatively low, no C3/C5 convertase is formed, C3 and C5 are not cleaved, and C3a and C5a are not generated.

The edema in HAE is caused not by the potent inflammatory mediators of the late events in complement activation, but by C2b generated during the early events, and by bradykinin generated through the uninhibited activation of the kinin system.

Answer 2

The only other complement component that should be decreased is C2, which is also cleaved by C1. C1 plays no part in the alternative pathway of complement activation, so complement activation by the alternative pathway is not affected. The terminal components are not affected either. The unregulated activation of the early complement components does not lead to the formation of the C3/C5 convertase (see Question 1), so the terminal components are not abnormally activated. The depletion of the early components of the classical pathway does not affect the response to the normal activation of complement by bound antibody because the amplification of the response through the alternative pathway compensates for the deficiency in C4 and C2.

Answer 3

This is not hard to explain; as we have already remarked, the alternative pathway of complement activation is intact and thus, although the classical pathway is affected by deficiencies in C2 and C4, these are compensated for by the potent amplification step from the alternative pathway.

Answer 4

Stanozolol is a well-known anabolic androgen that has been used illegally by Olympic competitors. For unknown reasons, anabolic androgens suppress the symptoms of HAE, and that is why stanozolol was prescribed to Richard. Patients, especially females, do not like to take these compounds because they cause weight gain, acne, and sometimes amenorrhea. Preparations of purified C1INH are now available, and intravenous injection of C1INH prepared from human donors is safe and very effective in halting the symptoms of the disease.

Answer 5

In practice, you would administer epinephrine immediately in any case, because most such emergencies are due to anaphylactic reactions and because epinephrine is a harmless drug. If the laryngeal edema is anaphylactic, it will

respond to the epinephrine. If it is due to hereditary angioedema, it will not. Anaphylactic edema is also likely to be accompanied by urticaria and itching, and the patient may have been exposed to a known allergen. Most patients know if they are allergic or have a hereditary disease, and they should be asked whether they have had a similar problem before.

Answer 6

HAE does not skip generations: it is therefore likely that its effects are dominant. It clearly affects both males and females, so it cannot be sex-linked. If the gene has a dominant phenotype, and Richard's two children are normal, then it follows they cannot have inherited the defective gene from their father, and their children cannot inherit the disease from them.

Richard has inherited his abnormal *C1INH* gene from his mother. Because he has a normal *C1INH* gene from his father, you might expect that he would have 50% of the normal level of C1INH. However, the tests performed by his immunologist revealed 16% of the normal level. In general, functional C1INH tests in HAE patients reveal between 5% and 30% of normal activity. How could this be explained? There are two possibilities: decreased synthesis (that is, less than 50% synthesis from only one gene); or increased consumption of C1INH as a result of increased C1 activation. Both explanations have been shown to be correct. Patients with HAE synthesize about 37--40% of the normal amount of C1INH, and C1INH catabolism is 50% greater than in normal controls.

Case 32

Answer 1

As Morris lives longer, his adaptive immunity against these common bacteria becomes stronger and he has come to rely less on innate immune mechanisms for protection against infection. Bacteria coated with antibodies can be phagocytosed independently of complement via the Fc receptors on phagocytes.

Answer 2

A similar result. The factor B would be rapidly destroyed and its rate of synthesis could turn out to be normal. The overproduction of C3b in the absence of factor I leads to an increased binding of factor B to C3b and its subsequent cleavage by factor D. Thus, factor B is being consumed excessively as a result of the deficiency in factor I. The lack of factor B, like the C3 deficiency, is secondary to the basic defect in factor I.

Answer 3

His serum levels of C3 and factor B rose to normal. C3b disappeared from his serum. The effect lasted for about 10 days.

Answer 4

Deficiency in factor H. Because factor H is needed for the cleavage of C3b by factor I in the blood, factor H deficiency should result in clinical symptoms identical with those of factor I deficiency. In fact this is true: several families with factor H deficiency have been studied, and they show symptoms indistinguishable from factor I deficiency.

Answer 5

He is producing large amounts of C3b, which binds to complement receptor 1 (CR1) on red blood cells (see Case 33) and leads to their agglutination by anti-C3.

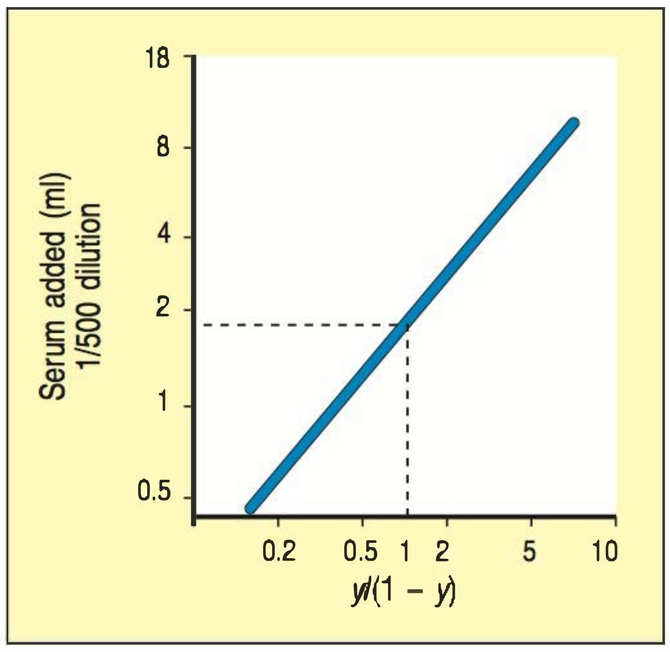
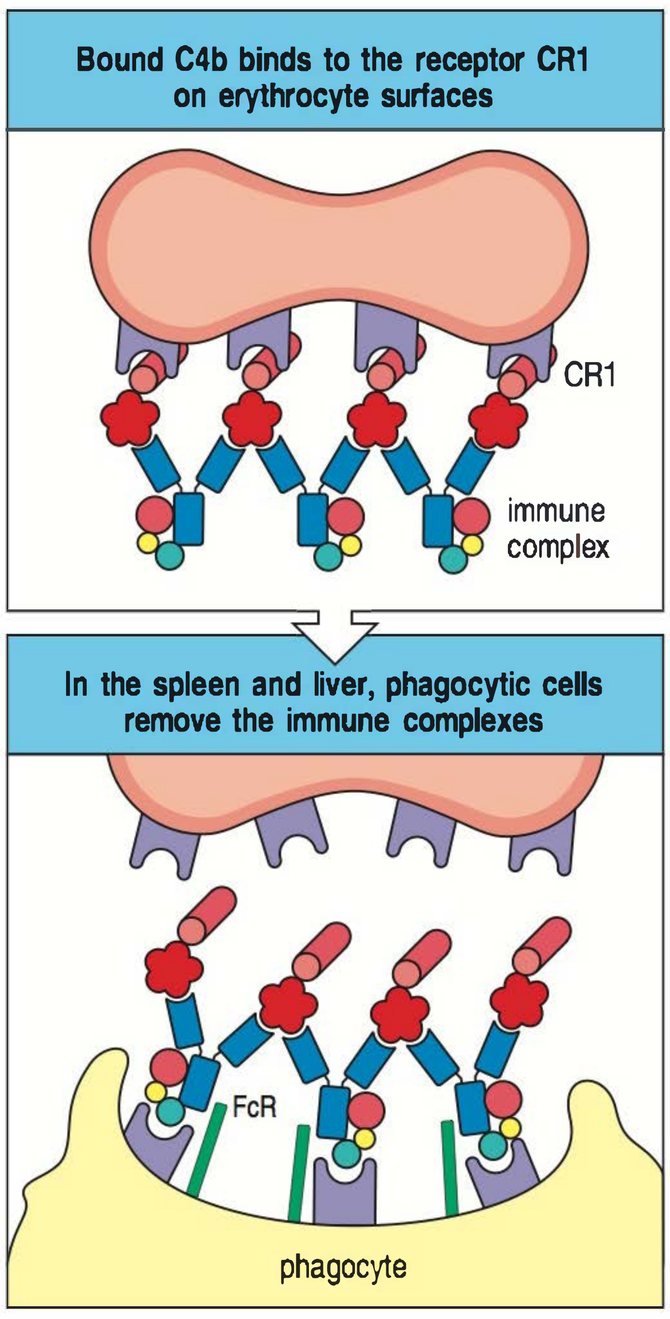


Fig. A33.1 Determination of CH_{50}. This is the quantity of complement required for 50% lysis of 5×10^8 optimally sensitized red blood cells in 1 hour at 37°C. Complement titers are expressed as the number of CH_{50} contained in 1 ml of undiluted serum. This is usually determined by plotting $\log_{10} y/(1-y)$ (where y is percentage lysis) against the logarithm of the amount of serum. This plot is linear near $y/(1-y) = 1$ (50% lysis). In the example shown, the serum contains 250 CH_{50} ml^{-1}.



Case 33

Answer 1

The CH_{50} is the quantity of complement required for 50% lysis of 5×10^8 optimally sensitized sheep red blood cells in 1 hour at 37°C. (The sheep red blood cells are usually sensitized with a rabbit antibody against the sheep cells.) The complement titer is expressed as the number of CH_{50} contained in 1 ml of undiluted serum from the patient. This is determined by plotting $\log y/(1-y)$ (where y is the percentage lysis) against the logarithm of the amount of serum. The plot is linear near $y/(1-y) = 1$ (50% lysis) (Fig. A33.1). Because this assay measures the functional integrity of all the complement components comprising the classical pathway, it is the single best screening test when a general diagnosis of complete complement deficiency is being considered.

Answer 2

The bacteria are temporarily vulnerable to killing by complement when they divide. At this time the bacterial membrane is exposed and is vulnerable to attack by the membrane-attack complex. The association between genetic deficiencies of membrane-attack complex proteins and neisserial infections illustrates that an important aspect of host defense against these infections is the killing of extracellular bacteria by complement-mediated lysis. *Neisseria* that escape killing enter a variety of cell types and establish an intracellular infection.

Answer 3

In humans, clearance of immune complexes from the blood is largely effected by their attachment to complement receptor 1 (CR1) on the surface of red blood cells. One of the ligands for CR1 is C4b (the other is C3b). All the C1 components are required for the formation of C4b, and if C4b is part of an immune complex, the binding of the complex by CR1 is facilitated (Fig. A33.3). Immune complexes bound to the surface of the red cell are transported to the liver and spleen, where C4b (or C3b) is converted by factor I to iC4b (or iC3b); this facilitates uptake of the immune complexes by phagocytes via complement receptor CR3 and others, and their destruction. In the absence of C4b, immune complexes are less efficiently attached to red cells and are therefore less efficiently cleared from the blood.

