Review Article

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Editors

THE IMMUNE SYSTEM

First of Two Parts

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THE immune system is an organization of cells and molecules with specialized roles in defending against infection. There are two fundamentally different types of responses to invading microbes. Innate (natural) responses occur to the same extent however many times the infectious agent is encountered, whereas acquired (adaptive) responses improve on repeated exposure to a given infection. The innate responses use phagocytic cells (neutrophils, monocytes, and macrophages), cells that release inflammatory mediators (basophils, mast cells, and eosinophils), and natural killer cells. The molecular components of innate responses include complement, acute-phase proteins, and cytokines such as the interferons. Acquired responses involve the proliferation of antigen-specific B and T cells, which occurs when the surface receptors of these cells bind to antigen. Specialized cells, called antigen-presenting cells, display the antigen to lymphocytes and collaborate with them in the response to the antigen. B cells secrete immunoglobulins, the antigen-specific antibodies responsible for eliminating extracellular microorganisms. T cells help B cells to make antibody and can also eradicate intracellular pathogens by activating macrophages and by killing virally infected cells. Innate and acquired responses usually work together to eliminate pathogens.

All these cells develop from pluripotent stem cells in the fetal liver and in bone marrow and then circulate throughout the extracellular fluid. B cells reach maturity within the bone marrow, but T cells must travel to the thymus to complete their development.

Adaptive immune responses are generated in the lymph nodes, spleen, and mucosa-associated lymphoid tissue. These are referred to as the secondary lymphoid tissues. In the spleen and lymph nodes, the activation of lymphocytes by antigen occurs in distinctive B- and T-cell compartments of lymphoid tissue. A striking morphologic feature of the B-cell area is the secondary follicle containing the germinal center, where B-cell responses occur within a meshwork of follicular dendritic cells. The mucosa-associated lymphoid tissues, including the tonsils, adenoids, and Peyer's patches, defend mucosal surfaces. Diffuse collections of lymphoid cells are present throughout the lung and the lamina propria of the intestinal wall.

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THREE LEVELS OF DEFENSE

To establish an infection, the pathogen must first overcome numerous surface barriers, such as enzymes and mucus, that either are directly antimicrobial or inhibit attachment of the microbe. Because neither the keratinized surface of skin nor the mucus-lined body cavities are ideal habitats for most organisms, microbes must breach the ectoderm. Any organism that breaks through this first barrier encounters the two further levels of defense, the innate and acquired immune responses.

IMMUNE RECOGNITION

The body can potentially respond to almost anything that can be bound by the receptors of either the innate or the acquired immune system. Molecules recognized by receptors on lymphocytes are generically referred to as antigens and can range from small chemical structures to highly complex molecules. Both the T-cell receptor and the antibody that is embedded in the B-cell membrane, the B-cell receptor, have binding sites[1,2] that are only 600 to 1700 Å^2. Therefore, these receptors recognize only a small part of a complex antigen, referred to as the antigenic epitope. For these reasons, complex antigens consist of a mosaic of individual epitopes.

Antigens that elicit immune responses are termed immunogens. Not all antigens are naturally immunogenic. Small, nonimmunogenic antigens are called haptens and must be coupled to larger immunogenic molecules, termed carriers, to stimulate a response.[3] Large protein antigens usually contain epitopes equivalent to carriers and haptens and are therefore inherently immunogenic. Carbohydrates, by contrast, must often be coupled to proteins in order to be immunogenic, as is the case for the polysaccharide antigens used in the *Haemophilus influenzae* type b vaccine. Even large protein antigens with adequate numbers of carrier epitopes can be made more immunogenic by combining them with an adjuvant — a substance

GLOSSARY

Allele: An alternative form of a gene.

Allogeneic: Genetically dissimilar individuals of the same species; usually used in the context of organ or cell transplantation (i.e., allografts).

Allotypes: Antigenic determinants that differ among individuals of the same species, such as the epitopes of the Rh blood group system or epitopes of the HLA system.

Anergy: A potentially reversible form of immunologic tolerance in which lymphocytes become functionally unresponsive.

Antibody-dependent cellular cytotoxicity (ADCC): The killing of antibody-coated target cells by Fc receptor–bearing leukocytes, including natural killer cells, macrophages, and neutrophils.

Apoptosis: A specific form of cell death mediated by enzymatic degradation of DNA and that, in contrast to necrosis, is not associated with signs of inflammation. Also called programmed cell death.

B1 cells: A minor population of B lymphocytes that secrete polyspecific low-affinity IgM antibodies. Most express CD5 on their cell surface and may be self-renewing.

B2 cells: The chief population of B lymphocytes, B2 cells arise from stem cells in the bone marrow, do not express CD5, and secrete highly specific antibody within the secondary lymphoid tissues.

CD antigen: Cell-surface antigens are classified according to the cluster of differentiation (CD), in which individual molecules are assigned a CD number on the basis of their reactivity to panels of monoclonal antibodies.

Chemokine: Chemotactic cytokines that regulate the transit of leukocytes from blood into tissues. Each type of leukocyte (e.g., neutrophil, lymphocyte, and eosinophil) bears chemokine receptors that guide it to particular chemokines in the tissue.

Clone: A group of genetically identical cells with a common ancestor.

Complementarity-determining region (CDR): The surface of the variable region of an antibody or a T-cell receptor that binds to antigen. The CDR consists of three subregions: CDR1, CDR2, and CDR3. Also known as the hypervariable region.

Costimulatory molecule: A molecule that provides additional ("second") signals for lymphocyte activation beyond those provided through the antigen receptor.

Cytokines: A large family of low-molecular-weight soluble proteins involved in regulating cellular activity, particularly (but by no means exclusively) within the immune system.

Cytotoxic T cell: A T lymphocyte (which usually expresses CD8) that kills its target cell on recognizing complexes of peptides and major-histocompatibility-complex molecules on the target-cell membrane.

Epitope: The structure on the antigen that is recognized by an antigen receptor (antibody or T-cell receptor).

Gene library: A collection of cloned genes from which individual genes of interest can be selected (for example, by antibody screening after the expression of the gene in bacteria).

Germ line: The genetic material carried by ova and sperm. The germ line contains the genes that parents transmit to their offspring.

Graft-versus-host disease: The consequence of an immune reaction of transplanted allogeneic lymphocytes (usually contained in a bone marrow graft) against alloantigens of the recipient (host).

Haplotype: Closely linked alleles on a single chromosome that are usually inherited as a group and determine a particular phenotype.

Helper T cell: A T lymphocyte (which usually expresses CD4) that secretes the various cytokines required for the functional activity of other cells in the immune system.

Idiotype: An antigenic determinant within the binding site of an antibody that is recognized by another antibody.

Immunologic memory: The ability of the immune system to recall an encounter with a specific antigen and to mount a quantitatively and qualitatively superior secondary immune response on reencountering the antigen, a process that involves the generation of memory T and B cells during the primary immune response.

Intrinsic affinity: The binding strength between a receptor (e.g., one Fab arm of an antibody) and a ligand (e.g., an antigenic epitope).

Isotypes: Antigenic determinants of immunoglobulin heavy chains that define classes of immunoglobulins, such as IgM and IgE, and subclasses, such as IgG1 and IgG2.

Knockout mouse: A mouse in which a particular gene has been intentionally deleted through homologous recombination.

Locus: The site or location of a gene in a chromosome.

Natural antibody: Antibody that occurs naturally without apparent antigenic stimulation from an infection or immunization. Often, these antibodies are polyspecific, low-affinity IgM antibodies secreted by B1 cells.

Natural killer (NK) cell: The cell of the innate response that recognizes and then kills abnormal cells — for example, infected cells or tumor cells that lack cell-surface major-histocompatibility-complex class I molecules.

Polymorphism: An allele with a frequency in a population of at least 1 percent.

Tolerance: Specific immunologic unresponsiveness that occurs either centrally, in the primary lymphoid organs (bone marrow and thymus) (central tolerance), or peripherally, at any other location in the body (peripheral tolerance), and is induced mainly by clonal deletion (involving apoptosis) or by clonal anergy.

Transgenic animal: An animal bearing a foreign gene (termed a transgene), which is usually spliced to a tissue-specific or cell-specific promoter. The transgene is inserted into a fertilized egg in vitro, and thus becomes integrated into the animal's germ line.

Type 1 (Th1) helper T cell: A helper T cell that secretes the cytokines interleukin-2 and interferon-γ (but not interleukin-4, 5, or 6), inhibits type 2 helper T cells, and is chiefly involved in cell-mediated immunity (i.e., the activation of macrophages and cytotoxic T cells).

Type 2 (Th2) helper T cell: A helper T cell that secretes the cytokines interleukin-4, 5, 6, and 10 (but not interleukin-2 or interferon-γ), inhibits type 1 helper T cells, and is chiefly involved in humoral immunity (i.e., the production of antibody by B cells).

that nonspecifically enhances antigen-specific immunity.[4] Many microorganisms inherently possess adjuvant activity in the form of immunostimulatory molecules such as lipopolysaccharide and muramyl dipeptide.

INNATE IMMUNE RESPONSES

Cellular Components of Innate Responses

The innate immune system consists of all the immune defenses that lack immunologic memory. Thus, a characteristic of innate responses is that they remain unchanged however often the antigen is encountered. These types of responses developed earlier in evolution than acquired responses. Nonetheless, defects in these evolutionarily primitive innate immune mechanisms, such as those that occur in chronic granulomatous disease (in which there is defective killing of phagocytosed microorganisms) can be fatal. (This subject will be discussed in more detail later in the Advances in Immunology series.)

Macrophages (derived from blood-borne monocytes) possess receptors for carbohydrates that are not normally exposed on the cells of vertebrates,[5] such as mannose, and therefore can discriminate between "foreign" and "self" molecules. In addition, both macrophages and neutrophils have receptors for antibodies and complement, so that the coating of microorganisms with antibodies, complement, or both enhances phagocytosis.[6] The engulfed microorganisms are subjected to a wide range of toxic intracellular molecules, including superoxide anion, hydroxyl radicals, hypochlorous acid, nitric oxide, antimicrobial cationic proteins and peptides, and lysozyme. Phagocytes also remove the body's own dead or dying cells. Dying cells in necrotic tissue release substances that trigger an inflammatory response, whereas cells that are dying as a result of apoptosis (programmed cell death resulting in the digestion of DNA by endonucleases) express molecules on their cell surface, such as phosphatidyl serine, that identify them as candidates for phagocytosis.[7]

A key cellular component of innate immunity — and one of the most intensely studied components during the past decade — is the interdigitating dendritic cell (Fig. 1).[8] Cells of this type, which include Langerhans' cells in skin, constantly but quietly endocytose extracellular antigens. However, they become activated and behave as antigen-presenting cells when pattern-recognition receptors on their surface recognize distinctive pathogen-associated molecular patterns on the surface of microorganisms.[9] Endogenous danger signals,[10] such as the release of interferon-α from virally infected cells or an increase in heat-shock proteins as a result of necrotic cell death, also activate dendritic cells. Molecules that act as pattern-recognition receptors on dendritic cells include the lipopolysaccharide receptor, the mannose receptor, and members of a family of molecules called toll. Pathogen-associated molecular patterns include yeast-cell-wall mannans, lipopolysaccharides on the surface of gram-negative bacteria, and teichoic acids, which are present on gram-positive bacteria.[9]

Activation causes dendritic cells to up-regulate the expression of B7 costimulatory molecules (also known as CD80 and CD86) on their surface. Costimulatory molecules are molecules that provide the signals necessary for lymphocyte activation in addition to those provided through the antigen receptor. These activated dendritic cells migrate to the local draining lymph node, where they present antigen to T cells. The antigen is processed intracellularly into short peptides by means of proteolytic cleavage before it is presented by major-histocompatibility-complex (MHC) molecules on the surface of dendritic cells.

There are two classes of MHC molecules, class I and class II. There are three main types of class I molecules, HLA-A, B, and C, and three main kinds of class II molecules, HLA-DP, DQ, and DR. The MHC class II molecules present the peptides to the T-cell receptor on the surface of helper T cells. Dendritic cells are particularly efficient at initiating (priming) immune responses for which immunologic memory has not been established — that is, they activate so-called naive T cells (Fig. 1). (This subject will be discussed in more detail later in the series.)

Unlike macrophages and neutrophils, eosinophils are only weakly phagocytic and, on activation, probably kill parasites mainly by releasing cationic proteins and reactive oxygen metabolites into the extracellular fluid. They also secrete leukotrienes, prostaglandins, and various cytokines.[11]

Basophils and mast cells have similar functional characteristics,[12] but there is little evidence that blood basophils develop into tissue mast cells.[13] Both types of cells possess high-affinity receptors for IgE (FcϵR)[14] and thereby become coated with IgE antibodies. These cells are important in atopic allergies such as eczema, hay fever, and asthma, in which allergen binding to the IgE cross-links the FcϵR. This event triggers the cell to secrete inflammatory mediators such as histamine, prostaglandins, and leukotrienes. (This subject will be discussed in more detail later in the series.)

