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Editors

THE IMMUNE SYSTEM

Second of Two Parts

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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 lymphocytes⁵⁹ 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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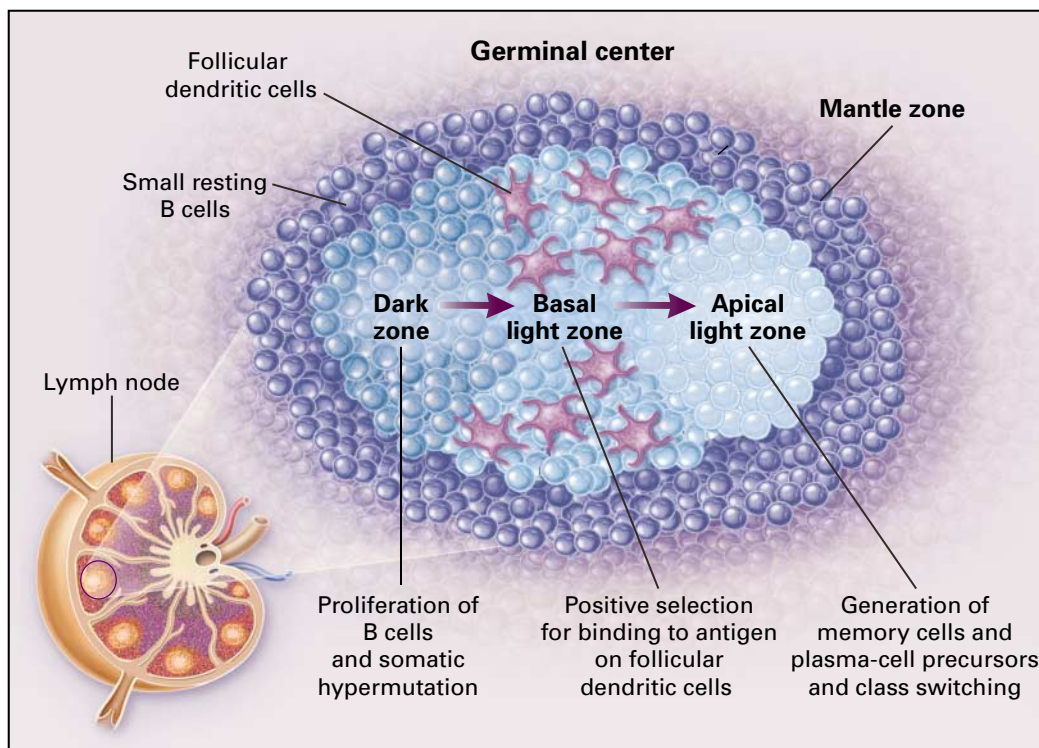


Figure 8. The Germinal Center.

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' pu-

rines 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 CD3 γ , CD3 δ , and two molecules of CD3 ϵ , 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-