Case 34

Answer 1

Anakinra is a recombinant protein that competitively blocks the binding of IL-1β to its receptor, IL-1R, and would thus be expected to mitigate the effects of the excess IL-1β produced in the cryopyrinopathies. Pretreatment with anakinra before exposure to cold has been shown to prevent the development of symptoms and the elevation of acute-phase reactants in patients with familial cold autoinflammatory syndrome, and it also improves symptoms and corrects biochemical abnormalities in Muckle–Wells syndrome. Children with NOMID/CINCA have shown similarly promising results, with resolution of fever, rash, and uveitis (inflammation of the middle layer of the eyeball, the uvea, comprising the choroid, iris, and cilary body), and the relief of excess pressure exerted by the CSF.

Fig. A33.3 Erythrocyte complement receptor CR1 helps to clear immune complexes from the circulation. Immune complexes bind to CR1 on erythrocytes, which transport them to the liver and spleen, where they are removed by macrophages expressing receptors for both Fc and bound complement components.

Answer 2
Colchicine inhibits the assembly of microtubules in cells by binding to β-tubulin subunits. In the context of autoinflammatory disease it is thought to act mainly by inhibiting the neutrophil response. It inhibits microtubule-dependent processes in the cell, including the division of neutrophil precursors and the secretion of pro-inflammatory mediators by mature neutrophils. In addition, colchicine has been shown to modulate the production of chemokines and pro-inflammatory prostanoids (prostaglandins and leukotrienes) by neutrophils, and it is also thought to inhibit neutrophil adhesion to the endothelium, a necessary step in the migration of neutrophils out of the blood and into tissues. Interestingly, it has also recently been shown to block IL-1β processing and secretion. Together, all of these actions are anti-inflammatory.

Answer 3
Caspase 1 is required to process the precursor forms of IL-1 and IL-18 proteolytically to produce mature active cytokines that can be secreted. It is now known that the cytokine IL-33 also requires processing by caspase 1. IL-33 is an IL-1-like cytokine that signals through the IL-1 receptor-related protein ST 2. IL-33, via ST 2, activates the transcription factor NFκB and mitogen-activated protein kinases (MAPKs), and drives the production of the T_H2-associated cytokines IL-4, IL-5, and IL-13. In addition to IL-33, one might expect that blood levels of cytokines that are induced by the action of IL-1β, such as IL-6, would be increased in response to caspase 1 activation.

Case 35

Answer 1
Patients with sJIA have an increased secretion of IL-1β (unlike in other forms of arthritis), and some show remarkable improvement when treated with anakinra. Unlike the periodic fever syndromes discussed in Case 34, however, the causative gene mutation in sJIA has not yet been identified.

Answer 2
Anti-IL-1 therapies have been on the market for the past few years. Anakinra is an IL-1 receptor antagonist. It is used for the treatment of rheumatoid arthritis and more recently for pediatric rheumatologic conditions such as sJIA. Because IL-1 is important in the normal response to infection, higher rates of serious infections or infectious complications in patients on anakinra would not be unexpected. Meta-analysis of four large anakinra trials showed overall a similar rate of infection compared with placebo; however, there was a modest increase in the infection rate for patients on the highest anakinra doses.

Tocilizumab was recently approved by the US Food and Drug Administration for rheumatoid arthritis, and studies using tocilizumab to treat sJIA look promising. Tocilizumab blocks the effects of IL-6 by binding to its receptor. Again, an increased risk of infections is the primary theoretical concern with tocilizumab. Trials of tocilizumab for patients with rheumatoid arthritis showed a small but significant increase in the infection rate, especially in patients on higher doses. Elevation of serum cholesterol has also been observed in individuals on tocilizumab. Large cohorts of patients on IL-1 and IL-6 inhibitors will need to be followed for years to ascertain their effects fully.

Answer 3
Understanding of the autoinflammatory disorders has advanced considerably over the past few years. Many of these disorders involve the inflammasome and subsequent IL-1 signaling. NLRP3, a component of the inflammasome, was found to be mutated in the cryopyrin-associated periodic fever syndromes

(CAPS) (see Case 34). The CAPS are a spectrum of disorders, with the mildest being familial cold autoinflammatory syndrome (FCAS). Patients with Muckle–Wells syndrome have an intermediate phenotype, and individuals with NOMID/CINCA (neonatal-onset multisystem inflammatory disease) develop rash, fever, poor growth, and characteristic facial features at or shortly after birth (see Case 34). These NLRP3 mutations result in an increased processing and secretion of IL-1/beta. More recently, the gene encoding the IL-1 receptor antagonist (*IL1RN*) was found to be mutated in DIRA (deficiency of IL-1 receptor antagonist). Patients with DIRA present early in life with severe rashes and bony deformities. Fortunately, patients with CAPS or DIRA respond to treatment with anakinra and other IL-1 antagonists.

Answer 4

It is still a mystery exactly why the fevers with sJIA occur daily or twice daily. The fever-inducing cytokine IL-1 drives IL-6 production. IL-1 has a very short half-life in serum; however, IL-6 levels can be measured and they correlate with fluctuations in the temperature curve. The rash may be affected by some of the same inflammatory mediators as well as by the fever itself. The rash tends to be more prominent with heat, included elevated room temperature. It can also be brought out after rubbing the skin or after trauma to the area.

Case 36

Answer 1

Rheumatoid factors can be found in the serum of patients with other immune-complex diseases. In Case 38 we encounter an example in the case of mixed essential cryoglobulinemia. Patients with hypergammaglobulinemia and chronic infection can also have circulating immune complexes and rheumatoid factor.

Answer 2

Mast cells express the IgG receptor FcγRIII. They use this receptor to take up IgG:antigen complexes. This causes the immediate release from mast cells of preformed TNF-α and IL-1. Mast cells are the only cells in which such cytokines are already preformed.

Answer 3

They upregulate the expression of the integrin CD11:CD18 (LFA-1) on the leukocytes, and this increase in integrin expression promotes the binding of the leukocytes to the blood vessel wall and their emigration from the blood vessels. The soluble IL-1 receptor antagonist anakinra (Kineret) has been used successfully in the treatment of rheumatoid arthritis.

Answer 4

The monoclonal antibody infliximab is a chimeric human–mouse immunoglobulin against which patients may develop antibodies. These antibodies would render the therapy useless and might even cause anaphylactic reactions. Better anti-TNF monoclonal antibodies are those that are completely humanized, such as adalimumab, and therefore do not elicit an immune response. A more general risk is that of the reactivation of a preexisting infection, such as tuberculosis, because TNF-α is normally important in containing infections. This risk applies to all classes of anti-TNF agents. Patients about to receive infliximab or another anti-TNF agent should have a tuberculin skin test to ensure they are free of tuberculosis. The activation of tuberculosis in the absence of TNF-α suggests that this cytokine is critical in activating macrophages to contain the latent infection.

Case 37

Answer 1
The serum levels of complement proteins C3 and C4 are lowered in SLE by the large number of immune complexes binding C3 and C4, triggering their cleavage. The depletion of these proteins is therefore proportional to the severity of the disease. Successful immunosuppressive therapy is reflected in an increase in the serum levels of C3 and C4. Measurement of either C3 or C4 is sufficient; it is not necessary to measure both, and C3 is most usually measured.

Answer 2
The objective of these tests was to establish whether Nicole had autoimmune hemolytic anemia, which occurs in SLE when there are antibodies against erythrocytes. Nicole did not have hemolytic anemia (see Case 41).

Answer 3
Because ultraviolet light provokes the onset of SLE and causes relapses.

Answer 4
She had not developed glomerulonephritis. If she had, her urine would have contained protein and red blood cells.

Answer 5
As a result of the constant stimulation of their B cells by autoantigens, patients with SLE have a greatly expanded B-cell population and consequently an increased number of plasma cells secreting immunoglobulin. A lymph node biopsy from Nicole would have exhibited follicular hyperplasia in the cortex and increased numbers of plasma cells in the medulla.

Answer 6
In the first place, a large multimolecular complex such as a nucleosome carries many separate epitopes, each of which can stimulate antibody production by a B cell specific for that epitope. Any of these antibodies can bind the nucleosome particle to form an immune complex. Such potentially autoreactive B cells probably exist normally in the circulation but, provided that T-cell tolerance is intact, they are never activated because this requires T cells to be reactive against the same autoantigen. SLE is probably caused by a failure of T-cell tolerance. T cells for each of the components of the complex antigen will not be needed to induce antibodies against its individual components. As Fig. A37.6 shows, a T cell that is specific for one protein component of a nucleosome could activate B cells specific for both protein and DNA components.

Case 38

Answer 1
The goal of treatment for symptomatic cryoglobulinemia associated with hepatitis C virus (HCV) infection is to eradicate the HCV or at least reduce the level of viral infection. There are no specific inhibitors of HCV replication currently available; however, interferon-α (IFN-α) is effective in treating HCV infection in some patients. Although the mechanism of action remains uncertain, there is evidence that in patients with chronic HCV, IFN-α clears the virus by preventing new infection of liver cells rather than by inhibiting viral replication within cells. There is also some evidence that IFN-α may tip the balance between the T_H1 and the T_H2 immune response in favor of a T_H1 response. Although IFN-α has been shown to improve many of the features