Natural killer cells destroy infected and malignant cells.[15] They recognize their targets in one of two ways. Like many other cells, they possess Fc receptors that bind IgG (FcγR). These receptors link natural killer cells to IgG-coated target cells, which they kill by a process called antibody-dependent cellular cytotoxicity. The second system of recognition that is characteristic of natural killer cells relies on the killer-activating receptors and killer-inhibitory receptors of these cells (Fig. 2). The killer-activating receptors recognize a number of different molecules present on the surface of all nucleated cells, whereas the killer-inhibitory receptors recognize MHC class I molecules, which are also usually present on all nucleated

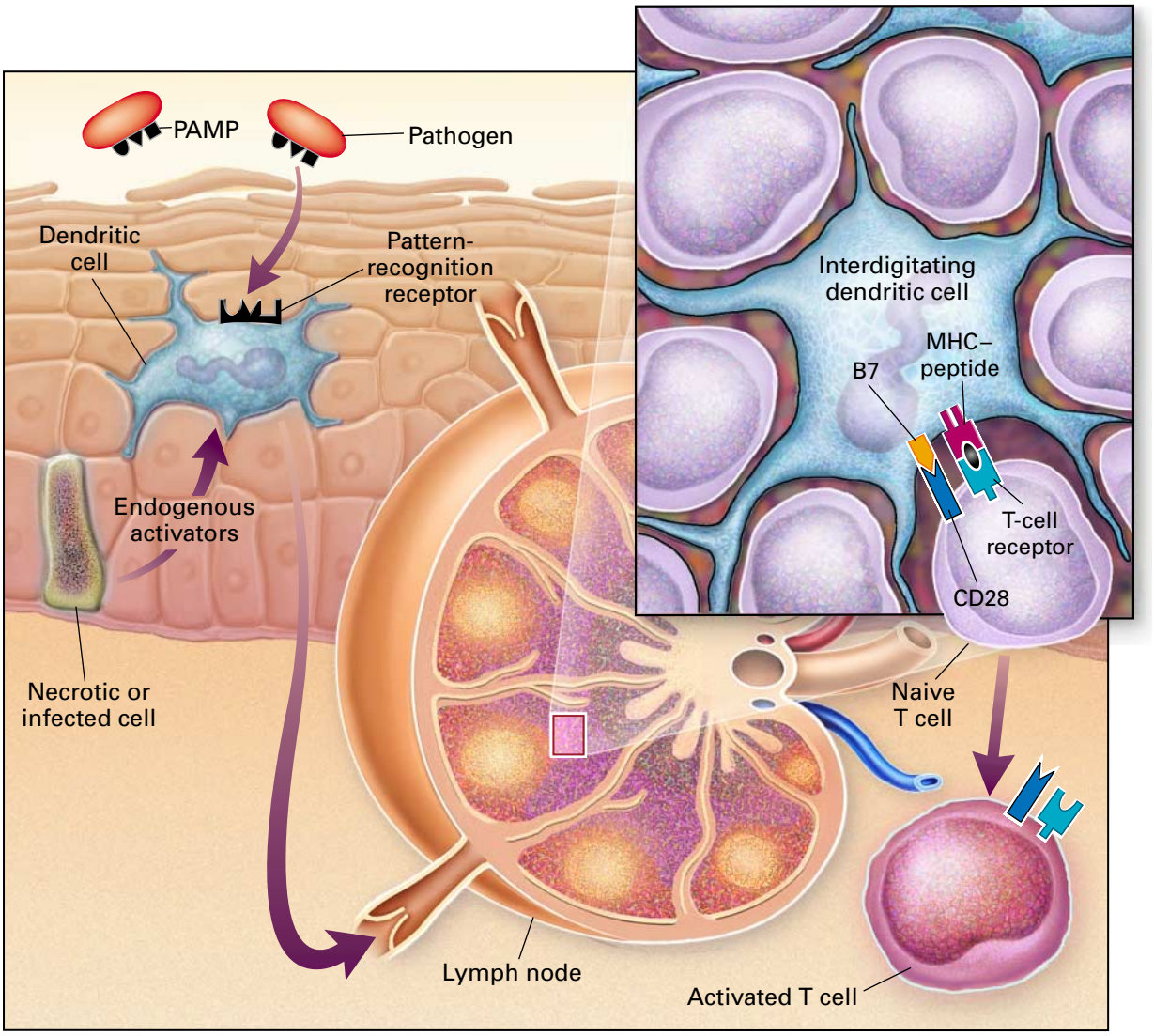

Figure 1. Function of Interdigitating Dendritic Cells.

Pathogen-associated molecular patterns (PAMPs) allow the pattern-recognition receptors on the dendritic cells and macrophages of the innate immune response to differentiate between potentially harmful foreign microorganisms and self constituents. These cells are also stimulated by endogenous activators such as interferon-α, heat-shock proteins, and tumor necrosis factor α that are released as a result of infection. The activated antigen-presenting cells then present a cell-surface complex of a major-histocompatibility-complex (MHC) molecule and peptide, derived by intracellular processing of the foreign antigen, to the T-cell receptors on the highly specific CD28-bearing naive T cells, which become activated in the acquired immune response. Activation also causes dendritic cells to enhance their expression of B7 costimulatory molecules.

cells.[16,17] If the killer-activating receptors are engaged, a "kill" instruction is issued to the natural killer cell, but this signal is normally overridden by an inhibitory signal sent by the killer-inhibitory receptor on recognition of MHC class I molecules (Fig. 2).

Although all nucleated cells normally express MHC class I molecules on their surface, they can sometimes lose this ability. This loss may occur as a result of either microbial interference with the expression mechanism — for example, after herpesvirus infection — or malignant transformation. Therefore, cells that lack MHC class I surface molecules are in some way abnormal. This lack of MHC class I molecules means that there is no inhibitory signal from the killer-inhibitory receptor, and the natural killer cell kills the abnormal target cell by inserting the pore-forming molecule perforin into the membrane of the target cell and then injecting it with cytotoxic granzymes.

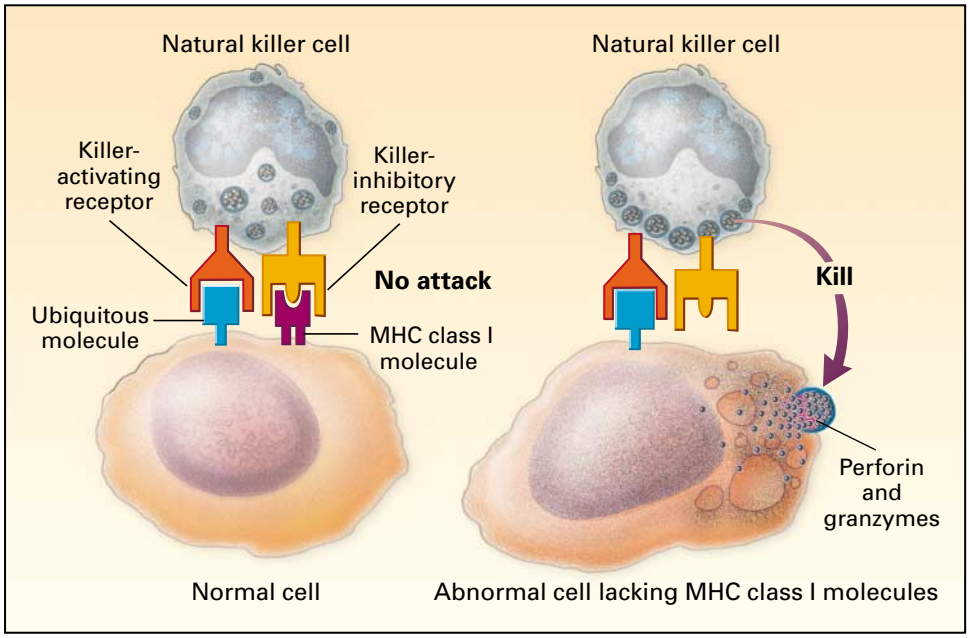

Figure 2. A System Used by Natural Killer Cells to Recognize Normal Cells and Cells That Lack Major-Histocompatibility-Complex Class I Surface Molecules.

Killer-activating receptors recognize a number of molecules present on the surface of normal, nucleated cells, and in the absence of an inhibitory signal from killer-inhibitory receptors, which recognize major-histocompatibility-complex (MHC) class I molecules, the receptors issue an order to the natural killer cells to attack and kill the other cell. The cytotoxic granules of the natural killer cells, which contain perforin and granzymes, become polarized at the interface with the target cell and are then released into the cell.

The role of erythrocytes and platelets in immune responses is sometimes overlooked, but because they have complement receptors, they play an important part in the clearance of immune complexes consisting of antigen, antibody, and components of the complement system.

Soluble Factors in Innate Defense

Innate responses frequently involve complement, acute-phase proteins, and cytokines. The early events of complement activation, which are based on an enzymatic amplifying cascade comparable to that seen in blood clotting, can be triggered by one of three pathways.[18] The classic pathway is activated by antigen–antibody complexes, the alternative pathway by microbial-cell walls, and the lectin pathway by the interaction of microbial carbohydrates with mannose-binding protein in the plasma.[19]

Irrespective of the source of activation, the outcome is the generation of a number of immunologically active substances. For example, a proteolytic-cleavage fragment of complement component C3, the C3b molecule, becomes deposited on the surface of microorganisms. This event enhances phagocytosis of the microbe, because phagocytic cells have cell-surface receptors for C3b. The complement fragments C3a, C4a, and C5a cause the release of inflammatory mediators from mast cells. C5a also acts as a powerful neutrophil chemoattractant. The complement components C5b, C6, C7, C8, and C9 form the membrane-attack complex, which perforates cell membranes and thereby leads to the death of the target cell.

The molecules collectively referred to as acute-phase proteins enhance resistance to infection and promote the repair of damaged tissue.[20] Plasma levels of these proteins change rapidly in response to infection, inflammation, and tissue injury. In addition to some complement components, the acute-phase proteins include C-reactive protein (a useful marker of inflammation, particularly in diseases such as rheumatoid arthritis), serum amyloid A protein, proteinase inhibitors, and coagulation proteins.

Cytokines constitute another group of soluble mediators. They act as messengers both within the immune system and between the immune system and other systems of the body, forming an integrated network that is highly involved in the regulation of immune responses.[21] The presence of a cytokine is sensed by a cell by means of specific cytokine receptors. However, the distinction between cytokines and cytokine receptors is sometimes blurred, because there

are soluble forms of cytokine receptors[22] and membrane-anchored forms of some cytokines.[23]

In addition to acting as messengers, some cytokines have a direct role in defense; for example, the interferons that are released by virally infected cells establish a state of viral resistance in the surrounding cells. Cytokines and their antagonists are increasingly being used as therapeutic agents. For example, a combination of interleukin-2 and interferon-α has proved valuable in the treatment of melanoma.[24] Infliximab, a chimeric monoclonal antibody against tumor necrosis factor α, has had strikingly beneficial effects in patients with rheumatoid arthritis.[25]

The Acute Inflammatory Response

Infection with a pathogen triggers an acute inflammatory response in which cells and molecules of the immune system move into the affected site. The activation of complement generates C3b, which coats the surface of the pathogen. The neutrophil chemoattractant and activator C5a is also produced, and together with C3a and C4a triggers the release of histamine by degranulating mast cells. This in turn causes the contraction of smooth muscles and a rapid increase in local vascular permeability. Substances released from the pathogen and from damaged tissues up-regulate the expression of adhesion molecules on vascular endothelium, alerting passing cells to the presence of infection. The cell-surface molecule L-selectin on neutrophils recognizes carbohydrate structures such as sialyl-Lewis^x on the vascular adhesion molecules.[26] The neutrophil rolling along the vessel wall is arrested in its course by these interactions. As the neutrophil becomes activated, it rapidly sheds L-selectin from its surface and replaces it with other cell-surface adhesion molecules, such as the integrins. These integrins bind the molecule E-selectin, which appears on the blood-vessel wall under the influence of inflammatory mediators such as bacterial lipopolysaccharide and the cytokines interleukin-1 and tumor necrosis factor α. Complement components, prostaglandins, leukotrienes, and other inflammatory mediators all contribute to the recruitment of inflammatory cells, as does an important group of chemoattractant cytokines called chemokines. (This subject will be discussed in more detail later in the series.) The activated neutrophils pass through the vessel walls, moving up the chemotactic gradient to accumulate at the site of infection, where they are well placed to phagocytose any C3b-coated microbes (Fig. 3). Mutations in the genes for a number of different adhesion molecules have been described in patients with leukocyte-adhesion deficiencies, some of which are associated with life-threatening infections.[27]

ACQUIRED IMMUNE RESPONSES

The development of lymphocytes and the myeloid lineage from primordial stem cells in the fetal liver and in bone marrow is guided by interactions with stromal cells (such as fibroblasts) and by cytokines (including stem-cell factor and various colony-stimulating factors).[28] The initial stages of lymphocyte development do not require the presence of an antigen, but once these cells express a mature antigen receptor, their survival and further differentiation become antigen-dependent.