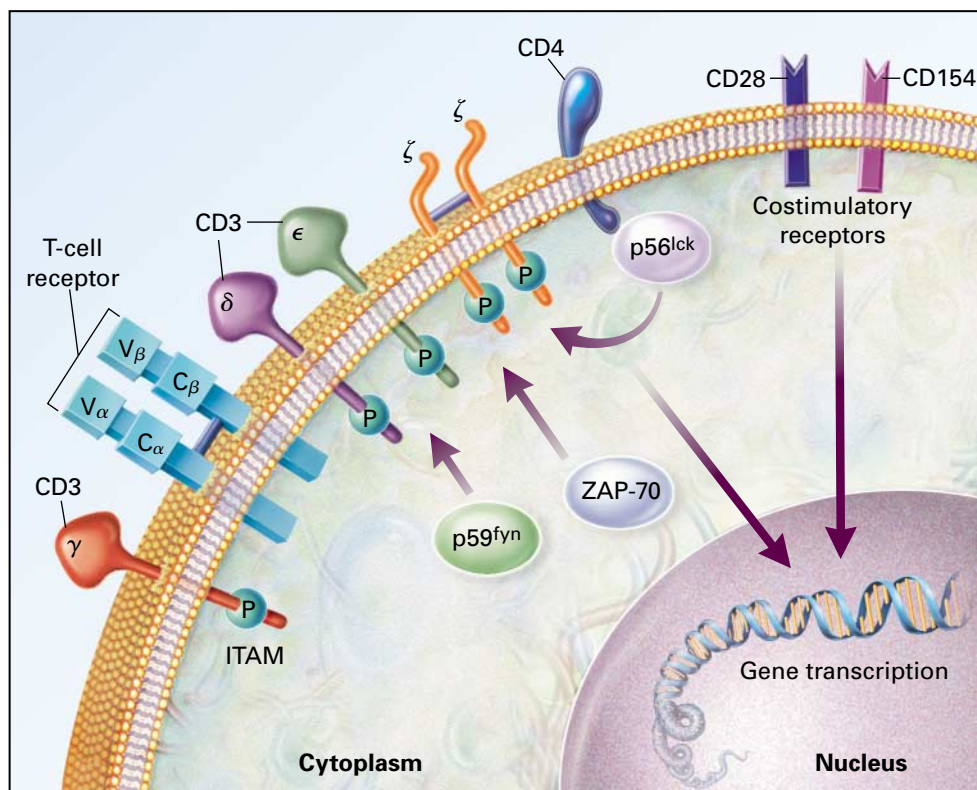


Figure 9. Activation of T Cells.

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 CD3 ϵ 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, Ig α (CD79a) and Ig β (CD79b), which transmit activation signals to the cell.⁸¹ Mirroring the cytoplasmic tails of the CD3 complex of T cells, Ig α and Ig β 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 neighbor-

ing 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 Fc γ 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. Cy-

tototoxic 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 non-classic 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 invader¹⁰⁴ (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