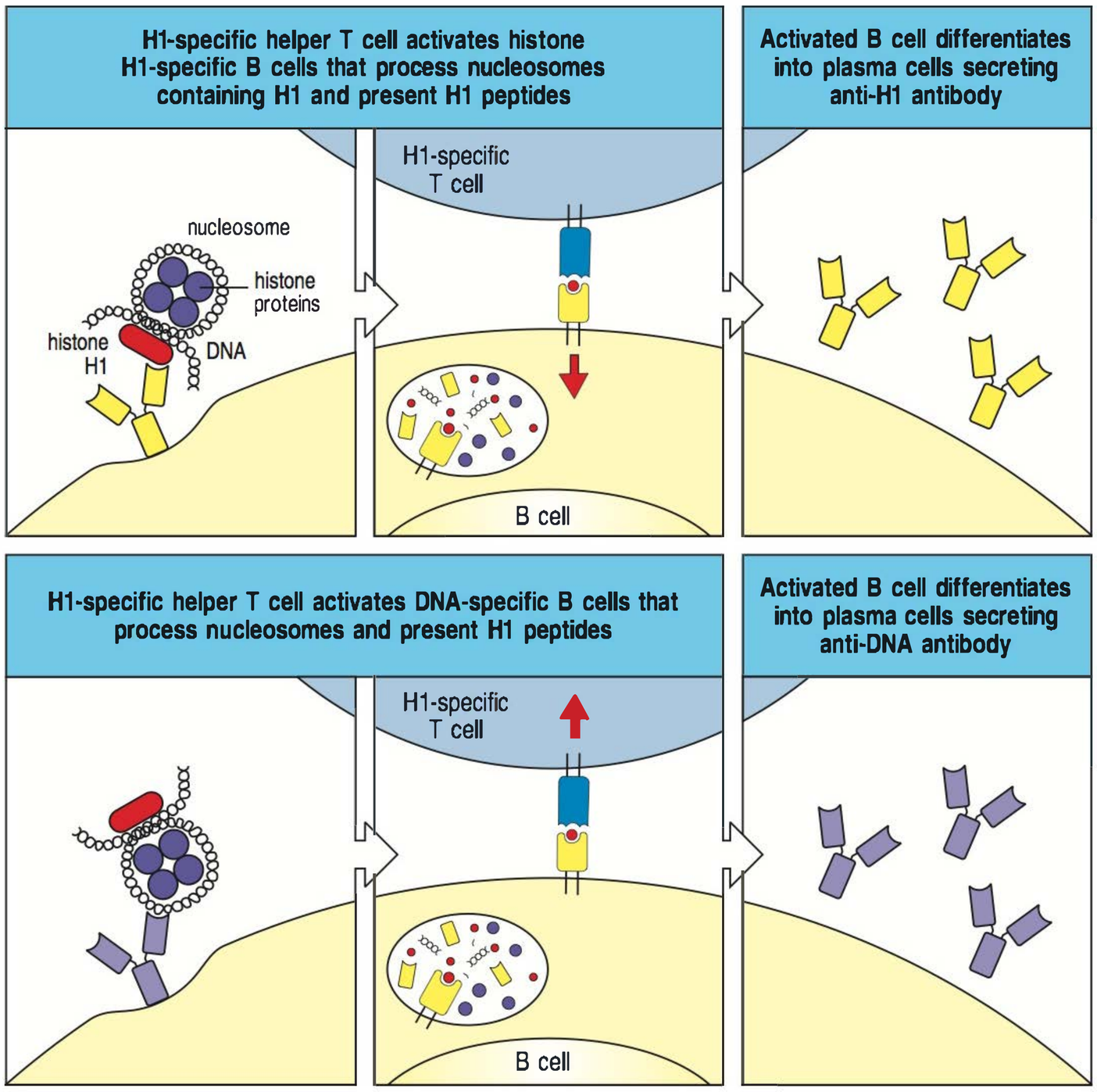


Fig. A37.6 Autoantibodies against various components of a complex antigen can be stimulated by an autoreactive helper T cell of a single specificity. In SLE, patients often produce autoantibodies against all of the components of a nucleosome, or of some other complex antigen. The most likely explanation is that all the autoreactive B cells have been activated by a single clone of autoreactive T cells specific for a peptide of one of the proteins in the complex. A B cell binding to any component of the complex through its surface immunoglobulin can internalize the complex, degrade it, and return peptides derived from the relevant protein to the cell surface bound to class II MHC molecules, where they stimulate helper T cells. These, in turn, activate the B cells. The figure illustrates this scheme for a T cell specific for the H1 histone protein of the DNA:protein complex comprising the nucleosome, and two B cells specific for the histone protein and double-stranded DNA, respectively.

of HCV-induced cryoglobulinemia, cessation of therapy often, unfortunately, results in a relapse of the disease. The combination of ribavirin with IFN-α improves both biochemical and virologic responses in chronic HCV infection and has been beneficial in symptomatic cryoglobulinemia. Ribavirin is a guanosine nucleoside analog that is active against many RNA and DNA viruses.

For severe systemic illness such as the intestinal vasculitis, plasmapheresis is an option for rapidly lowering the circulating titer of autoantibodies and immune complexes. Plasmapheresis involves withdrawal of blood from the patient's vein, separation of the plasma from blood cells, either by centrifugation or membrane separation, and reinfusion of the patient's blood cells, which are resuspended in donor plasma or another replacement solution (such as albumin, normal saline, or lactated Ringer's solution).

Answer 2

Rituximab is a chimeric anti-CD20 monoclonal antibody consisting of human IgG1 κ-chain constant regions and murine variable regions. The CD20 antigen is expressed constitutively on B-lineage cells. Rituximab bound to CD20-positive cells leads to the very efficient depletion of circulating B lymphocytes by a range of mechanisms. These include complement fixation, which results in formation of the membrane-attack complex, and antibody-dependent cell-mediated cytotoxicity (ADCC). The initial clinical application of rituximab was in the treatment of patients with B-cell lymphoma that was refractory to standard therapy; however, its use has now been extended to treat a range of autoimmune diseases, including autoimmune hemolytic anemia and cold agglutinin disease (see Case 41).

In Billy's case, rituximab might help his immune complex-mediated disease in several ways. The most straightforward interpretation is that rituximab causes

B-cell depletion, leading to decreased antibody production and a decrease in the level of circulating immune complexes. However, B cells are important not only in generating antibody responses but also in their role as antigen-presenting cells and cytokine-producing cells. Removal of B cells by rituximab may have additional benefits in symptomatic cryoglobulinemia.

A major concern about the use of rituximab is the risk of infections. In patients with HCV-related cryoglobulinemia it is possible that a decrease in anti-HCV antibody levels after B-cell depletion might allow uncontrolled replication of the HCV. Further studies need to be performed to determine whether this theoretical risk is important in patients.

Case 39

Answer 1

Platelets are an acute-phase reactant and are elevated as part of a systemic inflammatory response. Just as inflammatory cytokines increase the production of other markers of inflammation such as C-reactive protein, cytokines lead to increased platelet release and production in the bone marrow.

Answer 2

6-Mercaptopurine (6-MP) is metabolized to 6-thioguanine, which inhibits the synthesis of purine nucleotides required for DNA and RNA synthesis. 6-MP is toxic to rapidly dividing cells, which require nucleic acid synthesis. Like rapidly dividing cancer cells, lymphocytes mediating autoimmune and inflammatory responses are proliferating rapidly and can be targeted by antimetabolite drugs.

Answer 3

Some patients treated with infliximab generate neutralizing antibodies against the mouse portion of the chimeric monoclonal antibody, recognizing the drug as a foreign antigen. Because adalimumab is a fully humanized protein, the problem of neutralizing antibodies is greatly diminished.

Answer 4

Lymphocytes use cell-adhesion molecules such as α_4 integrin to bind to its ligand, VCAM-1, expressed on endothelial cells. This causes lymphocyte arrest and enables them to migrate from the vasculature to sites of inflammation, to which they are attracted by chemokines. Inhibiting T-cell homing to the gut in Crohn's disease reduces the extent of inflammation and diminishes disease symptoms.

Case 40

Answer 1

The oligoclonality of the immunoglobulins in the cerebrospinal fluid reflects the activation of a limited number of B-cell clones that have gained entry into the central nervous system after the breakdown of the blood–brain barrier. Only those B cells that recognize antigen via their surface immunoglobulin receptor and receive a stimulatory signal from an activated T cell will proceed to synthesize and secrete immunoglobulins.

Answer 2

Corticosteroids and cyclophosphamide (a powerful cytotoxic drug) inhibit T-cell proliferation and thus interfere with the secretion of cytokines that

drive the inflammation and further T-cell activation. The mechanism of action of IFN-β is not known. More recently, a monoclonal antibody, natalizumab (Tysabri), that targets the α_4 integrin subunit has been reapproved in the United States for a restricted subset of patients with MS, after being withdrawn from the market in 2005 because three patients developed progressive multifocal leukoencephalopathy due to the JC virus. This drug is aimed at blocking the movement of leukocytes from the blood into sites of inflammation.

Answer 3

The patients got markedly worse. IFN-γ upregulates the expression of MHC class II molecules and thus enhances antigen presentation. In addition, it drives the differentiation of T_H1 cells, which are involved in the pathogenesis of MS.

Answer 4

Proteins eaten as part of food have long been known not to elicit routine immune responses. The reason seems to be that there are antigen-specific mechanisms in the gut for suppressing peripheral immune responses to antigens delivered by mouth. One is that when T cells in gut-associated lymphoid tissues are presented with orally delivered protein antigens in the absence of an infection, a lack of co-stimulatory signals induces the T cells to become anergic. Another involves the development of regulatory T cells (see Case 18), which can actively suppress antigen-specific responses after rechallenge with antigen. Such cells produce cytokines, including interleukin-4 (IL-4), IL-10, and TGF-β, which inhibit the development of T_H1 responses and are associated with low levels of antibody and virtually absent inflammatory T-cell responses. However, attempts to treat MS in humans by feeding the MBP antigen have proved unsuccessful.

Answer 5

EAE cannot be induced in mice lacking CD28. CD28 on T cells is the receptor for the B7 co-stimulatory molecules, which are essential for the activation of naive antigen-specific T cells, including T cells that recognize MBP. In contrast, mice in which the cell-surface protein CTLA-4, another receptor for B7 molecules, has been knocked out develop EAE more readily than their normal littermates. This is because CTLA-4 binds B7 molecules about 20 times more strongly than does CD28, and normally delivers an inhibitory signal to the activated T cell. In CTLA-4 knockout mice, this inhibitory signal is missing and so the T cells are more readily activated.

Case 41

Answer 1

A plasma exchange (plasmapheresis). In this procedure the patient's blood is repeatedly removed 300–500 ml at a time and the blood is centrifuged (in this case at 37°C, at which temperature the IgM antibodies would be eluted from the red blood cells). The cells are resuspended in normal plasma and infused back into the patient. This is a relatively efficient procedure for removing IgM antibodies because 70% of IgM is in the plasma compartment and only 30% of IgM is in the extravascular compartment. In contrast, only 50% of IgG is in the vascular compartment and 50% is in the extravascular space.

Answer 2

They usually do not cause anemia because they do not fix complement (C1q) and there are no Fc receptors for these immunoglobulin classes on cells of the macrophage lineage, which bear only Fcγ receptors. Fcε receptors are

expressed on mast cells and B cells, and their engagement would not result in the destruction of red blood cells.