The Structure of Antigen-Specific Molecules

The B-Cell Receptor and Soluble Antibodies

Antibodies consist of two identical heavy chains and two identical light chains that are held together by disulfide bonds.[29] The N terminal of each chain possesses a variable domain that binds antigen through three hypervariable complementarity-determining regions (Fig. 4). The C terminal domains of the heavy and light chains form the constant regions, which define the class and subclass of the antibody and govern whether the light chain is of the κ or λ type. The amino acid sequence of the constant region of the heavy chains specifies five classes of immunoglobulins (IgG, IgA, IgM, IgD, and IgE), four subclasses of IgG, and two subclasses of IgA. These classes and subclasses have different functions. Each type of antibody can be produced as a circulating molecule or as a stationary molecule. The latter type has a hydrophobic transmembrane sequence that anchors the molecule in the B-cell membrane, where it functions as the B-cell receptor.

All immunoglobulins are glycoproteins and contain 3 to 13 percent carbohydrate, depending on the class of the antibody. The carbohydrate is essential in maintaining the structure of the antibody. The basic antibody "monomeric unit" (which is biochemically a tetramer) is bivalent, with two antigen-binding arms of identical specificity. Each of these arms can be cleaved proteolytically in the laboratory to yield individual monovalent antigen-binding fragments (Fab) (Fig. 4).[30] Another part of the immunoglobulin molecule, the Fc region, contains most of the constant region of the heavy chains. The secretory IgA at mucosal surfaces is a tetravalent "dimer," whereas circulating IgM is a decavalent "pentamer." These IgA and IgM polymers are stabilized by a polypeptide, the J (joining) chain. Secretory IgA also contains a molecule called secretory component, which may protect the IgA against proteolytic cleavage within the gastrointestinal tract.

The T-Cell Receptor

Unlike antibodies, T-cell receptors are produced only as transmembrane molecules. They consist of α/β or γ/δ heterodimers; each α, β, γ, and δ chain contains a variable domain and a constant domain. As in the antibody molecule, the variable domains contain three complementarity-determining regions (Fig. 4), which in the case of the α/β T-cell receptor

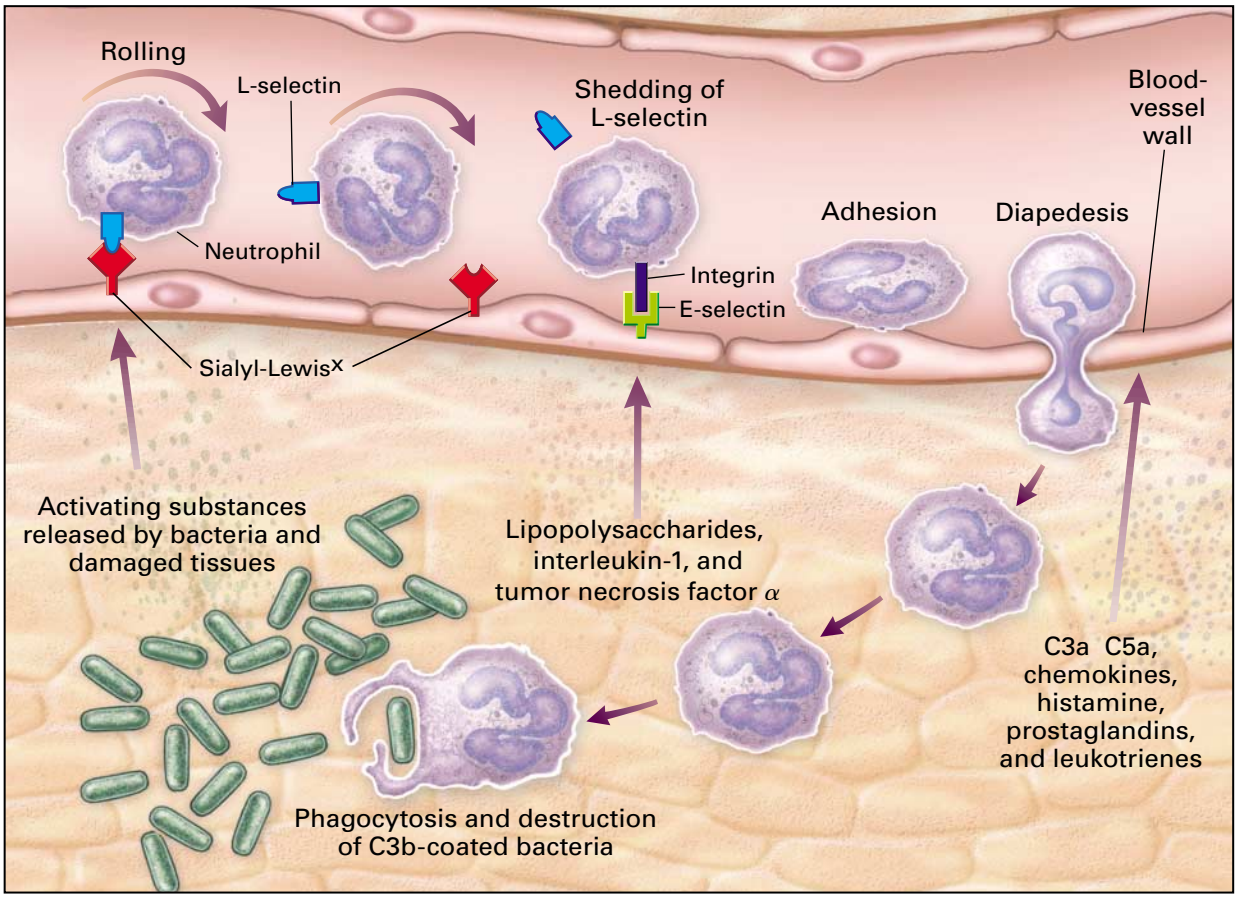

Figure 3. The Acute Inflammatory Response.

Neutrophils are among the first cells to arrive at the scene of an infection and are important contributors to the acute inflammatory response. As the neutrophil rolls along the blood-vessel wall, the L-selectin on its surface binds to carbohydrate structures such as sialyl-Lewis^x on the adhesion molecules on the vascular endothelium, and its progress is eventually halted. As the neutrophil becomes activated, it replaces L-selectin with other cell-surface adhesion molecules, such as integrins. These molecules bind E-selectin, which is present on the blood-vessel wall as a result of the influence of inflammatory mediators such as bacterial lipopolysaccharides and the cytokines interleukin-1 and tumor necrosis factor α. The activated neutrophil then enters the tissues, where it is attracted to the infection site by a number of chemoattractants. The neutrophil can then phagocytose and destroy the C3b-coated bacteria.

recognize a complex formed by a peptide seated within the groove of an MHC molecule.[2,31] Most γ/δ T cells do not recognize antigen in the form of peptide–MHC complexes, although MHC-like ("nonclassic MHC") molecules such as CD1 may present certain antigens (particularly lipids and glycolipids) to some γ/δ T cells. Other γ/δ T cells do recognize antigen directly, just as antibody molecules do.[32]

The Diversity of Antigen Receptors

It has been estimated that lymphocytes are capable of producing about 10^{15} different antibody variable regions (B cells) and a similar number of T-cell–receptor variable regions. Remarkably, the vast diversity of the immune repertoire originates from fewer than 400 genes. This extraordinary feat is achieved by unique recombination processes that cut, splice, and modify variable-region genes.[33]

The genetic components that encode the immunoglobulins lie on three chromosomes: the *IGH* cluster (named for the heavy chain and located on chromosome 14), the *IGK* cluster (named for the κ light chain and located on chromosome 2), and the *IGL* cluster (named for the λ light chain and located on chromosome 22). Within the *IGH* cluster are four types of gene segments: *V* (variable), *D* (diversity), *J* (joining), and *C* (constant). The *IGK* and *IGL* clusters lack *D* segments. All these segments contain multiple genes; in the *IGH* cluster, for example, there are about 50 functional *V* segments.

The T-cell receptor genes have a similar organization and also contain *V, D, J,* and *C* segments. The

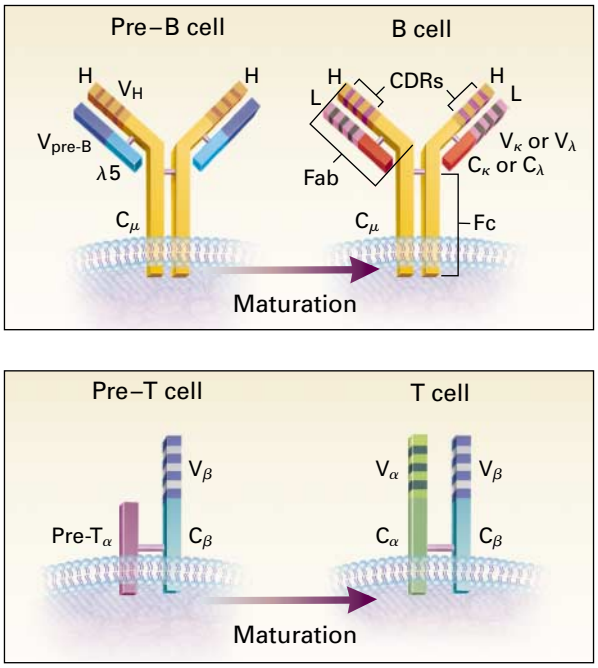

Figure 4. Structure of Immature and Mature B-Cell and T-Cell Antigen Receptors.

The immature pre–B cells and pre–T cells express preliminary versions of the antigen receptor. At this stage, the B-cell receptor comprises a pair of heavy (H) chains, each with a variable (V) and a constant (C_μ) region identical to those found in the mature receptor, and a pair of surrogate light chains, termed $V_{pre\text{-}B}$ and λ5. As the B cell develops, the surrogate light chains are replaced by conventional light (L) chains of either the κ or λ type, each with a variable and a constant region. This mature IgM molecule acts as the B-cell receptor for antigen, usually together with IgD B-cell receptors with the same antigen specificity. The variable regions of the heavy and light chains each contain three hypervariable complementarity-determining regions (CDRs). The CDRs make contact with the antigen. One of the two antigen-binding arms (Fab) of the bivalent antibody molecule is indicated. The circulating version of the antibody contains the same four chains but lacks the transmembrane sequence that anchors the B-cell receptor in the lymphocyte membrane. With respect to T cells, the immature T-cell receptor consists of a β chain identical to that found in the mature receptor and a pre-T_α chain that comprises only a constant region. This segment is replaced by an α chain to form the mature T-cell receptor, and each chain consists of a variable region and a constant region. For the sake of simplicity, the antigen receptors are shown without their associated signal-transduction units.

three loci, *TCRA/D* (on chromosome 14), *TCRB* (on chromosome 7), and *TCRG* (on chromosome 7), correspond to the α and δ chains, β chain, and γ chain, respectively, of the T-cell receptor. In contrast to the *TCRB* and *TCRD* loci, the *TCRA* and *TCRG* loci do not contain *D* segments. And, as in the case of immunoglobulin genes, each locus contains multiple *V, D,* and *J* genes; on *TCRA,* for example, there are 70 to 80 *V* genes and about 60 *J* genes.

The laying down of genetic instructions for the variable region involves the recombination of genes from the *V, D,* and *J* segments. The recombination process joins one gene segment of each type (e.g., *VDJC* in the case of the immunoglobulin heavy chain) to form a linear coding unit for each chain of the receptor. Each lymphocyte uses a different combination of these gene segments to form the genetic code of its antigen receptor. The recombination process is subject to splicing inaccuracies that cause slight variations in the nucleotides at the *VDJ* junctions. Furthermore, the enzyme terminal deoxyribonucleotidyltransferase can insert additional nucleotides around the *VDJ* junctions before they are ligated. Both the splicing errors and the added nucleotides further increase diversity[34] and impart to each B-cell or T-cell clone a molecularly unique receptor (Fig. 5).

A series of nucleases and ligases do the cutting and pasting of the gene segments. Defects in the recombination-activating genes *RAG-1* and *RAG-2,*[35] which encode two of the enzymes that mediate the recombination of variable-region genes in both B cells and T cells, are responsible for one form of severe combined immunodeficiency; affected patients are unable to produce functional lymphocytes bearing antigen receptors.[36] (This subject will be discussed in more detail later in the series.)

The sequences of T-cell receptors generally remain unaltered during cell division, but this is not the case with the B cell, which, in the germinal centers of secondary lymphoid organs, can undergo further rearrangements of *V* genes by a process referred to as receptor editing.[37] This mechanism enables self-reactive B cells to see the error of their ways and redeem themselves by replacing one variable-region gene with a new variable-region gene. In this process, the existing *V* gene in the rearranged *VDJ* sequence is replaced by another *V* gene segment. The constant region specifies the class of the antibody (e.g., IgM or IgG), and during the immune response, the *VDJ* unit in B cells can join with different constant-region genes to alter the class of antibody in a process called class switching.[38]

Clonal Selection

There are no more than a few thousand lymphocytes specific for each antigen. Since each B cell is programmed to express only one of the vast number of potential antibodies, all the antigen-receptor molecules on a given lymphocyte have the same specificity. Such clones of lymphocytes are selected to participate in an immune response if they bear a receptor that can bind the relevant antigen, a process called clonal selection. The antigen-selected cells proliferate, leading to a rapid increase in the number of B or T cells that can recognize the antigen. Most responses involve many different clones — that is, they are polyclonal — because even relatively simple an-

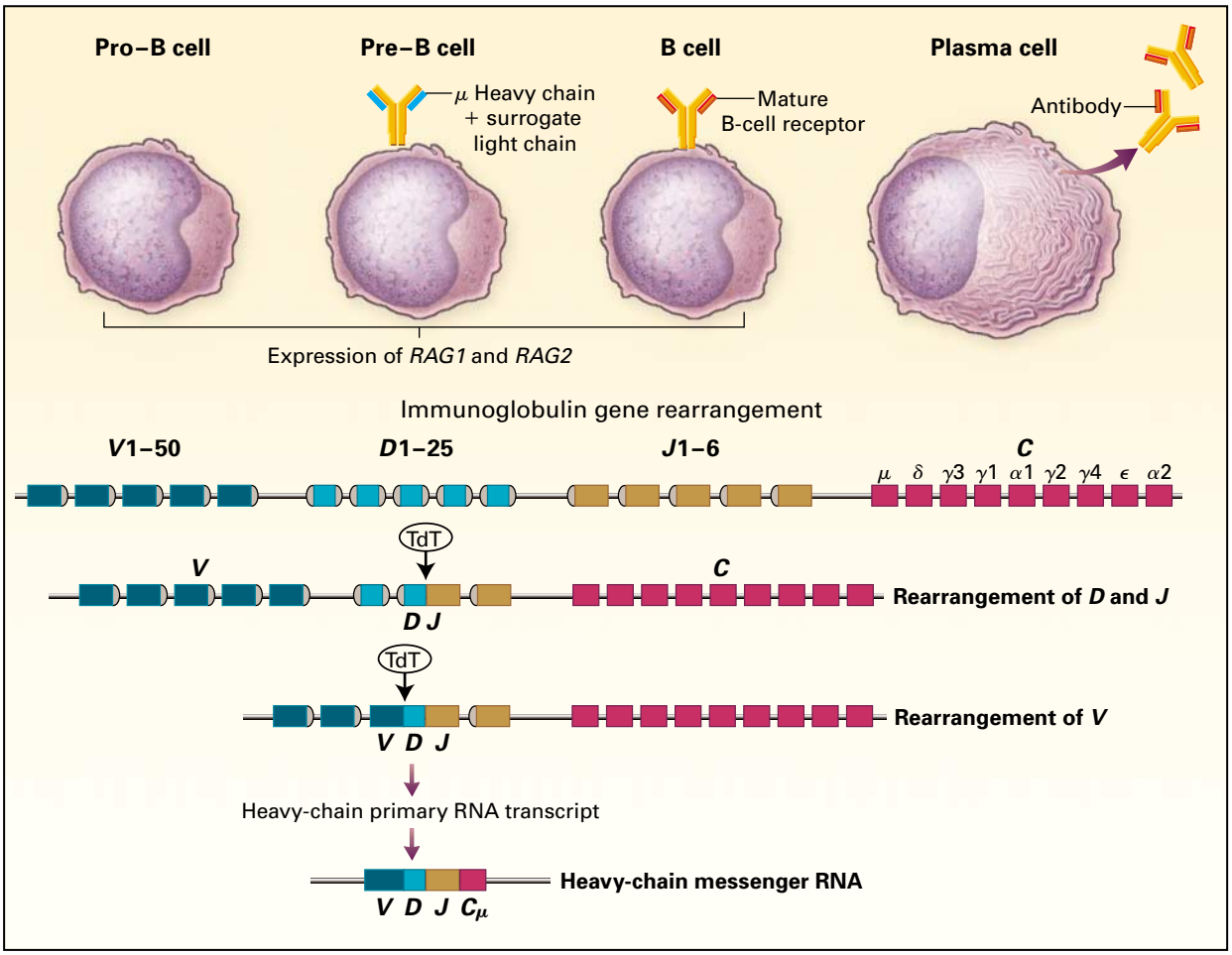

Figure 5. Diversity of Antigen Receptors.

The enormously diverse specificities of the antigen receptors are produced by gene rearrangements during the early developmental stages of the lymphocyte. The events involved in generating a coding sequence for the immunoglobulin heavy chain are shown. Early in B-cell development, pro–B cells mature into pre–B cells, at which stages they express the recombination-activating genes *RAG1* and *RAG2*. The recombinases encoded by these genes mediate the random rearrangement of 1 of 25 diversity (*D*) gene segments next to any 1 of 6 joining (*J*) gene segments. This is followed by the rearrangement of any 1 of 50 variable (*V*) gene segments next to the already rearranged *DJ* segment. Different B cells will rearrange a different segment in each pool, thereby creating one level of diversity. Further diversity is brought about by splicing inaccuracies and by the incorporation of nucleotides mediated by the enzyme terminal deoxyribonucleotidyltransferase (TdT). The heavy-chain primary RNA transcript is processed into messenger RNA (mRNA), with splicing of the rearranged *VDJ* segment next to the constant (*C*) region gene. This mRNA will encode a heavy chain that appears on the surface of the pre–B cell together with the surrogate light chain, which is encoded by genes that do not undergo rearrangement. As the pre–B cell continues to mature, the immunoglobulin light-chain genes undergo rearrangement; the resulting light chain replaces the surrogate light chain, and thereby produces a mature IgM B-cell receptor on the cell surface. The B-cell receptors at this stage also usually include IgD antibodies with the same specificity as the IgM molecule, produced by alternative splicing of the rearranged *VDJ* to either the C_μ or the C_δ gene. The expression of *RAG1* and *RAG2* is then switched off. After encountering an antigen, and in the presence of costimulatory signals, the B cell further differentiates into a plasma cell, which secretes high levels of the specific antibody (or into a memory B cell). The same general principles regarding the rearrangement process apply to the generation of α/β and γ/δ T-cell receptors. The gene segments in the figure are not drawn to scale.

tigens bear several different epitopes (Fig. 6), each with the capacity to bind to a unique clone.

Unlike the genes for T-cell receptors, the genes encoding B-cell receptors undergo a process of somatic hypermutation. This process occurs during B-cell proliferation within the germinal centers of secondary lymphoid tissues. The changes in amino acids in the antibody that result from this process fine-tune the recognition of antigen by B-cell receptors and determine the strength of binding (affinity) of the antibody. The stronger the binding to antigen, the greater the chance the B cell has of surviving and

multiplying — a classic Darwinian mechanism of selecting cells that produce high-affinity antibodies. The result of clonal selection is a population of B cells with high affinity and exquisite antigen specificity for the immunizing antigen, as well as a memory of the encounter.

The proliferation of naive lymphocytes during the first encounter with an antigen, the primary immune response, generates both effector T and B cells (cytotoxic and helper T cells and antibody-secreting plasma cells) and memory T and B cells. The memory cells enable a quantitatively and qualitatively superior secondary immune response to be mounted after a subsequent encounter with the same antigen. Naive and memory T cells can, to some extent, be distinguished, because they often express different versions of the CD45 molecule (a tyrosine phosphatase that regulates cellular activation) on their surface; CD45RA is expressed on naive cells, and CD45RO is expressed on memory cells.[39] Because memory cells are increased in number relative to naive cells and because memory cells are also more readily triggered, the secondary response is more rapid than the primary immune response. It produces a larger number of lymphocytes and, in the case of B cells, induces greater levels of antibody that has a greater affinity for the antigen than the antibody of the primary response.

The concept of vaccination is based on the fact that deliberate exposure to a harmless version of a pathogen generates memory cells but not the pathologic sequelae of the infectious agent itself. In this way, the immune system is primed to mount a secondary immune response with strong and immediate protection should the pathogenic version of the microorganism be encountered in the future.

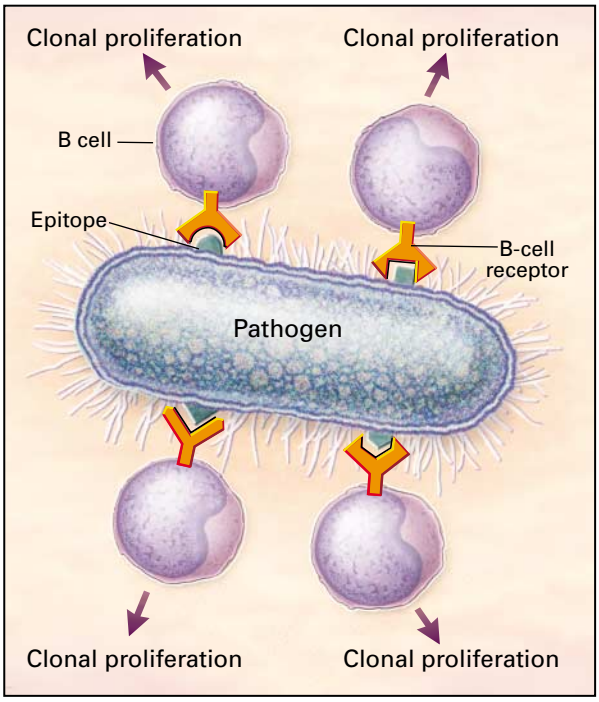

Figure 6. Recognition of Epitopes by B Cells.

Using the antibody molecule as its receptor, the B cell recognizes epitopes on the surface of the antigen. If it is stimulated by this contact, the B cell proliferates, and the resulting clones can secrete antibody whose specificity is the same as that of the cell-surface receptor that bound the epitope. Responses usually involve several different clones of lymphocytes and are therefore referred to as polyclonal. Although not shown here, for each epitope there may be several different lymphocyte clones with different B-cell receptors, each of which recognizes the epitope in a slightly different way and therefore with a different binding strength (affinity).

Major Populations of B Cells

The B cells that develop earliest during ontogeny are referred to as B1 cells. Most B1 cells express CD5, an adhesion and signaling cell-surface molecule. They are the source of the so-called natural antibodies, which are IgM antibodies and are frequently polyreactive (i.e., they recognize several different antigens, often including common pathogens and autoantigens). In most cases, natural antibodies have a relatively low affinity.[40,41]

Most B cells lack the CD5 molecule, and because they develop slightly later in ontogeny, they are referred to as B2 cells. Before they encounter antigen, mature B2 cells coexpress IgM and IgD antibodies on their cell surface, but by the time they become memory cells, they have usually switched to the use of IgG, IgA, or IgE as their antigen receptors. Complexes of antibodies with a newly encountered antigen and complement are localized in the follicular dendritic cells (a different type of cell from the interdigitating dendritic cell) within secondary lymphoid tissues. This event initiates the formation of the germinal centers, which are discrete areas within the spleen and lymph nodes where B-cell responses occur. Within these germinal centers, B2 cells that encounter the antigen undergo immunoglobulin class switching and begin to produce IgG, IgA, or IgE, and somatic hypermutation of their antigen-receptor genes occurs. Memory cells and plasma-cell precursors are also generated in the germinal centers. The final stages of differentiation of B2 cells into antibody-secreting plasma cells occur within the secondary lymphoid tissues but outside the germinal centers. Although generally short-lived, with a half-life of only a few days, some plasma cells survive for weeks, especially within the bone marrow.[42]

T Cells and the Thymus

Stem cells continuously migrate from the bone marrow to the thymus, where they develop into T cells.[43] Recent evidence suggests that, despite the partial de-

generation of the thymus that occurs at puberty, T cells continue to develop in the thymus throughout life.[44] T cells with α/β T-cell receptors initially remain in the thymus, where they are subjected to a series of selection procedures (Fig. 7).[45] Unlike the antibody molecule, which acts as the antigen receptor on B cells and recognizes antigen in its native (natural) state, the α/β T-cell receptor recognizes short peptides that result from the intracellular processing of protein antigens, which are presented to the T-cell receptor by MHC molecules on the cell surface. The amino acids recognized by the T-cell receptor derive from both the MHC molecule and the antigenic peptide. Thus, the T-cell receptor recognizes an individual's own MHC molecules (self) together with peptides derived from foreign antigens.