Answer 3

The internal thiol ester that binds covalently to the IgM autoantibody is situated in the C3d and C4d fragments. The complement components C3b and C4b that are bound to the autoantibody may be digested by Factor I to C3c + C3d and C4c + C4d. C3c and C4c would be released from the antigen:antibody complex and thus antibodies against them would not agglutinate the red blood cells.

Answer 4

There is almost certainly no T-cell antigen receptor for this carbohydrate antigen, so that help to move the B cells into follicles would not occur and specific B cells would undergo apoptosis in the T-cell zone (41.4). Alternatively, the B cells might be rendered anergic by soluble antigen or undergo programmed cell death through the interaction of Fas and Fas ligand.

Although the precise mechanism of autoimmunization awaits elucidation, the molecular characterization of the autoantigen I and of the host cell adherence receptors for *Mycoplasma pneumoniae* have indicated collectively that the disorder has its origin in the interaction of the infective agent with carbohydrate attachment sites on host cells. The mycoplasma adheres to the ciliated bronchial epithelium, and also to red cells and a variety of other cells via ligands that consist of long carbohydrate chains of I antigen type that are capped with sialic acid (see Fig. 41.5). When bound to the carbohydrate chain that contains the I-antigen sequence, the mycoplasma may act as a carrier and the I antigen may act as a hapten.

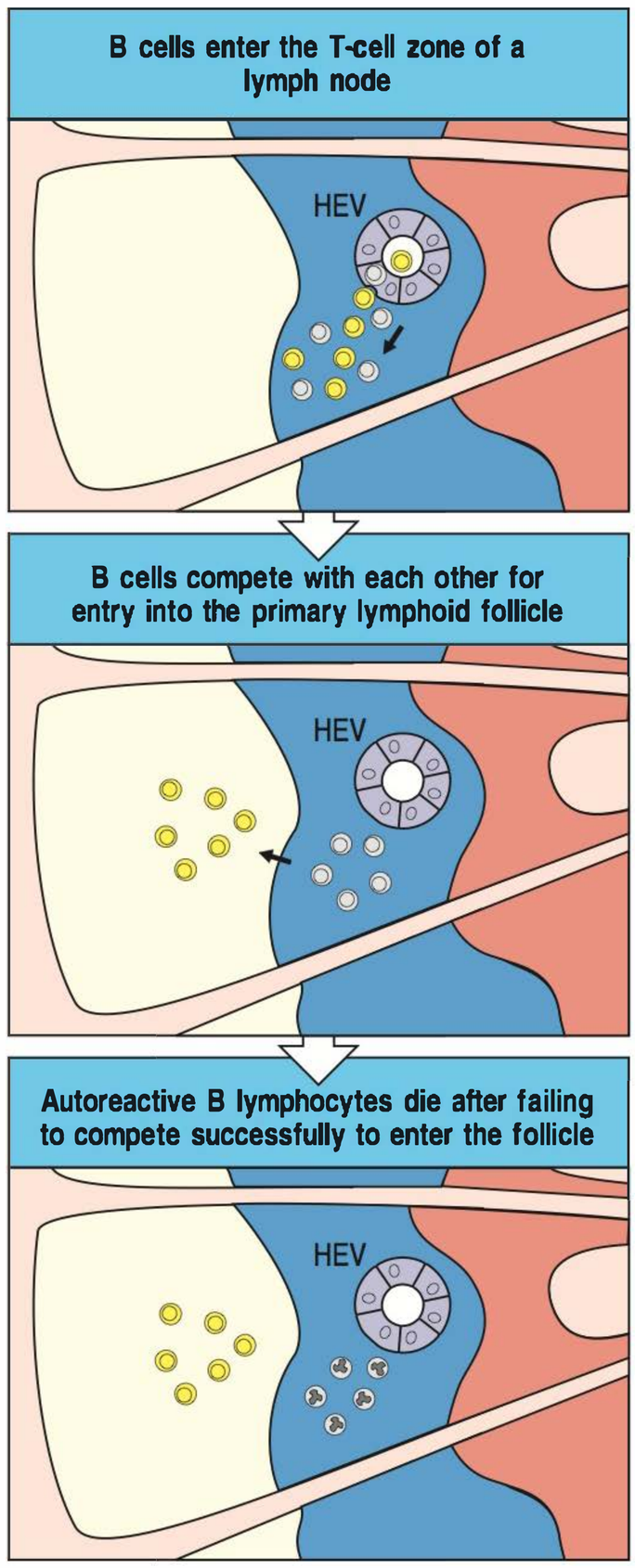


Fig. A41.4 Autoreactive B cells do not compete effectively to enter primary lymphoid follicles in peripheral lymphoid tissue. In the top panel, B cells are seen entering the T-cell zone of a lymph node through high endothelial venules (HEVs). Those with reactivity to foreign antigens are shown in yellow, and autoreactive cells are shown in gray. The autoreactive cells fail to compete with B cells specific for foreign antigens for exit from the T-cell zone and entry into primary follicles (middle panel). This is because B cells reactive to foreign antigens receive signals from antigen-specific T cells that promote their activation and survival. In contrast, the autoreactive B cells fail to receive survival signals and undergo apoptosis in the T-cell zone (bottom panel).

Case 42

Answer 1

It would be likely to last 1–2 weeks. The infant has the disease because maternal IgG antibodies against the acetylcholine receptor have crossed the placenta from the maternal circulation to the fetal circulation. The infant is not synthesizing these autoantibodies; he or she has acquired the disease passively by transfer of the antibodies (Fig. A42.1). The maternal IgG antibodies bind to the acetylcholine receptors in the baby, and the complex of the receptor with bound IgG antibodies is internalized into the cell and degraded. Within 10–15 days all the maternal IgG antibodies against the acetylcholine receptor are adsorbed from the babies' blood and the symptoms abate.

Answer 2

Azathioprine is an immunosuppressive agent. It is converted in the liver to 6-mercaptopurine, which inhibits DNA synthesis. Thus the growth of rapidly dividing cells, such as B cells and T cells, is inhibited and the immune response is suppressed. Unfortunately the effects of azathioprine are not specific. It suppresses not only the formation of antibodies against the acetylcholine receptor but also all other immune responses. Patients taking azathioprine become susceptible to infections. If used for very prolonged periods it is associated with the development of lymphomas. The reasons for this are not well understood.

Answer 3

Fig. A42.3 summarizes ways in which infectious diseases can break self tolerance and induce, or worsen, an autoimmune disease. An infectious agent may

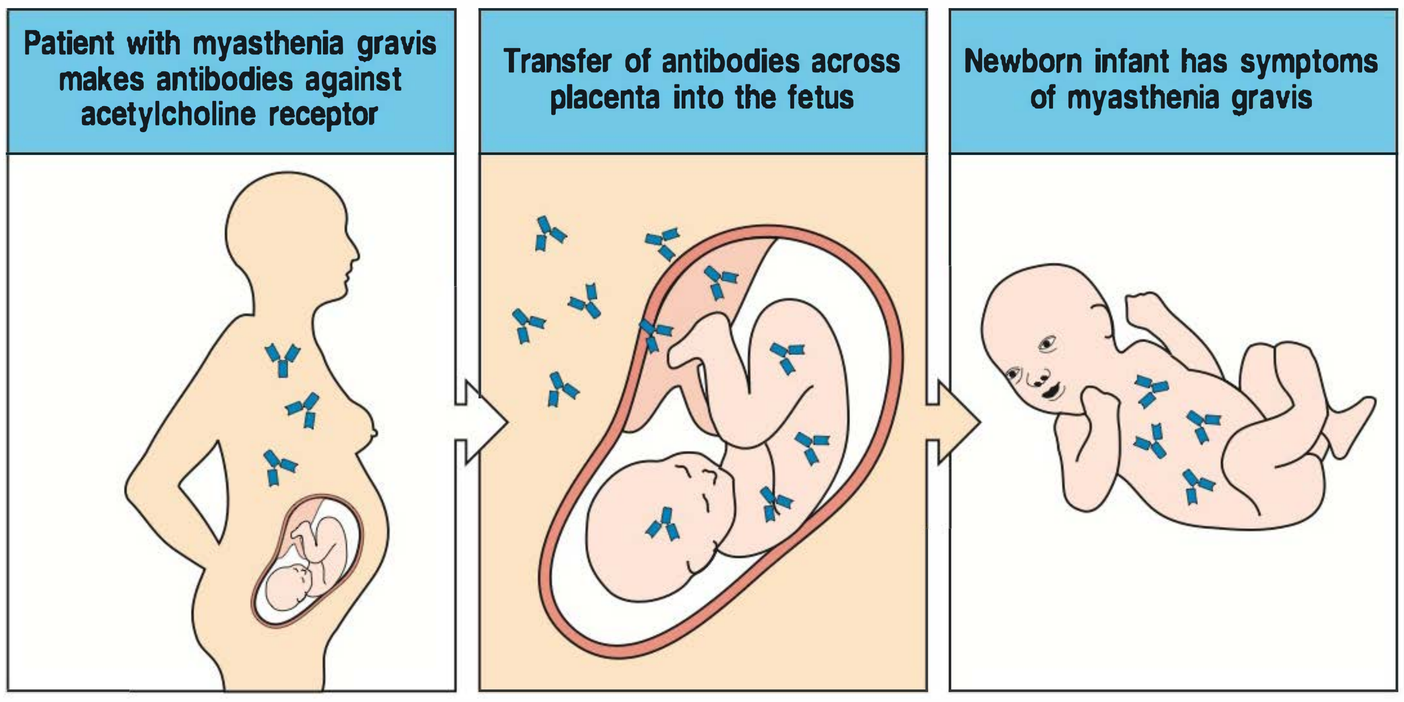


Fig. A42.1 Antibody-mediated autoimmune disease can appear in the infants of affected mothers as a consequence of transplacental transfer of IgG autoantibodies.