Since MHC molecules are highly polymorphic, the desirable immature T cells in each person in an outbred population are those that can recognize self MHC molecules but that are not autoreactive. This objective is achieved by thymic education, a process that involves both positive and negative selection.[46-49] Cells are positively selected if they express a T-cell receptor capable of interacting with the MHC com-

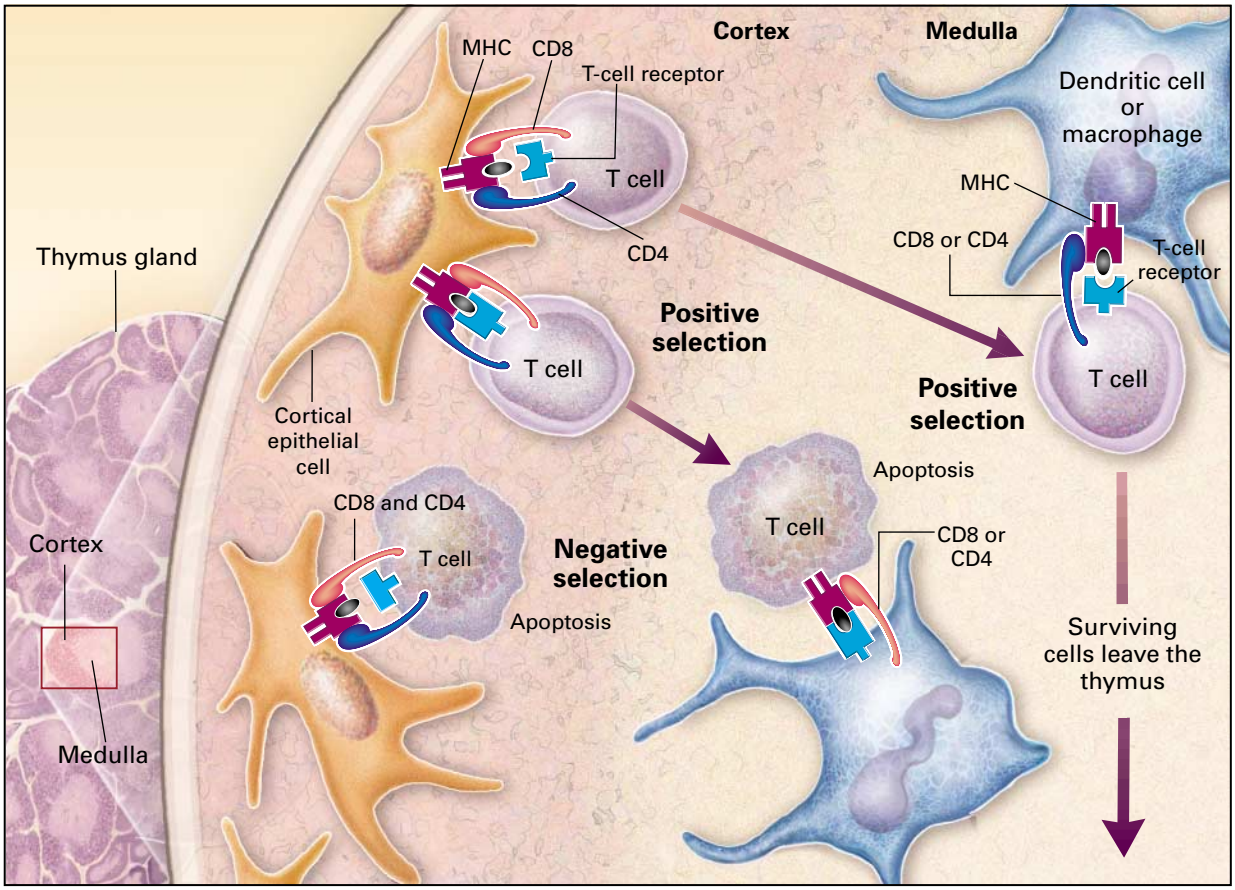

Figure 7. Positive and Negative Selection in the Thymus.

T cells need to detect foreign antigens presented by self major-histocompatibility-complex (MHC) molecules. Part of the T-cell receptor recognizes the foreign peptide, and part of it recognizes the self MHC molecule. The random nature of T-cell–receptor gene rearrangements means that only a minority of T cells are capable of performing this task. Many of the immature CD4 and CD8 double-positive T cells are useless because their T-cell receptors do not recognize self MHC molecules at all. These T cells eventually undergo apoptosis. Cells whose T-cell receptors have various affinities for binding self MHC molecules (usually containing a self peptide) are positively selected on cortical epithelial cells. However, many of these cells are potentially harmful because their T-cell receptors have a high affinity for a complex of self peptide and a self MHC molecule (or even an MHC molecule alone). These autoimmune T cells are eliminated by the induction of apoptosis when they interact with dendritic cells and macrophages in the thymic medulla (negative selection). This leaves T cells with only a weak affinity for self MHC molecules. These cells form the pool of T cells that are exported from the thymus as single-positive (CD4 or CD8) cells. In the periphery they have the potential to recognize a complex of foreign peptide plus self MHC molecules and to become activated if the affinity of the interaction exceeds a certain threshold.

plexes on the person's own epithelial cells in the thymic cortex. Positive selection switches off the signal for spontaneous apoptosis that is otherwise triggered naturally in developing T cells. More than 95 percent of T cells are not selected at this stage and therefore die in the thymus. In contrast, negative selection involves the induction of apoptosis in any lymphocyte that expresses a T-cell receptor with a high affinity for the complex of a self peptide plus a self MHC molecule on dendritic cells and macrophages in the thymic medulla. (This subject will be discussed in more detail later in the series.)

During thymic education, the expression of a large number of T-cell–surface molecules is switched on and off in a highly regulated manner. Some of these, and many other cell-surface molecules with a role in immune responses, were originally characterized on the basis of their reactivity to panels of monoclonal antibodies. The antibodies produced by various laboratories were said to form a cluster when they could be grouped together because they recognized the same cell-surface molecule. This led to a nomenclature in which a given molecule was assigned a "cluster of differentiation," or CD, number — for example CD1, CD2, and CD3. This CD nomenclature has become the standard way of referring to these cell-surface molecules.

The CD4 and CD8 molecules are of particular note with regard to T-cell development; together with the CD3 group of molecules, they form an essential part of the T-cell–receptor complex. CD4 binds to an invariant part of the MHC class II molecule, whereas CD8 binds to an invariant part of the MHC class I molecule. CD4 T cells usually act as helper T cells and recognize antigens presented by MHC class II molecules, whereas CD8 T cells are usually cytotoxic and recognize antigen presented by MHC class I molecules. Early in T-cell development in the thymus, immature T cells express both CD4 and CD8.[50] If they have an appropriate T-cell receptor, these double-positive immature T cells have the potential to recognize an antigen-derived peptide presented by either MHC class I or MHC class II molecules. As T cells mature in the thymus, however, the expression of one of these molecules is lost, resulting in single-positive CD4 or CD8 T cells that recognize a peptide presented only by MHC class II or MHC class I molecules, respectively.

MHC class I molecules are expressed on all nucleated cells. This allows infected cells to signal their plight to cytotoxic CD8 T cells and establish intimate intercellular contacts by presenting the complex of foreign peptide and MHC molecule to the T-cell receptor of the effector cell. Since MHC class II molecules signal CD4 helper T cells to secrete cytokines, the effector function of the helper T cell does not always depend on the establishment of intimate contact with the cell that will respond to the cytokine. This explains why the immune system needs only a few kinds of specialized "professional antigen-presenting cells" (dendritic cells, B cells, and activated macrophages) to express MHC class II molecules.

A minority of T cells in the thymus use γ and δ chain genes to produce a T-cell receptor. These γ/δ T cells rapidly leave the thymus, and some may also develop outside the thymus, possibly in the gut.[51] Thymus-derived γ/δ T cells migrate to various locations throughout the body, including the epithelium of the gastrointestinal tract, where they are thought to contribute to mucosal defenses. The range of their specificities remains to be fully characterized, but they include both proteinaceous and nonproteinaceous antigens from mycobacteria and other infectious organisms. In addition, they have an important immunoregulatory role because they influence antibody production and immunoglobulin class switching by B cells and modify T-cell responses.[32] Precisely how they mediate these immunoregulatory functions also remains to be established.

Tolerance Mechanisms

Negative selection constitutes one form of immunologic tolerance in that it removes from the immune system T cells that recognize any of the body's own antigens within the thymus. Tolerance of T cells induced in the thymus and of B cells in the bone marrow is called central tolerance,[52] but another mechanism to prevent autoimmunity is necessary, because most tissue-specific antigens are not present in the thymus or bone marrow or are present in amounts too small for the induction of tolerance.

Mechanisms occurring elsewhere in the body, which are collectively referred to as peripheral tolerance, supplement central tolerance.[53] They are thought to be based largely on incomplete activation signals given to the lymphocyte when it encounters self antigen in the periphery, a phenomenon that leads to a state of specific unresponsiveness termed anergy (associated with impaired intracellular signaling) or to apoptosis.[54] (This subject will be discussed in more detail later in the series.)

Many autoreactive B cells undergo clonal deletion or become anergic as they mature within the bone marrow. Negative selection occurs in the bone marrow if B cells encounter high levels of self antigen, either in the soluble phase or as cell-membrane constituents. However, the deletion of self-reactive B cells may be more rigorously enforced in the germinal centers of secondary lymphoid tissues such as the spleen.[55] Because B cells recognize native antigen, there is no role for MHC molecules in any of these processes. For self antigens that are present at relatively low levels, immunologic tolerance is often maintained only within the T-cell population. This is sufficient to maintain tolerance because it denies the help essential for antibody production by self-reactive B cells.

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CORRECTION

The Immune System (First of Two Parts)

The Immune System (First of Two Parts) . The ligand for integrin-mediated arrest and adhesion of neutrophils to the vascular endothelium is intercellular adhesion molecule 1 (ICAM-1), not E-selectin as shown in Figure 3 and stated in the text on page 42, line 36 of the left-hand column.

N Engl J Med 2000;343:1132-a

Review Articles

Advances in Immunology

IAN MACKAY, M.D., AND FRED S. ROSEN, M.D.,
Editors

THE IMMUNE SYSTEM

Second of Two Parts

PETER J. DELVES, PH.D., AND IVAN M. ROITT, PH.D.

LYMPHOCYTES AND LYMPHOID TISSUE

The complexity of the cellular interactions that occur during acquired immune responses requires specialized microenvironments in which the relevant cells can collaborate efficiently. Because only a few lymphocytes are specific for a given antigen, T cells and B cells need to migrate throughout the body to increase the probability that they will encounter that particular antigen. In their travels, lymphocytes spend only about 30 minutes in the blood during each cycle around the body.[56]

Immune responses to blood-borne antigens are usually initiated in the spleen, and responses to microorganisms in tissues are generated in local lymph nodes, but most pathogens are encountered after they are inhaled or ingested. Antigens entering the body through mucosal surfaces activate cells in the mucosa-associated lymphoid tissues. Responses to intranasal and inhaled antigens occur in the palatine tonsils and adenoids.[57] Antigens from the gut are taken up by specialized epithelial cells, the microfold (or M) cells.[58] These cells transport the antigen across the epithelium to Peyer's patches, the chief sites for the induction of mucosal responses to ingested antigen.

Intraepithelial lymphocytes[59] interspersed between the epithelial cells in the gut encounter antigen being transported by microfold cells. Most of these intraepithelial lymphocytes are CD8-positive α/β T cells with the appearance of large granular lymphocytes; approximately 15 percent are γ/δ T cells.[60] The function of intraepithelial lymphocytes remains to be firmly established, but the α/β T cells in this population may assist in the production of mucosal IgA and some γ/δ T cells may participate in the induction of immunologic tolerance to antigens at mucosal surfaces. However, the specificity of many intestinal γ/δ T cells for microbial antigens indicates their role as sentinels of the gut.

After an immune response is induced in Peyer's patches, the lymphocytes enter the blood and travel to mucosal effector sites, such as the lamina propria, where large amounts of secretory IgA are produced.[61] To some extent these migratory pathways constitute a common mucosal immune system in which responses induced in one location can be replicated at other mucosal sites throughout the body. For example, intranasal exposure to antigen can result in the production of secretory IgA in the mucosal tissues of the reproductive tract.[62,63]

Lymphocytes enter lymph nodes, tonsils, and Peyer's patches from the blood by crossing through specialized postcapillary venules referred to as high endothelial venules.[64] This passage is mediated by adhesion molecules, some of which are constitutively expressed and others of which are induced by cytokines. For example, the constitutively expressed L-selectin on lymphocytes binds to a number of adhesion molecules on the high endothelial venules.[65] This interaction induces the expression of lymphocyte-function–associated antigen 1 (LFA-1) on lymphocytes, which facilitates the adhesion of the cells; the next step is migration of the lymphocytes across the endothelium into lymphoid tissue.

Lymphocytes from the blood enter the spleen, which lacks high endothelial venules, through the marginal zone. Thereafter, T cells travel mainly to the periarteriolar lymphoid sheaths, whereas B cells head to the lymphoid follicles.[66] The entry and exit of lymphocytes in nonlymphoid tissues, such as lung and liver, are also regulated.[56]

Germinal centers, the sites where hypermutation and class switching of immunoglobulin genes occur and memory B cells and plasma-cell precursors are generated,[67,68] are a feature of the secondary lymphoid follicles that appear in lymphoid tissue during the immune response (Fig. 8). They consist of a mesh of follicular dendritic cells that sustain rapidly dividing B cells. Surrounding these germinal centers is a mantle zone consisting of small, resting B cells. CD4 T cells and macrophages are also present in germinal centers. The organization of these structures provides a microenvironment that maximizes the generation of antibody responses by bringing all the relevant cells into intimate contact.

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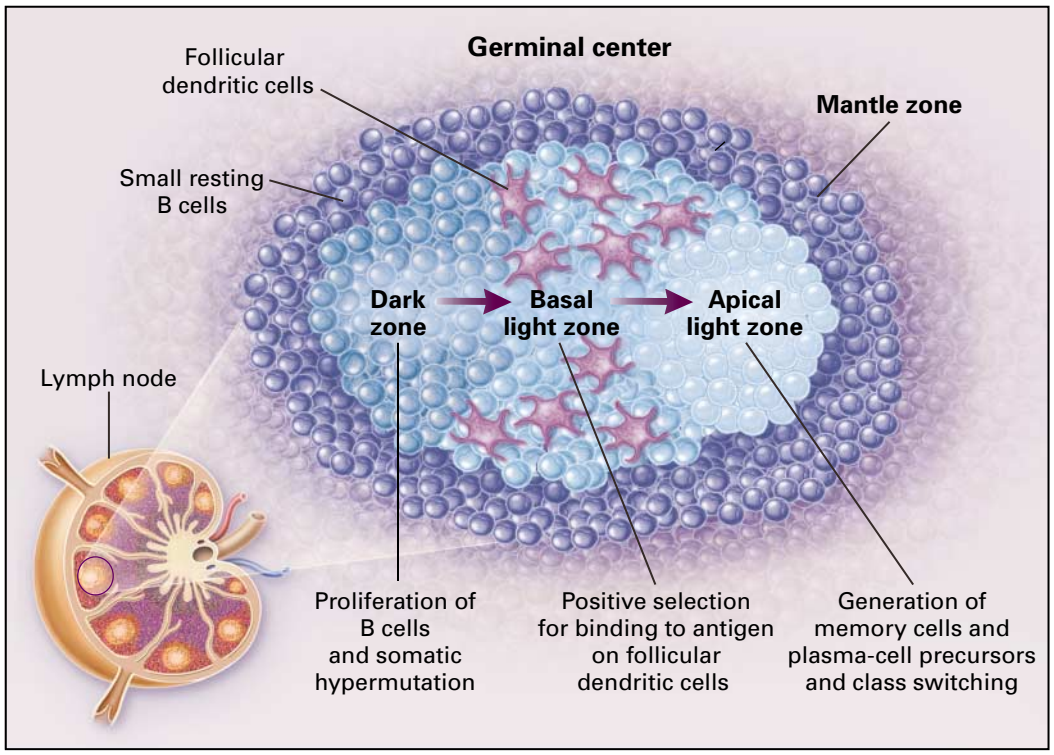

Figure 8. The Germinal Center.

During the initiation of the acquired immune response, germinal centers form in the secondary lymphoid tissues in order to create a microenvironment where all the necessary antigen-specific and innate antigen-presenting cells can interact. Several cytokines, such as interleukin-2, 4, 6, and 10 and transforming growth factor β, and various cell-surface molecules, including CD40, CD19, CD21, and B7, are critically important for these interactions. Antigen-stimulated proliferation of B cells occurs in the dark zone and is accompanied by the fine-tuning of specificity resulting from somatic hypermutation of the immunoglobulin variable-region genes. On reaching the basal light zone, high-affinity antigen-specific B cells are positively selected as a result of their interaction with antigen–antibody complexes on the surface of follicular dendritic cells. B cells that are not positively selected undergo apoptosis and are phagocytosed by tingible-body macrophages. The positively selected cells migrate to the apical light zone, where proliferation continues, class switching occurs, and memory cells and plasma-cell precursors are generated.

MOLECULAR ASPECTS OF THE IMMUNE RESPONSE

An antigen is recognized on the basis of shape: the shape of an epitope complements that of the antibody-combining site or the shape of a peptide–MHC complex complements the shape of the combining site on the T-cell receptor. The complementarity-determining regions of secreted antibodies and antigen receptors on lymphocytes bind noncovalently to the structures they recognize. The intermolecular forces involved in the binding come into play only when the complementary molecular structures are in relatively close proximity. For small antigens, the binding site on the antibody may be a pocket or cleft, but in most cases it more closely resembles an undulating surface.[69]

Antibodies, whether in their secreted form or acting as the B-cell receptor, can sometimes recognize a continuous peptide sequence. Usually, however, they recognize discontinuous epitopes, composed of amino acids that are brought together when the protein folds into its native structure.[69] Some epitopes on an antigen fit particularly well with the combining sites available in the B-cell repertoire, and the population of antibodies against these epitopes tends to dominate the polyclonal response against that antigen (Fig. 6).

The epitopes recognized by α/β T-cell receptors are, by contrast, linear peptides derived by intracellular proteolysis of the antigen. These peptides are transported to the cell surface within the peptide-binding groove of an MHC molecule, as will be described in more detail later in the series.

Although antibodies and T-cell receptors can accurately distinguish between closely related antigens, they sometimes cross-react with apparently unrelated antigens, either because the two antigens happen to share an identical epitope or because two different epitopes have similar shapes and charges. Such cross-reactions underlie the concept of molecular mimicry, whereby epitopes on microbial agents stimulate the production of antibodies (or the proliferation of T cells) that react with self antigens. Molecular mimicry may be a cause of autoimmune disease.[70,71] An example is poststreptococcal rheumatic fever, which is caused by antibodies induced by an epitope on streptococcal M protein that cross-react with a similar epitope on cardiac myosin.

Some antigens — the T-cell–independent antigens — can stimulate B cells without assistance from T cells.[72,73] Among these are polysaccharides or polymerized flagellin, which have numerous repeating epitopes. These arrays bind avidly to the B-cell receptors, and in conjunction with activation signals that can be provided by a variety of cell types, they activate B cells without the need for help from CD4 T cells. T-cell–independent antigens do not induce the formation of germinal centers and are therefore unable to induce the generation of memory B cells or the somatic hypermutation that results in the production of high-affinity antibodies. The extent of class switching from IgM to other classes of antibodies is also severely limited in the absence of cytokines from helper T cells. For these reasons, T-cell–independent antigens predominantly give rise to low-affinity IgM antibodies.

Unlike polysaccharides, most antigens are unable to stimulate B cells in the absence of help from CD4 T cells and are therefore referred to as T-cell–dependent antigens. When these antigens are bound by B-cell receptors, they are internalized and processed by the B cell into short peptides, which are brought to the cell surface by MHC class II molecules. Neighboring CD4 T cells that recognize these peptide–MHC complexes become activated and express costimulatory molecules such as CD154 (also called CD40 ligand) on their surface. When the CD154 on the activated T cell binds to its receptor, CD40, on the B cell, a signal is generated that prompts the B cell to begin the processes of somatic hypermutation and immunoglobulin class switching.

Help is also provided by various cytokines, such as interleukin-2, 4, and 5, released from the helper T cells. Dendritic cells and macrophages, by presenting peptide–MHC class II complexes, can also activate helper CD4 T cells, and through this pathway the activated T cells also express costimulatory molecules and release immunostimulatory cytokines. Certain sequences of microbial DNA, particularly those containing unmethylated CpG motifs (cytosine–guanosine dinucleotide sequences flanked by two 5′ purines and two 3′ pyrimidines), can stimulate B cells directly. They also have adjuvant properties, which are mediated by an activating effect on dendritic cells and macrophages.[74,75]

Once the immune system is stimulated by an immunogenic epitope, additional epitopes on the antigen may be drawn into the response as a result of the general up-regulation of antigen processing and presentation. This effect, referred to as epitope spreading,[76] may spill over to other antigens (intermolecular spreading). Its clinical relevance is that in some autoimmune diseases, notably systemic lupus erythematosus, a structural complex of several independent molecules, as occurs in the nucleosome, may provoke a broad spectrum of autoantibodies.

Cryptic epitopes that are normally not recognized efficiently by the immune system may be revealed by a change in the processing of antigen caused by the stimulation of antigen-presenting cells by proinflammatory cytokines, as might occur during the processing of myelin basic protein.[77] Moreover, the processing of antigen by B cells may generate peptides that are not produced by dendritic cells or macrophages. For example, the model antigen hen-egg lysozyme has been used to demonstrate that antigen processed by dendritic cells focuses the immune response on a limited number of epitopes, whereas B cells may increase the diversity of the T-cell response by presenting a broader range of processed peptides in association with their MHC molecules.[78]

The continual mutation of microorganisms causes a phenomenon called antigenic drift. The mutants pose problems for the memory component of the immune system. An even greater risk ensues from the exchange of genetic material between related organisms, causing antigenic shift.[79] Very few, if any, memory cells that were generated during exposure to the native organism may be able to recognize the new variant. An example of the devastating effects of antigenic shift is the influenza pandemics that have killed large numbers of people as the virus has swept relatively unchallenged across the globe.

ACTIVATION AND REGULATION OF LYMPHOCYTES

T-cell receptors are associated on the surface of T cells with the CD3 complex of molecules that transmit activation signals into the cell when the T-cell receptor binds antigen. This complex consists of CD3γ, CD3δ, and two molecules of CD3ϵ, together with a disulfide linked ζ chain homodimer.[80] Cross-linking of the T-cell receptor, which occurs when it binds to peptide–MHC complexes on cell surfaces, initiates the activation signals. Aggregation of the receptor leads to phosphorylation of tyrosines in the cytoplasmic tails of the CD3 complex, and the transduction of the downstream signal to the nucleus that follows such phosphorylation initiates the transcriptional ac-

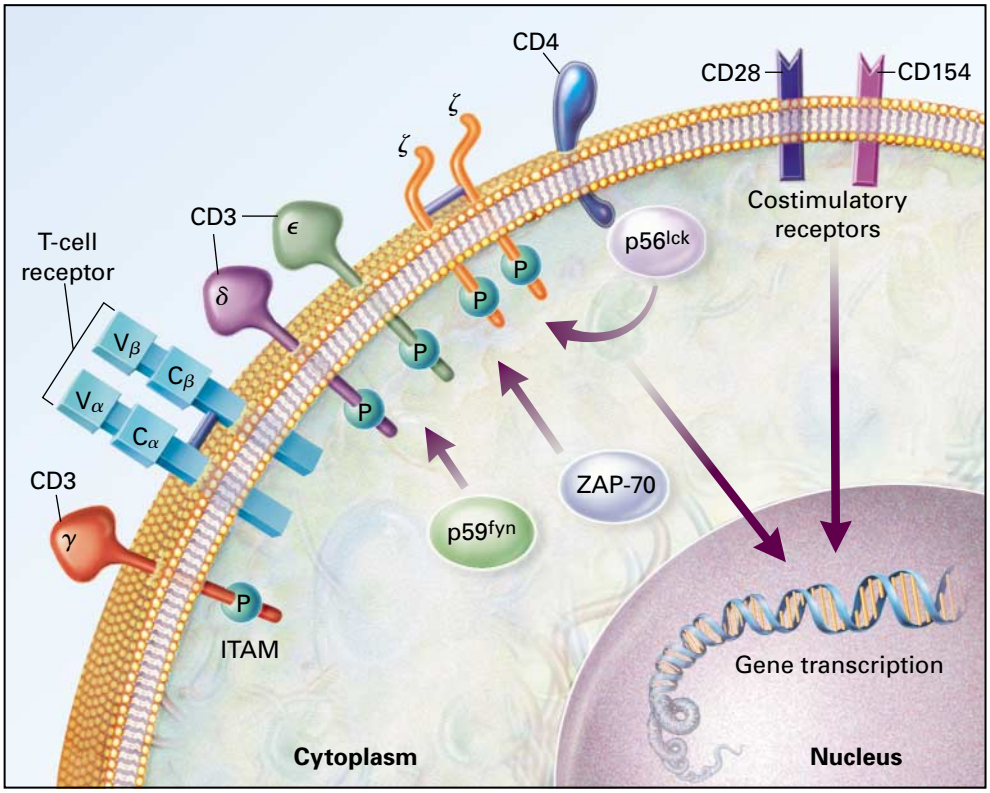

Figure 9. Activation of T Cells.