Fig. A42.3 Ways in which infectious agents can break self tolerance. Because some antigens are sequestered from the circulation, either behind a tissue barrier or within the cell, it is possible that an infection that breaks cell and tissue barriers may expose hidden antigens (first column). A second possibility is that the local inflammation in response to an infectious agent may trigger the expression of MHC molecules and co-stimulators on tissue cells, inducing an autoimmune response (second column). In some cases, infectious agents may bind to self proteins. Because the infectious agent induces a helper T-cell response, any B cell that recognizes the self protein will also receive help. Such responses should be self-limiting once the infectious agent has been eliminated, because at this point the T-cell help will no longer be provided (third column). Infectious agents may induce either T-cell or B-cell responses that can cross-react with self antigens. This is termed molecular mimicry (fourth column). T-cell polyclonal activation by a bacterial superantigen could overcome clonal anergy, allowing an autoimmune process to begin (fifth column). There is little evidence for most of these mechanisms in human autoimmune disease. EAE, experimental autoimmune encephalomyelitis.

expose hidden antigens, or may increase the expression of MHC molecules and co-stimulators on tissue cells so as to induce an autoimmune response. B ells already primed to make an autoantibody may receive help from nearby T cells activated by an infection, especially if pathogens become attached to self molecules. Pathogens may induce responses that cross-react with self molecules. Bacterial and viral superantigens can overcome clonal anergy and break tolerance to self antigens. At present, relatively little is known about the induction of human autoimmune disease, and there are only a few examples in which the evidence for any one of these mechanisms is strong.

Case 43

Answer 1

The monomeric IgG in the gamma globulin administered intravenously binds to high-affinity Fc receptors on macrophages and causes the release of immunosuppressive cytokines such as transforming growth factor-β (TGF-β), interleukin (IL)-10 and the IL-1 receptor antagonist. In patients with pemphigus vulgaris, clinical improvement after intravenously administered gamma globulin is noted in about 6 weeks, and decreased antibody titers are found after 6 months.

Answer 2

The reasons for this difference are not understood. We know that the IgG1 antibody reacts with a different epitope of desmoglein-3 from that targeted by the IgG4 antibody, but this in itself does not provide an explanation. IgG1

Mechanism	Disruption of cell or tissue barrier	Infection of antigen-presenting cell	Binding of pathogen to self protein	Molecular mimicry	Superantigen
Effect	Release of sequestered self antigen; activation of nontolerized cells	Induction of co-stimulatory activity on antigen-presenting cells	Pathogen acts as carrier to allow anti-self response	Production of cross-reactive antibodies or T cells	Polyclonal activation of autoreactive T cells
Example	Sympathetic ophthalmia	Effect of adjuvants in induction of EAE	? Interstitial nephritis	Rheumatic fever ? Diabetes ? Multiple sclerosis	? Rheumatoid arthritis

fixes complement, and thus we must infer that the proteinases generated by complement activation do not digest desmoglein-3.

Answer 3

Isotype switching is required to make the IgG4 antibodies in addition to or in lieu of the IgG1 antibodies. Switching to IgG4 is stimulated by the cytokine IL-4 released by activated T_H2 cells. If we take T cells from asymptomatic individuals who make IgG1 antibodies and T cells from patients who make IgG4 antibodies and stimulate them with desmoglein-3, we might find that the patients' T cells make more IL-4 (and thus undergo more isotype switching) than those of the asymptomatic individuals. Higher levels of IL-4 production in patients' T cells are indeed found in such an experiment.

Answer 4

Cyclophosphamide (Fig. A43.4) is an alkylating agent that interferes with DNA synthesis and therefore stops cell division. Although it has many bad side effects such as anemia, thrombocytopenia, and hair loss, it is effective in halting lymphocyte cell division, and thus suppresses immune reactions.

Answer 5

Pneumocystis jirovecii is an opportunistic pathogen that is a frequent cause of pneumonia in immunosuppressed patients. In this case, the high-dose corticosteroid treatment suppressed T-cell functions and trafficking, causing increased susceptibility to this potentially serious infectious agent.

Case 44

Answer 1

The 33-mer peptide has a predominance of proline residues, and this structure is crucial to its resistance to digestion by gastrointestinal enzymes. A bacterial propyl endopeptidase could potentially catalyze the breakdown of this peptide, thereby preventing it from interacting with TTG. Removing the 33-mer and its antigenic epitopes from the subepithelial space would eliminate the interaction of HLA DQ2-bound peptides with T cells and would halt the inflammatory process.

Answer 2

Testing is recommended in asymptomatic children who are in high-risk groups (type 1 diabetes mellitus, autoimmune thyroiditis, Down syndrome, Turner syndrome, Williams syndrome, IgA deficiency, and first-degree relatives of patients with celiac disease) starting at age 3 years, as long as they have not been put on a gluten-free diet. The reasoning behind testing asymptomatic children is that celiac disease can be clinically difficult to detect—but there is reason to believe that ongoing inflammation in the gut has deleterious effects and so it should be diagnosed and treated as early as possible.

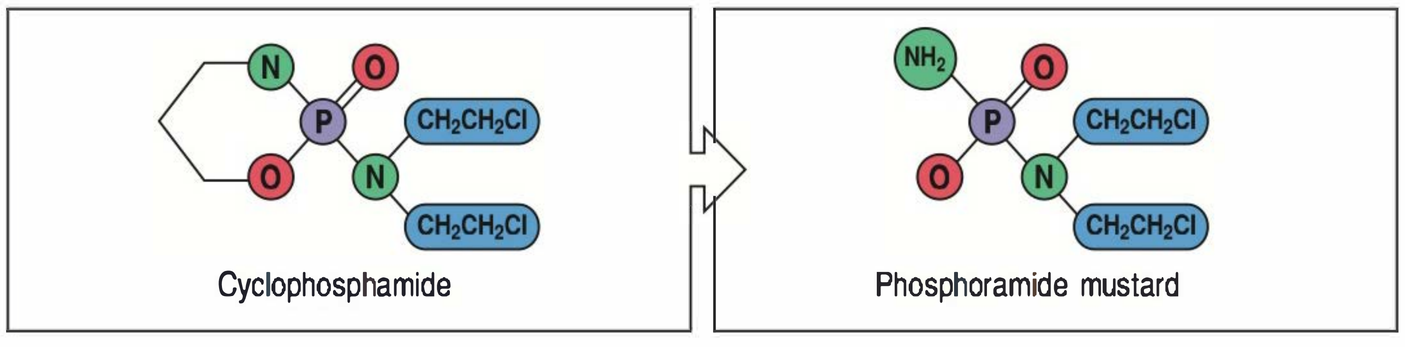


Fig. A43.4 The structure and metabolism of the cytotoxic drug cyclophosphamide. Cyclophosphamide is administered as a stable 'pro-drug', which is transformed enzymatically in the body to phosphoramide mustard, a powerful and unstable DNA alkylating agent.

An initial negative screen for celiac disease does not preclude its emergence later in some people. Repeat screening for type 1 diabetics and patients with Down syndrome and first-degree relatives with celiac disease should be performed periodically. The optimal frequency of such testing has not yet been clarified. An alternative to interval testing is to HLA type the high-risk individuals. In the absence of HLA DQ2 or DQ8, celiac disease is extremely rare.

Answer 3

A level of 220 p.p.m. gluten had been arbitrarily designated as 'gluten free,' but there is emerging evidence that even very small amounts of gluten are toxic to people with celiac disease and a new limit of 20 p.p.m. is being considered. The amount of gluten in foods is not the only challenge to patients; unclear labeling also poses a major obstacle to maintaining a gluten-free diet.

Answer 4

Oats do not contain gluten, but they can be cross-contaminated with wheat as a result of storage in silos that hold both types of grains. Patients newly diagnosed with celiac disease should not eat oats, but the grain can be reintroduced in small amounts once the disease is in remission.

Case 45

Answer 1

Cell-mediated cytotoxic activity against EBV-infected cells is the main method of controlling EBV replication. Although EBV-specific antibodies are produced in a normal infected host, they do not seem to have a major role in controlling the virus. Patients who lack the ability to produce specific antibody, but who have intact T-cell cytotoxic responses, are in most instances able to fight the infection effectively.

Answer 2

Bone marrow transplantation is most often performed in patients whose immune systems have either been destroyed with high doses of chemotherapy or in patients with primary immune deficiency. A major complication that can arise after a bone marrow transplant from a donor that is not fully HLA-matched to the recipient is graft-versus-host disease (GVHD; see Case 11). In GVHD, mature T lymphocytes in the bone marrow graft are activated and begin to attack the host's tissues. To prevent this, bone marrow grafts are often treated to remove mature T cells. If a bone marrow donor has been infected with EBV, they will have a small number of transformed B cells carrying the virus (about one in a million B cells). In the donor, these B cells are being 'held in check' by cytotoxic cells; there is an equilibrium between cell division and death of EBV-infected B cells. If mature T cells are removed from a marrow graft, but B cells are left, then the transformed B cells will escape from surveillance by cytotoxic T cells and might begin to proliferate at a high rate. Removing both mature B and T cells from donor bone marrow results in a lower rate of EBV lymphoproliferative disease after transplantation. An alternative explanation is that if a transplant recipient is infected with EBV, the destruction of their immune system before transplantation removes enough cytotoxic cells to tip the balance in favor of the donor transformed B cells.

Although treatment of EBV-related lymphoproliferative disease after transplantation is now largely based on the use of anti-CD20 monoclonal antibody (rituximab), the potential utility of adoptive immunotherapy has been also demonstrated. EBV-specific cytotoxic T cells are used directly from donor blood or can be expanded *in vitro* by culture with EBV-transformed donor cells. The cytotoxic T cells are then transfused into the transplant recipient

with lymphoproliferative disease and can kill the dividing donor B cells. This treatment has achieved remission of EBV lymphoproliferative disease in up to 90% of patients in various studies so far. The major complication of this treatment is acute or chronic GVHD.

Answer 3

Many adults who have been exposed to EBV in childhood have circulating in their blood effector cytotoxic T cells specific for EBV. When a blood sample is cultured and then infected with EBV, the B cells will become infected and the effector cytotoxic T cells will then immediately destroy the infected cells. Transformed B cells can be prepared from these individuals either by removing T cells from the blood sample before culture or by adding inhibitors of T-cell activation such as cyclosporin A. It is exceedingly unlikely that a fetus will have been infected by EBV, and so their blood sample is unlikely to contain any activated EBV-specific cytotoxic T cells.

Answer 4

EBV activates B cells polyclonally; that is, without respect to the antigen specificities of the infected cells. A significant percentage (5–10%) of circulating B lymphocytes bear antigen receptors of low affinity for several cross-reacting carbohydrate, nucleotide, or glycoprotein antigens. If infected with EBV and activated, these cells will begin to secrete polyspecific IgM antibodies. Many of these antibodies will bind relatively nonspecifically to erythrocytes of other species such as horse, ox, cow, or sheep and are thus called heterophile ('other-loving') antibodies. They are found in approximately 90% of patients with EBV.

Answer 5

These patients have no B cells, the cellular host of EBV.

Case 46

Answer 1

Most IgG antibody against the Rh antigen is of the IgG3 or IgG1 subclass; these are the IgG subclasses that bind most tightly to the high-affinity Fcγ receptor (FcγRI; CD64). Red blood cells coated with anti-Rh antibody adhere tightly to the Fc receptors of macrophages in the red pulp of the spleen, the Kupffer cells of the liver, and elsewhere (Fig. A46.1). The macrophages destroy the antibody-coated red cells (Fig. A46.2).

Answer 2

If the mother and father are ABO identical, the fetus has a high likelihood of also being ABO compatible with its mother. If fetal blood enters the maternal circulation, it is likely to last much longer if the mother has no ABO alloantibodies against the fetal cells. For example, if the fetal red blood cells were of type B, they would be quickly hemolyzed if the mother were of red blood cell type A; she would have anti-B alloantibodies. Rapid destruction of the fetal red blood cells by hemolysis would impede alloimmunization of the mother.

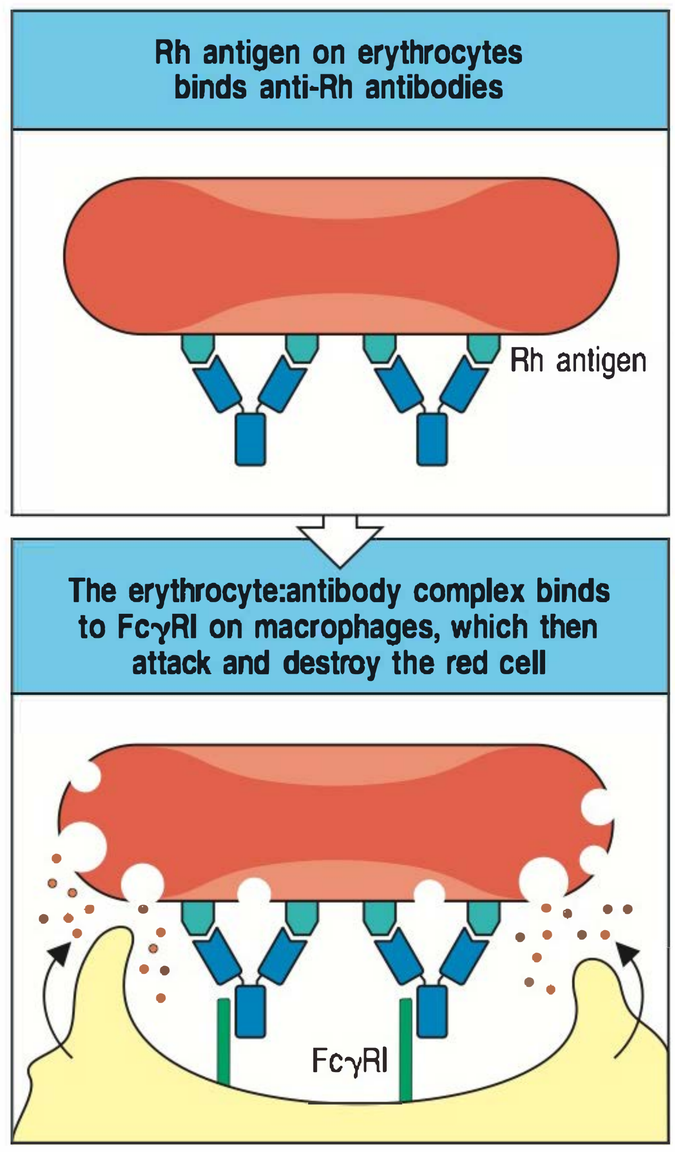


Fig. A46.1 IgG antibodies in a complex with their antigen bind via their Fc portions to the high-affinity Fcγ receptor on the surface of phagocytic cells. The Fc portions of IgG antibodies, including subclasses IgG3 and IgG1, bind the high-affinity Fc receptor FcγRI on macrophages when the antibody is complexed with an antigen (in this case a red blood cell). The binding of the Fc receptors helps to activate the macrophage, which then attacks the bound red cell.

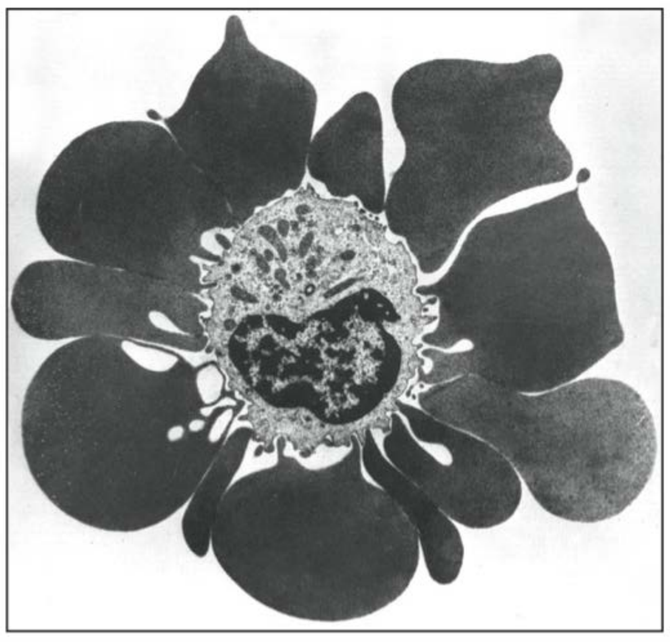


Fig. A46.2 A rosette of Rh-positive (Rh^+) red cells. The red cells are coated with anti-Rh antibodies, which adhere to the Fc receptors on a macrophage (central cell). The macrophage is pitting the red blood cells and destroying them. Photograph courtesy of J. Jandl.

Answer 3
Rh-negative cells were used because they cannot be hemolyzed by the IgG Rh antibodies that have crossed the placenta.

Answer 4
You shouldn't, because the amount given (300 μg of IgG) is insufficient to cause the fetus harm. This amount of antibody raises the maternal titer in the indirect Coombs test to less than 1:4, a titer of Rh antibody that cannot cause significant hemolysis in the fetus. If the maternal titer is more than 1:4 she has been alloimmunized by her fetus.

Answer 5
She has IgM anti-Rh antibodies; they agglutinate Rh-positive cells in saline, unlike IgG anti-Rh antibodies. Because IgM antibodies do not cross the placenta, you would have no immediate concern about her pregnancy. However, she should be tested repeatedly to be sure that she is not developing a positive Coombs test, signifying the presence of IgG antibodies.

Case 47

Answer 1
Superantigens, but not conventional antigens, can activate naive T cells. Superantigens will thus induce the proliferation of lymphocytes from neonates and from the thymus, because previous exposure to the antigen and expansion of the number of antigen-reactive cells is not required. Superantigens do not require processing by accessory cells and are thus able to induce the proliferation of purified T cells in the presence of paraformaldehyde-treated monocytes, which lack the capacity to process antigen. Direct binding of a labeled protein to cells positive for MHC class II, or its co-precipitation with MHC class II molecules, confirms it as a superantigen.

Answer 2
During the evolution of an adaptive immune response to conventional antigen, a cascade of events must occur over a relatively long period. The antigen has to be internalized, processed, and presented as peptide:MHC complexes by antigen-presenting cells. The complexes are recognized only by those T cells bearing a T-cell receptor specific for the antigen-derived peptides—a fraction of a percent (less than 0.1%) of the entire T-cell pool. These few antigen-specific T cells must then proliferate and bystander cells must be recruited before an effective response can be mounted. In contrast, superantigen-induced immune activation is independent of antigen processing, thus bypassing the first step, and immediately activates a sizeable fraction of T cells. A very small number of superantigen molecules is sufficient to activate a T cell with the appropriate V_β region in its receptor (fewer than 10 molecules per T cell). Activation results in a massive secretion of T-cell cytokines, which include IL-2, IFN-γ, TNF-α, and lymphotoxin. In addition, superantigens can directly activate monocytes and dendritic cells by cross-linking their surface MHC class II molecules. Cross-linking is effected by superantigens bound to T-cell receptor β chains and/or because a number of superantigens, including TSST-1, have two distinct binding sites for MHC class II molecules. Cross-linking of MHC class II molecules causes a rapid and massive release of cytokines such as IL-1, TNF-α, IL-6, CXCL8, and IL-12. This is associated with the upregulation of B7 co-stimulatory molecules on these cells, which, together with cytokine action, further amplifies T-cell activation by superantigen. Thus, minute amounts of superantigen are sufficient to rapidly activate a large number of T cells and monocytes/macrophages, resulting in an

amplification loop and in a massive outpouring of cytokines, which leads to the rapid appearance of clinical symptoms.

Answer 3

Liver injury may occur as a result of decreased organ perfusion during hypotension. However, immunologic mechanisms may also contribute to injury. Hepatocytes express Fas, a cell-surface molecule crucial for the induction of apoptosis (programmed cell death). T-cell activation by superantigens and the massive release of cytokines results in the upregulation of the natural ligand for Fas—FasL—on the surface of circulating lymphocytes. Cross-linking of Fas on hepatocytes by FasL on circulating lymphocytes results in the triggering of apoptosis in hepatocytes. In addition, circulating cytokines such as TNF-α are also capable of triggering cell death and can result in liver injury.

Answer 4

Protection against toxic shock is conferred by antibodies against the superantigen, which neutralize it before it can cause disease. To stimulate an antibody response, the superantigen must be recognized, internalized, and processed by superantigen-specific B cells, which then present the antigenic peptides to antigen-specific T cells. These are activated to become helper T cells that can in turn stimulate the production of superantigen-specific antibodies on reexposure to the superantigen. Antibodies against other antigens that cross-react with the superantigen may also confer protection.

In humans, there is evidence that during and after TSST-associated illness, $V_\beta 2$ T cells become anergic and thus cannot provide help to superantigen-specific B cells. Patients with TSS therefore fail to develop TSST-1-specific antibody. So Claire is, unfortunately, likely to be at risk of another episode of TSS. Hopefully, she will eventually develop anti-TSST-1 antibodies.