The activation of T cells involves a highly complex series of integrated events that result from the cross-linking of the antigen receptor on the cell surface. Because the antigen receptors have extremely short cytoplasmic tails, they are associated (in T cells) with the CD3 and ζ chain signal-transduction molecules bearing cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs), which are subject to phosphorylation (P) by protein kinases such as $p56^{lck}$, $p59^{fyn}$, and ZAP-70 (for simplicity, only one of the CD3ϵ molecules is shown). The initial stages of activation also involve the binding of $p56^{lck}$ to the cytoplasmic tail of CD4 (in helper T cells) or CD8 (in cytotoxic T cells). These events lead to downstream signaling involving a number of different biochemical pathways and ultimately to the transcriptional activation of genes involved in cellular proliferation and differentiation. Signals from costimulatory receptors such as CD28 and CD154 must also be present in order to activate the lymphocyte; in the event that signals are sent only from the antigen-receptor signal-transducing molecules, anergy or apoptosis will occur.

tivation of various gene sequences, including those encoding cytokines that stimulate and regulate the proliferation of T cells (Fig. 9). The B-cell receptor also associates with two molecules, Igα (CD79a) and Igβ (CD79b), which transmit activation signals to the cell.[81] Mirroring the cytoplasmic tails of the CD3 complex of T cells, Igα and Igβ also undergo phosphorylation, an essential feature of the transduction of signals to the nucleus of the B cell.

Signaling by means of the antigen receptors alone, in the absence of costimulatory signals, does not activate lymphocytes. Instead, such isolated signals lead to anergy or apoptosis. The additional signals required for the activation of lymphocytes come from various costimulatory molecules on the surface of neighboring cells and soluble mediators such as cytokines. Molecules on the surface of T cells that bind to costimulatory molecules on antigen-presenting cells include CD28, whose ligand is B7; CD154 (the CD40 ligand), which binds to CD40; and CD2, the ligand for CD58.[82-84] Essentially, the molecules constitute receptor–ligand pairs that are required for activating and regulating lymphocytes.

Activated dendritic cells are particularly potent stimulators of naive T cells, because they express large amounts of the costimulatory B7 and CD40 molecules.[85] The need for these molecules to participate in the activation of the immune response can be exploited clinically. Agents such as the CD28–immunoglobulin fusion molecule, which interferes with

the CD28–B7 interaction, have great potential in limiting the rejection of transplanted organs by interfering with the activation of T cells.[86]

Ligation of CD40 (constitutively expressed by B cells) by CD154 (induced on antigen-stimulated CD4 T cells) activates protein kinases within the B cell that mediate antibody class switching. Defects in the gene encoding CD154 occur in the X-linked hyper-IgM syndrome, in which affected patients have low or undetectable levels of IgG, IgA, and IgE but normal or elevated levels of IgM.[87] (This subject will be discussed in more detail later in the series.)

Another receptor for costimulation is CD45, a phosphatase enzyme with a critical role in the activation of both T cells and B cells.[88] The costimulatory molecules that activate CD45 have yet to be fully defined, but they may include CD22, an adhesion receptor on the surface of B cells.

Cytokines, including interleukin-1, interleukin-6, and tumor necrosis factor α, also provide costimulatory signals.[89] However, not all signals from cytokines and cell-surface molecules are stimulatory. Interleukin-10 and transforming growth factor β often provide negative signals.[90,91] Similarly, ligation of the T-cell–surface molecule CTLA-4 by B7, in contrast to the ligation of CD28 by B7, provides a down-regulating signal,[92] as does ligation of the Fcγ receptor for IgG on B cells.[93,94]

EFFECTOR FUNCTIONS OF T CELLS

CD4 T cells are mainly cytokine-secreting helper cells, whereas CD8 T cells are mainly cytotoxic killer cells. CD4 T cells can be divided into two major types.[95] Type 1 (Th1) helper T cells secrete interleukin-2 and interferon-γ but not interleukin-4, 5, or 6. Type 2 (Th2) helper T cells secrete interleukin-4, 5, 6, and 10 but not interleukin-2 or interferon-γ (Fig. 10).

Cytokines (Table 1) have a central role in influencing the type of immune response needed for optimal protection against particular types of infectious agents, and they may also normally reduce allergic and autoimmune responses.[96,97] For example, the release of interleukin-12 by antigen-presenting cells stimulates the production of interferon-γ (immune interferon) by Th1 cells. This cytokine efficiently activates macrophages, enabling them to kill intracellular organisms. To generalize and simplify somewhat, the production of cytokines by Th1 cells facilitates cell-mediated immunity, including the activation of macrophages and T-cell–mediated cytotoxicity; on the other hand, Th2 cells help B cells produce antibodies.

The elimination of virally infected cells falls to the CD8 cytotoxic T cell. The infected cell marks itself as a target for the cytotoxic T cell by displaying peptides derived from intracellular viral protein on its surface. These viral peptides are bound to the peptide-binding regions of class I MHC molecules. Cytotoxic T cells bind to this viral peptide–MHC complex and then kill the infected cell by at least two different pathways. They can insert perforins into the target-cell membrane, which produce pores through which granzymes are passed from the cytotoxic T cells into the target cell. At least one of these proteolytic enzymes activates the caspase enzymes that mediate apoptosis in the target cell. Alternatively, cytotoxic T cells can bind the Fas molecule on the target cell using their Fas ligand, a process that will also activate caspases within the target cell and ultimately induce apoptosis.[98] These killing strategies not only deprive the virus of the host enzymes needed for replication but also deny it a sanctuary within the cell. Any released virus is immediately susceptible to the effects of antibody.

In addition to killing infected cells directly, CD8 T cells also produce a number of cytokines, including tumor necrosis factor α and lymphotoxin. Interferon-γ, another product of CD8 cells, reinforces antiviral defenses by rendering adjacent cells resistant to infection.[99] To a minor extent, CD4 cells of both the Th1 and Th2 populations can become cytotoxic on recognizing peptides presented by MHC class II molecules.[100,101]

Rearrangement of T-cell receptor genes does not occur in most natural killer cells and these cells thus do not express specific T-cell receptors. They use broad-spectrum, less-specific receptors to identify target cells in innate cytotoxic immune responses.[102] However, natural killer T cells, a distinct population of cells with certain phenotypic characteristics of natural killer cells, express CD4 and intermediate levels of T-cell receptors with a highly restricted repertoire. These cells recognize antigen presented by the nonclassic MHC molecule CD1 and may have an immunoregulatory role, because they can secrete interleukin-4 and interferon-γ.[103]

IMMUNE PROTECTION BY ANTIBODIES

When B cells undergo terminal differentiation into plasma cells, they acquire the ability to produce and secrete high levels of antibodies. These antibodies can be directly protective if they sterically inhibit the binding of a microorganism or toxin to the corresponding cellular receptor. In most instances, however, antibodies do not function in isolation but instead marshal other components of the immune system to defend against the invader[104] (Fig. 11).

The classic pathway of complement activation is triggered by contact of its first component, C1q, with closely associated Fc regions of IgM or IgG on the surface of a cell.[18] The binding of these arrays to C1q leads to the generation of C3b and other biologically active complement components. Enhanced phagocytosis in the presence of IgM or IgG antibacterial antibodies can occur after binding of the newly generated C3b to receptors on neutrophils and

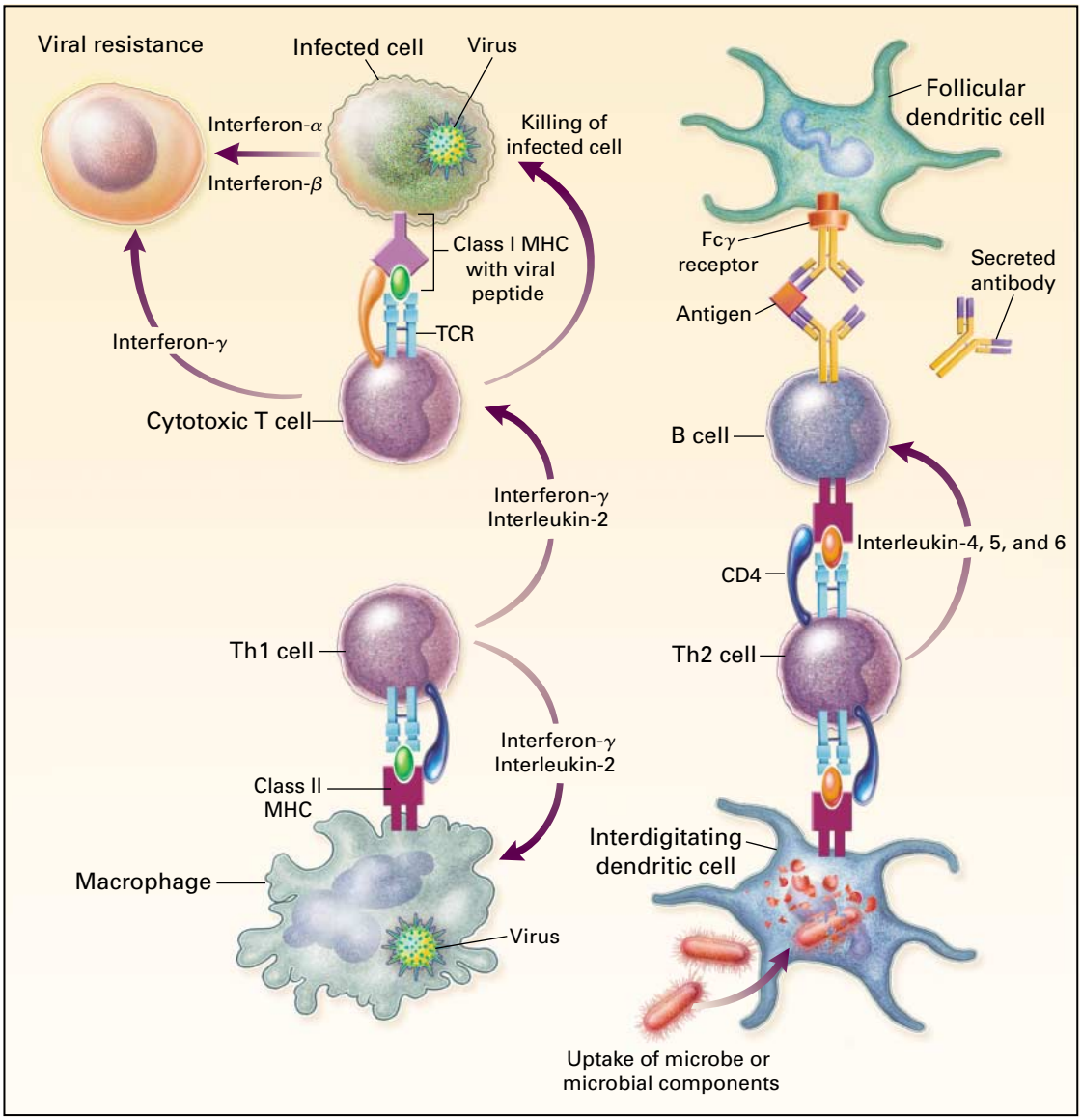

Figure 10. An Overview of Lymphocyte Responses.

T cells characteristically possess T-cell receptors (TCRs) that recognize processed antigen presented by major-histocompatibility-complex (MHC) molecules, as shown on the left-hand side of the figure. Most cytotoxic T cells are positive for CD8, recognize processed antigen presented by MHC class I molecules, and kill infected cells, thereby preventing viral replication. Activated cytotoxic T cells secrete interferon-γ that, together with interferon-α and interferon-β produced by the infected cells themselves, sets up a state of cellular resistance to viral infection. As shown on the right-hand side of the figure, helper T cells are generally positive for CD4, recognize processed antigen presented by MHC class II molecules, and can be divided into two major populations. Type 1 (Th1) helper T cells secrete interferon-γ and interleukin-2, which activate macrophages and cytotoxic T cells to kill intracellular organisms; type 2 (Th2) helper T cells secrete interleukin-4, 5, and 6, which help B cells secrete protective antibodies. B cells recognize antigen either directly or in the form of immune complexes on follicular dendritic cells in germinal centers.

macrophages. In the absence of complement, microorganisms coated with IgG, IgA, or IgE antibody bind to the corresponding Fc receptors (FcγR, FcαR, or FcϵR) on phagocytic cells. IgG and IgE antibodies can mediate antibody-dependent cellular cytotoxicity, an extracellular killing process in which cells bearing Fc receptors for these classes of antibody become linked to antibody-coated target cells or parasites.[105] Natural killer cells, monocytes, macrophages, and neutrophils can all function in IgG-mediated antibody-dependent cellular cytotoxicity; macrophages, eosinophils, and platelets are involved in IgE-medi-