Case 48

Answer 1

The absence of delayed-type hypersensitivity to a wide range of antigens unrelated to *M. leprae* is called anergy. This should not be confused with T-cell or B-cell anergy, although it might operate by similar mechanisms. In tuberculoid leprosy, there is a strong delayed-type hypersensitivity to *M. leprae* and no anergy. The existence of anergy in the lepromatous form of leprosy but not in the tuberculoid form is most probably due to the presence of regulatory CD8 T cells in lepromatous leprosy. The CD8 T cells secrete the cytokines IL-10 and LT and thereby suppress antigen presentation by macrophages. These cytokines not only influence the T_H1 versus T_H2 phenotype, as discussed in the Case, but can also suppress T-cell responses to other unrelated antigens. IL-10 and LT suppress not only the *M. leprae*-specific T cells but also neighboring T cells, leading to global hyporesponsiveness, which was manifested in Ursula's case as anergy to candida and mumps antigens. However, Ursula's case is somewhat atypical; in many patients with lepromatous leprosy the unresponsiveness is confined to *M. leprae*, and responses are made to other antigens. Other pathogens use the IL-10 pathway to produce anergy; the Epstein–Barr virus, for example, produces a viral protein, vIL-10, that is homologous to human IL-10. Measles virus induces anergy by binding to CD46 on monocytes and inhibiting their production of IL-12.

Answer 2

The immune response in patients with lepromatous leprosy is skewed toward the T_H2 phenotype, leading to a disseminated infection. Because tuberculoid

leprosy involves a T_H1 response and significantly reduced symptoms, we would like to switch the response to the T_H1 phenotype. Cytokines with the potential to inhibit T_H2 and induce a T_H1 response are IL-2, IFN-γ, and IL-12. Local injection of IFN-γ has been shown to lead to partial reversal of anergy and reduction of lesions. IFN-γ has also been shown to be effective in the treatment of similar diseases, such as leishmaniasis. In the visceral form of leishmaniasis, the T-cell response is also skewed to the T_H2 phenotype. This is in contrast to the cutaneous form of leishmaniasis, which is accompanied by a T_H1 response. IL-12 might also be beneficial, because it can induce T_H1 cells and does not activate T_H2 cells.

Answer 3

In lepromatous leprosy, a humoral immune response driven by T_H2 cells predominates, with vigorous antibody production, leading to hypergammaglobulinemia as observed in Ursula. The cytokines produced in T_H2 responses lead to enhanced immunoglobulin production. IL-4 induces isotype switching to IgE and increased production of IgG4 and IgE. IL-10 stimulates the production of IgG1 and IgG3, whereas IL-5 stimulates immunoglobulin production globally. It is therefore not uncommon to find hypergammaglobulinemia in patients who are producing a vigorous T_H2 response to an antigen.

Answer 4

Ursula's T_H2 response to *M. leprae* leads to increased production of IL-4 and IL-10. When she encounters a new antigen, her immune system will be awash with IL-4, triggering a T_H2 response to that antigen. This T_H2 response with its associated IL-4 production leads to an IgE response. Because asthma and other atopic diseases are T_H2-driven diseases involving IgE production, Ursula has a higher risk of developing asthma.

Case 49

Answer 1

John's hoarseness resulted from angioedema of the vocal cords. His wheezing was due to forced expiration of air through bronchi that had become constricted. In this case, constriction resulted from the release by activated mast cells of histamine and leukotrienes that caused the smooth muscles of the bronchial tubes to constrict.

Answer 2

John's parents were instructed to avoid feeding him any food containing peanuts and to read the labels of packaged foods scrupulously to avoid anything containing peanuts. They were advised to inquire in restaurants about food containing peanuts. Because green peas, also a legume, contain an antigen that cross-reacts with peanuts and might also incite an anaphylactic reaction, peas were withdrawn from John's diet. A Medi-Alert bracelet, indicating his anaphylactic reaction to peanuts, was ordered for John. The parents were also given an Epi-Pen syringe pre-filled with epinephrine to keep at home or while traveling, in case John developed another anaphylactic reaction.

Answer 3

Epinephrine acts at β_2-adrenergic receptors in smooth muscle surrounding blood vessels and bronchi. It has opposing effects on the two types of muscle. It contracts the muscle surrounding the small blood vessels, thereby constricting them, stopping vascular leakage, and raising the blood pressure. It relaxes that of the bronchi, making breathing easier.

Answer 4
Histamine and tryptase are released by activated mast cells; high levels in the blood indicate the massive release from the mast cells that occurs during an anaphylactic reaction.

Answer 5
Immediately after a systemic anaphylactic reaction the patient is unresponsive in a skin test owing to the massive depletion of mast-cell granules and failure of the blood vessels to respond to mediators. This is called tachyphylaxis and lasts for 72–96 hours after the anaphylactic reaction. For this reason, John had to come back to the Allergy Clinic a few days later for his tests.

Answer 6
Increased incidence of peanut allergy has been related to the increasing topical use of peanut-oil-based creams to treat dry skin in infants. Although peanut oil is not the culpable allergen, it is often contaminated by allergenic peanut proteins.

Answer 7
It has been shown that depletion of IgE by the administration of a humanized mouse anti-human IgE antibody that binds circulating IgE, but not mast-cell-bound IgE, results in protection from peanut anaphylaxis. This therapy works because it results in the eventual depletion of mast-cell-bound IgE, which is in equilibrium with serum IgE. It is safe because the anti-IgE antibody does not trigger mast-cell activation.

Case 50

Answer 1
During inspiration, the negative pressure on the airways causes their diameter to increase, allowing an inflow of air. During expiration, the positive expiratory pressure tends to narrow the airways. This narrowing is exaggerated when the airway is inflamed and bronchial smooth muscle is constricted, as in asthma. This causes air to be trapped in the lungs, with an increase in residual lung volume at the end of expiration. Breathing at high residual lung volume means more work for the muscles and increased expenditure of energy; this results in the sensation of tightness in the chest. The high residual lung volume is also the cause of the hyperinflated chest observed on the chest radiograph. The peribronchial inflammation in asthma causes bronchial marking around the airways.

Answer 2
Chronic allergic asthma is not simply due to constriction of the smooth muscles that surround the airway: it is largely due to the inflammatory reaction in the airway, which consists of cellular infiltration, increased secretion of mucus, and swelling of the bronchial tissues. This explains the failure of bronchodilators, which dilate smooth muscles, to maintain an open airway and their failure to completely reverse the decreased air flow during Frank's acute attacks. Steroids are therefore given to combat the inflammatory reaction of the late-phase response.

Answer 3
Allergic individuals have a tendency to respond to allergens with an immune response skewed to the production of T_H2 cells rather than T_H1. The cells produce the interleukins IL-4 and IL-13, cytokines that induce IgE production in humans. T_H2 cells also make IL-5, which is essential for eosinophil maturation.

Furthermore, activated T cells and bronchial epithelial cells secrete CCL11 (formerly known as eotaxin), which attracts eosinophils in the airways. The production of IL-4 and IL-5 by T_H2 cells responding to allergens in atopic individuals explains the frequent association of IgE antibody response and eosinophilia in these patients.

Answer 4

IgE-mediated hypersensitivity to an allergen is tested for by injecting a small amount of the allergen intradermally. In allergic individuals, this is followed within 10–20 minutes by a wheal-and-flare reaction at the site of injection (see Fig. 50.5), which subsides within an hour. The wheal-and-flare reaction is due mainly to the release of histamine by mast cells in the skin. This increases the permeability of blood vessels and the leakage of their contents into the tissues, resulting in the swollen wheal; dilation of the fine blood vessels around the area produces the diffuse red 'flare' seen around the wheal. This reaction is almost completely inhibited by antagonists of the histamine type 1 receptor, the major histamine receptor expressed in the skin.

Answer 5

The recurrence of the redness and swelling at the site of previous immediate allergic reactions represents the late-phase response characterized by a cellular infiltrate.

Answer 6

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen can induce wheezing in certain patients. This is classically seen in patients with Sampter's triad: asthma, nasal polyps, and NSAID sensitivity. NSAIDs inhibit the enzyme cyclooxygenase (COX). Normally, the actions of COX lead to the synthesis of prostaglandins from arachidonic acid. COX inhibition leads to shunting of the arachidonic acid precursor away from prostaglandin synthesis and into the leukotriene synthesis pathway (see Fig. 50.7). The increased leukotriene biosynthesis leads to bronchial smooth muscle constriction and cell proliferation, plasma leakage, mucus hypersecretion, and eosinophil migration, culminating in symptoms of wheezing and asthma exacerbation. Leukotriene E4 levels can be measured in the urine. In patients with aspirin sensitivity, E4 levels are higher at baseline and rise an additional fivefold after aspirin ingestion before returning to baseline as the aspirin-induced wheezing resolves.

Answer 7

Repeated administration of relatively high doses of allergen by subcutaneous injection is thought to favor antigen presentation by antigen-presenting cells that produce IL-12. This results in the induction of T_H1 cells rather than T_H2 cells. The presence of T_H1 cells tends to lead to an IgG antibody response rather than an IgE response because the T_H1 cells produce IFN-γ, which prevents further isotype switching to IgE. The IgG antibody competes with the IgE antibody for antigen. Furthermore, IgG bound to allergen inhibits mast-cell activation (via FcεRI) and B-cell activation (via surface immunoglobulin) by allergen because of inhibitory signals delivered subsequent to the binding of Fcγ receptors on these cells. This is thought to be one mechanism damping down the allergic response. Another is no further boosting of IgE production because IL-4 and IL-13 are not secreted. Existing IgE levels themselves may not fall by much, because IFN-γ does not affect B cells that have already switched to IgE production.

Answer 8

Most human allergy is caused by a limited number of inhaled protein allergens that elicit a T_H2 response in genetically predisposed individuals. These

allergens are relatively small, highly soluble protein molecules that are presented to the immune system by the mucosal route at very low doses. It has been estimated that the maximum exposure to ragweed pollen allergens is less than 1 μg per year. It seems that transmucosal presentation of very low doses of allergens favors the activation of IL-4-producing T_H2 cells and is particularly efficient at inducing IgE responses. The dominant antigen-presenting cell type in the respiratory mucosa expresses high levels of co-stimulatory B7.2 molecules. Expression of B7.2 on antigen-presenting cells is thought to favor the development of T_H2 cells. In contrast, injection of antigen subcutaneously in large doses, as occurs on vaccination, results in antigen uptake in the local lymph nodes by a variety of antigen-presenting cells and favors the development of T_H1 cells, which inhibit antibody switching to IgE.

Case 51

Answer 1

Corticosteroids bind to steroid receptors in inflammatory cells such as T cells and eosinophils. The steroid:receptor complex is translocated into the nucleus, where it can control gene expression, including the expression of cytokine genes, by binding to control elements in the DNA. In addition, corticosteroids increase the synthesis of the inhibitor of the transcription factor NFκB, which controls the expression of multiple cytokine genes. One effect is to inhibit the synthesis of cytokines and the release of preformed mediators and arachidonic acid metabolites. Although topical steroids are very effective, excessive or prolonged use of powerful steroids can lead to local skin atrophy.

Answer 2

The immunosuppressant cyclosporin A acts primarily on T cells and interferes with the transcription of cytokine genes. The drug binds to an intracellular protein, cyclophilin, and this complex in turn inhibits calcineurin, which normally dephosphorylates nuclear factor of activated T cells (NFAT), a major cytokine gene transcription factor. FK506, or tacrolimus, is another immunosuppressant with a spectrum of activity similar to that of cyclosporin. Tacrolimus binds to the cytoplasmic protein FK506-binding protein, and this complex also inhibits calcineurin. Tacrolimus has a smaller molecular size and higher potency than cyclosporin A and, perhaps because of these features, it seems to be effective as a topical formulation.

Answer 3

Patients with atopic dermatitis have defective local innate cell-mediated immunity, which is required for the control of herpesvirus and vaccinia virus infections: T_H2 cytokine expression in the affected skin inhibits the production of antimicrobial peptides by keratinocytes. Cell-mediated adaptive immune responses involve T_H1 CD4 cells and CD8 cytotoxic cells; patients with atopic dermatitis have selective activation of T_H2 rather than T_H1 cells, as shown by their reduced delayed-type hypersensitivity skin reactions. They also have decreased numbers and function of CD8 cytotoxic T cells. Furthermore, monocytes from patients with atopic dermatitis secrete increased amounts of IL-10 and prostaglandin E_2 (PGE_2). Both IL-10 and PGE_2 inhibit the production of the T_H1 cytokine IFN-γ, and IL-10 also inhibits T-cell-mediated reactions.

Answer 4

Scratching causes tissue damage that stimulates the keratinocytes to secrete cytokines and chemokines (IL-1, IL-6, CXCL8, GM-CSF, and TNF-α). IL-1 and TNF-α induce the expression of adhesion molecules such as E-selectin, ICAM-1, and VCAM-1 on endothelial cells, which attract lymphocytes, macrophages, and eosinophils into the skin. These infiltrating cells secrete cytokines

and inflammatory mediators that perpetuate keratinocyte activation and cutaneous inflammation.

Answer 5

The skin of more than 90% of patients with atopic dermatitis is colonized by *Staphylococcus aureus*. Recent studies suggest that *S. aureus* can exacerbate or maintain skin inflammation in atopic dermatitis by secreting a group of toxins known as superantigens, which cause polyclonal stimulation of T cells and macrophages (see Case 47). T cells from patients with atopic dermatitis preferentially express T-cell receptor β chains $V_\beta 3$, 8, and 12, which can be stimulated by staphylococcal superantigens, resulting in T-cell proliferation and increased IL-5 production. Staphylococcal superantigens can also induce expression of the skin homing receptor (CLA) in T cells, which is mediated by IL-12. In addition, nearly half of patients with atopic dermatitis produce IgE directed against staphylococcal superantigens, particularly SEA, SEB, and toxic shock syndrome toxin-1 (TSST-1). Basophils from patients with atopic dermatitis who produce antitoxin IgE release histamine on exposure to the relevant toxin. These findings suggest that local production of staphylococcal exotoxins at the skin surface could cause IgE-mediated histamine release and thereby trigger the itch–scratch cycle that exacerbates the eczema.

Answer 6

A mouse model of atopic dermatitis suggests that sensitization directly through the skin can result in allergen-induced asthma. In this model, patch application of allergen to the shaved skin of a normal mouse results in an eczematous dermatitis and subsequent allergen-specific airway hypersensitivity such that exposure to allergen by inhalation causes airway hyperresponsiveness typical of the asthmatic state.

There is epidemiologic evidence that sensitization of infants to food allergens through the skin may predispose to food allergy, and that unlike oral exposure to food allergens it does not induce tolerance, but rather results in IgE antibody formation that can cause anaphylaxis.

Case 52

Answer 1

The symptoms were caused by the activation of complement-generated C3a, which releases histamine from mast cells and causes hives. The swelling around the mouth and eyelids is a form of angioedema. There is a more complete discussion of the role of the complement and the kinin systems in the pathogenesis of angioedema in Case 31.

Answer 2

Gregory almost certainly had developed vasculitis in the small blood vessels of his brain, and this compromised oxygen delivery to his brain.

Answer 3

He had red cells and albumin in his urine, which indicated an inflammation of the small blood vessels in his kidney glomeruli. He also developed purpura in his feet and ankles. Purpura (which is the Latin word for purple) indicates hemorrhage from small blood vessels in the skin that are inflamed and have become plugged with clots. A skin biopsy of one lesion showed the deposition of IgG and C3 around the small blood vessels, suggesting that an immune reaction was taking place.

Answer 4
You would expect to see massive follicular hyperplasia, polyclonal B-cell activation, and many mature plasma cells in the medulla. The massive B-cell activation in the lymph nodes leads to an overflow of plasma cells from the medulla of the nodes into the efferent lymph. It is otherwise very, very rare to find plasma cells in the blood, as were found in Gregory's blood. They find their way to the bloodstream via the thoracic duct. The enlargement of the spleen was almost certainly due to hyperplasia of the white pulp. Some plasma cells probably enter the blood from the hyperplastic follicles in the spleen.

Answer 5
The acute-phase reaction is caused by interleukin (IL)-1 and to a greater extent by IL-6, which are released from monocytes that have been activated by the uptake of immune complexes. The acute-phase response consists of marked changes in protein synthesis by the liver. The synthesis of albumin drops sharply, as does the synthesis of transferrin. The synthesis of fibrinogen, C-reactive protein, amyloid A, and several glycoproteins is rapidly upregulated. The precise advantage to the host of the acute-phase reaction is not well understood, but it is presumably a part of innate immunity, which aids host resistance to pathogens before the adaptive immune system becomes engaged.

Answer 6
His serum C1q level was decreased. This almost always indicates complement consumption by immune complexes via the classical pathway. (In hereditary angioedema (see Case 31) the C1q level is normal; in this disease, complement is activated because of a defect in an inhibitor and not by the formation of immune complexes.) The level of C3 in Gregory's serum was also lowered, a further indication of complement consumption (see Case 32).

Answer 7
No! The skin test is positive when there are IgE antibodies bound to the mast cells in the skin. Gregory did not have IgE antibodies against penicillin, as confirmed by the negative RAST test. Serum sickness is caused by complement-fixing IgG antibodies.

Case 53

Answer 1
Pentadecacatechol can be transferred from the initial point of contact to other areas of the skin by the fingernails after scratching the itchy lesion at the primary site of hapten introduction. This is why it is essential to cut the fingernails short and thoroughly wash off the skin and scalp to remove the chemical and prevent further spread.

Answer 2
The half-life of some of the proteins haptenated by pentadecacatechol can be quite long. CD4 memory T cells will continue to be activated as long as the haptenated peptides are being generated. In Paul's case this went beyond the third week after contact with poison ivy.

Answer 3
Once an individual has been sensitized, the reaction often becomes worse with each exposure, as each reexposure not only produces the hypersensitivity reaction but generates more effector and memory T cells. Memory T cells that mediate delayed hypersensitivity reactions, such as contact sensitivity

Antigen	Consequence
Delayed-type hypersensitivity	
Proteins: Insect venom Mycobacterial proteins (tuberculin, lepromin)	Local skin swelling: Erythema Induration Cellular infiltrate Dermatitis
Contact hypersensitivity	
Haptens: Pentadecacatechol DNFB Metal ions: Nickel Chromate	Local epidermal reaction: Erythema Cellular infiltrate Contact dermatitis
Gluten-sensitive enteropathy (celiac disease)	
Gliadin	Villous atrophy in small bowel Malabsorption

Fig. A53.7 Some type IV hypersensitivity reactions. Depending on the source of antigen and its route of introduction, these clinical conditions have different names and consequences. DNFB, dinitrofluorobenzene.

to poison ivy and the tuberculin test, can persist for most of the life of the individual.

Answer 4

You could perform a patch test. In this test a patch of material impregnated with the hapten is applied to the skin under seal for 48 hours. The area is then examined for redness, swelling, and vesicle formation. Alternatively, peripheral blood mononuclear cells can be incubated with the hapten and T-cell proliferation assessed 6–9 days later.

Answer 5

The risk of Brian's developing poison ivy sensitivity is at least as high as that for a normal child. This is because antibody plays no discernible role in the genesis of delayed hypersensitivity reactions, and T-cell function is normal in X-linked agammaglobulinemia. In fact, clinical observations suggest that boys with X-linked agammaglobulinemia may develop more severe forms of poison ivy sensitivity. It has been suggested that, in the absence of antibody, more hapten is available for conjugation with self proteins and that, in the absence of antigen presentation by β cells, the T-cell response is skewed more towards T_H1 cells.

Answer 6

The artificially induced tuberculin reaction is a good model of a delayed hypersensitivity reaction. This skin test detects infection with the bacterium *Mycobacterium tuberculosis*, or previous immunization against tuberculosis with the live attenuated vaccine BCG. Small amounts of tuberculin, a protein derived from *M. tuberculosis*, are injected subcutaneously; a day or two later, a sensitized person develops a small, red, raised area of skin at the site of injection. In countries where BCG is administered routinely to babies, the tuberculin test can be used to test for T-cell function. This is because antigen-specific memory T cells are long-lived, and the sensitivity to tuberculin will persist throughout life. In the USA, children are not immunized with BCG. However, they all receive a full course of diphtheria and tetanus vaccines, which in each case contain purified protein toxoids as the antigen. Contact sensitivity to these two antigens can be used to test T-cell function. Alternatively, antigen derived from the yeast-like fungus *Candida albicans*, which is a normal inhabitant of the body flora, can be used to induce a delayed hypersensitivity reaction in the skin.

Answer 7

Some of the commoner environmental causes of delayed hypersensitivity reactions are insect bites or stings, which introduce insect venom proteins under the skin, and skin contact with chemicals in the leaves of some plants, or with metals such as nickel, beryllium, and chromium (Fig. A53.7). Nickel sensitivity is quite common and often occurs at the site of contact with nickel-containing jewelry. Contact sensitivity to beryllium has been well documented in factory workers engaged in manufacturing fluorescent light bulbs. Celiac disease (see Case 44) is a type of delayed sensitivity reaction seen in people who are allergic to the protein gliadin, a constituent of wheat grains and flour. Patients with celiac disease therefore have to avoid all food products containing wheat flour.