TABLE 1. EXAMPLES OF CYTOKINES AND THEIR CLINICAL RELEVANCE.*

CYTOKINE	CELLULAR SOURCES	MAJOR ACTIVITIES	CLINICAL RELEVANCE
Interleukin-1	Macrophages	Activation of T cells and macrophages; promotion of inflammation	Implicated in the pathogenesis of septic shock, rheumatoid arthritis, and atherosclerosis
Interleukin-2	Type 1 (Th1) helper T cells	Activation of lymphocytes, natural killer cells, and macrophages	Used to induce lymphokine-activated killer cells; used in the treatment of metastatic renal-cell carcinoma, melanoma, and various other tumors
Interleukin-4	Type 2 (Th2) helper T cells, mast cells, basophils, and eosinophils	Activation of lymphocytes, monocytes, and IgE class switching	As a result of its ability to stimulate IgE production, plays a part in mast-cell sensitization and thus in allergy and in defense against nematode infections
Interleukin-5	Type 2 (Th2) helper T cells, mast cells, and eosinophils	Differentiation of eosinophils	Monoclonal antibody against interleukin-5 used to inhibit the antigen-induced late-phase eosinophilia in animal models of allergy
Interleukin-6	Type 2 (Th2) helper T cells and macrophages	Activation of lymphocytes; differentiation of B cells; stimulation of the production of acute-phase proteins	Overproduced in Castleman's disease; acts as an autocrine growth factor in myeloma and in mesangial proliferative glomerulonephritis
Interleukin-8	T cells and macrophages	Chemotaxis of neutrophils, basophils, and T cells	Levels are increased in diseases accompanied by neutrophilia, making it a potentially useful marker of disease activity
Interleukin-11	Bone marrow stromal cells	Stimulation of the production of acute-phase proteins	Used to reduce chemotherapy-induced thrombocytopenia in patients with cancer
Interleukin-12	Macrophages and B cells	Stimulation of the production of interferon-γ by type 1 (Th1) helper T cells and by natural killer cells; induction of type 1 (Th1) helper T cells	May be useful as an adjuvant for vaccines
Tumor necrosis factor α	Macrophages, natural killer cells, T cells, B cells, and mast cells	Promotion of inflammation	Treatment with antibodies against tumor necrosis factor α beneficial in rheumatoid arthritis
Lymphotoxin (tumor necrosis factor β)	Type 1 (Th1) helper T cells and B cells	Promotion of inflammation	Implicated in the pathogenesis of multiple sclerosis and insulin-dependent diabetes mellitus
Transforming growth factor β	T cells, macrophages, B cells, and mast cells	Immunosuppression	May be useful therapeutic agent in multiple sclerosis and myasthenia gravis
Granulocyte–macrophage colony-stimulating factor	T cells, macrophages, natural killer cells, and B cells	Promotion of the growth of granulocytes and monocytes	Used to reduce neutropenia after chemotherapy for tumors and in ganciclovir-treated patients with AIDS; used to stimulate cell production after bone marrow transplantation
Interferon-α	Virally infected cells	Induction of resistance of cells to viral infection	Used to treat AIDS-related Kaposi's sarcoma, melanoma, chronic hepatitis B infection, and chronic hepatitis C infection
Interferon-β	Virally infected cells	Induction of resistance of cells to viral infection	Used to reduce the frequency and severity of relapses in multiple sclerosis
Interferon-γ	Type 1 (Th1) helper T cells and natural killer cells	Activation of macrophages; inhibition of type 2 (Th2) helper T cells	Used to enhance the killing of phagocytosed bacteria in chronic granulomatous disease

*AIDS denotes acquired immunodeficiency syndrome.

ated antibody-dependent cellular cytotoxicity. The cytotoxic mechanisms, which come into play when the target cell is too large for phagocytosis, include perforins, granzymes, and in some cases, reactive oxygen intermediates.[106]

Adults produce 3 to 4 g of secretory IgA per day. This form of IgA occurs selectively in saliva, colostrum, and other fluids. It is synthesized by plasma cells underlying mucosal surfaces and then transported across the epithelium by the polyimmunoglobulin Fc receptor.[107] On the luminal side, the released antibodies prevent the adhesion of microbes to the surface of host cells. A second type of epithelial Fc receptor, FcRn, has a number of functions,[108] including transferring maternal IgG across the placenta. This mechanism offers important protection to the fetus before the full development of its own immune system. FcRn also specifically transfers IgG from breast milk (which also contains IgA and IgM) across the intestinal epithelium of the neonate.

REGULATION OF THE IMMUNE RESPONSE

A successful immune response eliminates the inciting antigen, and once rid of this stimulus, the response returns to a near basal level. In addition to purging itself of antigen, the immune system uses several other mechanisms, initially to fine-tune and later to down-regulate its activity. IgG itself can switch off the response to its corresponding antigen, a process analogous to negative feedback loops in the en-

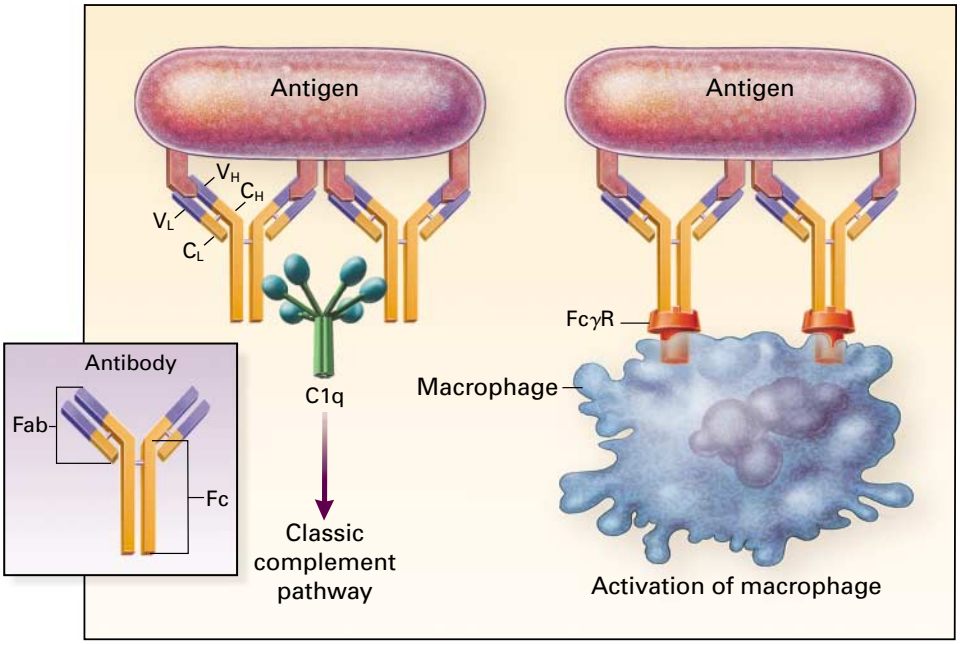

Figure 11. Role of Antibodies.

Antibodies rarely act in isolation. Their usual role is to focus components of the innate immune system on the pathogen, and the activation of these destructive forces normally requires coordinating events that occur after Fab heavy- and light-chain variable regions (V_H and V_L) of the antibody are bound to antigen, leading to the display of multiple exposed Fc regions. The figure shows two examples of this process: the activation of the classic complement pathway after binding of C1q to Fc, and the activation of phagocytosis after the cross-linking of Fc receptors and binding of the FcγR on the macrophage.

docrine system. This suppression of IgG production occurs when the FcγR and the B-cell receptor on the same cell are linked by immune complexes containing the relevant antigen. The result is the transmission of inhibitory signals into the nucleus of the B cell.[94]

Cytokines participate in another level of regulatory control. For example, the secretion of interferon-γ by Th1 cells inhibits Th2 cells, and the secretion of interleukin-10 by Th2 cells reciprocally inhibits Th1 cells.[95] Exploitation of such regulatory interactions between subgroups of T cells may lead to new therapeutic approaches for a variety of diseases. For example, the secretion of interleukin-4 by Th2 cells stimulates the production of IgE, so that devising a way of shifting the system to induce a Th1-cell–mediated response could ameliorate atopic allergy. Immunoregulation also involves interactions of the immune system with both the endocrine and nervous systems, with cross-talk between these systems involving hormones, cytokines, and neurotransmitters.

IMMUNOLOGIC TECHNOLOGY

The use in immunologic research of mice in which selected genes are either expressed (transgenic mice) or eliminated (knockout mice) has provided many valuable insights.[109] Transgenic animals are created by the injection of DNA containing the desired gene into the pronucleus of a fertilized egg. The eggs are then implanted into the uterus of a receptive female mouse, where they develop into fully formed transgenic infants. Tissue-specific and inducible promoters allow the expression of the transgene to be controlled. For example, studies in animal models of type 1 (autoimmune) diabetes use the insulin promoter to direct expression of transgenes to beta cells in the islets of Langerhans.[110]

In knockout animals a disabled version of a gene is created by the introduction of mutations with recombinant DNA methods. The disabled gene contains sequences incorporated by the investigator that promote the selection of cells in which the mutant gene has homologously replaced the normal gene (sequences endowing the cell with resistance to a particular drug are useful for this purpose). The defective gene is introduced into embryonal stem cells, and if it integrates into the locus occupied by the cell's normal copy of the gene (i.e., if it knocks out the normal gene), the stem cell becomes a carrier of the mutant gene and lacks the normal gene. These rare mutant cells are selected with use of drug-resistance markers, injected into blastocysts, and implanted in receptive females for further differentiation and development.

One initially surprising finding obtained in knockout mice was that the deletion of several molecules that had been thought to have obligatory roles in the immune system had only a minor effect on immune responses. These dispensable genes include interleukin-6 and 11. The implication of these findings is that there must be functional overlap between certain cytokines, such as between interleukin-1 and tumor necrosis factor α or between interleukin-6 and 11; if one cytokine is defective or absent, the other can act in its place. However, such redundancy is not absolute, because each cytokine has unique as well as shared properties.

The ability to create transgenic and knockout mice has also led to the development of animal models of autoimmune and immunodeficiency diseases. For example, using a transgene for a foreign protein such as hen's-egg lysozyme, the antigen effectively becomes a self antigen because it appears very early in the ontogeny of the transgenic animal. In this kind of experiment, B cells are not made tolerant of hen's-egg lysozyme that is expressed as a membrane-associated self antigen on thyroid epithelium, implying that tolerance of cell-associated thyroid antigens depends on the induction of tolerance in helper T cells.[111] Another approach for studying autoimmune mechanisms is to monitor transgenic antigen receptors (B-cell receptors or T-cell receptors) with specificity for self antigens to see whether tolerance or autoimmune abnormalities develop in the animals.[112] This approach was used to show that B cells with the genetic potential to produce high-affinity rheumatoid factor develop central tolerance, whereas B cells that can produce low-affinity rheumatoid factor do not.

The remarkable specificity of the antibody molecule has been exploited in many areas of biomedicine. Several clinically useful monoclonal antibodies, such as muromonab-CD3 (OKT3), used in thwarting rejection of organ allografts, have been produced.[113,114] It is sometimes advantageous to use antibody fragments, either Fab or the bivalent $F(ab')_2$ produced by proteolytic cleavage, or single-chain variable fragments (scFv) generated by recombinant DNA technology. Such fragments are particularly useful if the aim is to penetrate a tumor mass. Most monoclonal antibodies are of rodent origin and are therefore recognized as foreign by the human immune system, severely compromising their efficacy. However, with the use of recombinant-DNA technology, these antibodies can be "humanized" by the replacement of most of the rodent amino acid sequences with human sequences; only the amino acids that come into contact with the antigen need to be retained from the rodent sequence.

Recombinant-DNA technology can also be used to create a library containing millions of bacteriophages, each bearing a different antibody of a single specificity on its surface and containing the genes for that antibody.[115] When the contents of this library are poured onto antigen-coated plastic dishes, phages bearing the relevant antibody will bind to the antigen, and those that do not can be washed away. This technique permits antibodies of the desired specificity to be selected from the library, and the genes encoding the variable regions can be used to produce large amounts of recombinant antibody of virtually any desired specificity.[116] The use of phage libraries constructed from randomly paired heavy- and light-chain human antibody genes allows the direct selection of human antibodies for therapeutic use against, for example, tumor-associated antigens and self antigens such as CD4. Such specificities might otherwise be unobtainable as a result of clonal deletion of anti-self lymphocytes. If necessary, individual amino acid replacements in the binding site can be engineered to increase the affinity of the antibody.

Antibodies can be tagged with enzymes, fluorochromes, or isotopes for use in a wide range of clinical applications, including immunoassays, diagnostic phenotyping, histochemistry, in vivo imaging, and immunotherapy. If an antibody molecule is itself the target (for example, for measurement of autoantibodies in patients with autoimmune disease), labeled anti-immunoglobulins (secondary antibodies) may be tagged instead. For therapeutic applications against tumor cells or autoreactive cells, antibodies can be conjugated with cytotoxic molecules to produce immunotoxins.[117] An extra dimension can be provided by linking two different antibodies, or antibody fragments, to produce immunotherapeutic bispecific antibodies.[118] Undoubtedly, these approaches have greatly expanded the investigative, diagnostic, and therapeutic armamentarium of immunologists and clinicians.

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