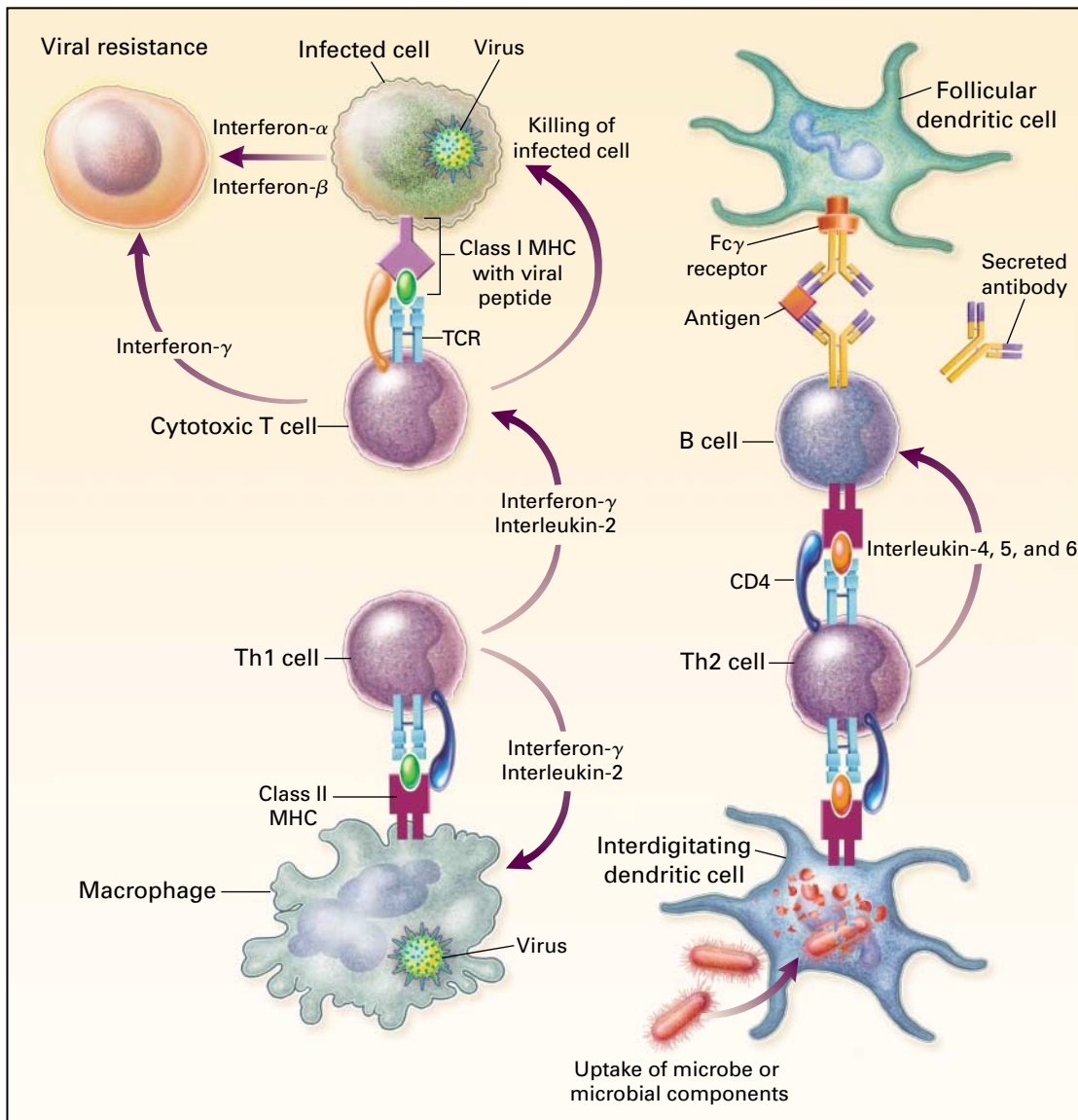


Figure 10. An Overview of Lymphocyte Responses.

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 (Fc γ R, Fc α R, or Fc ϵ R) 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-

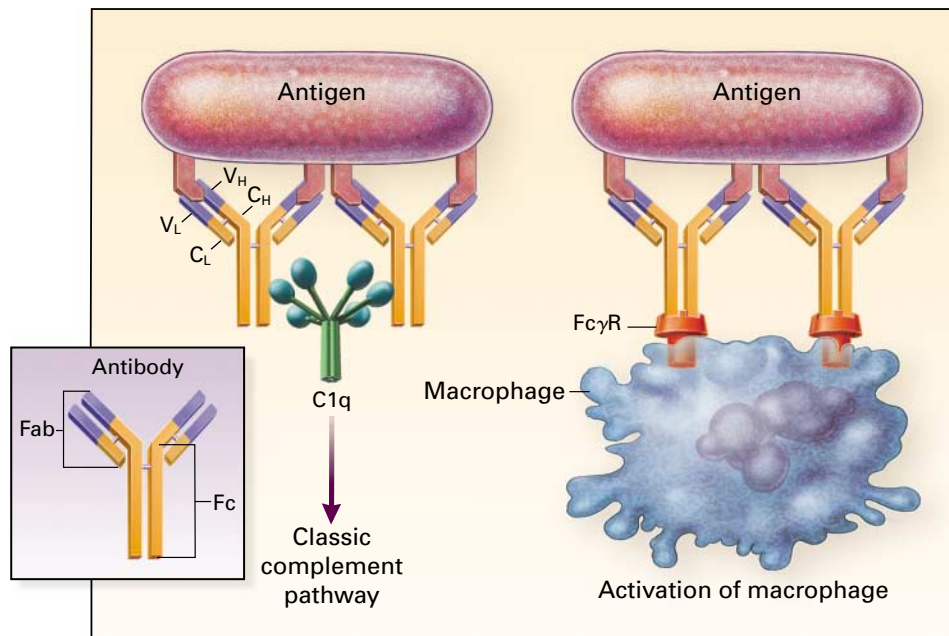


Figure 11. Role of Antibodies.

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 $Fc\gamma R$ on the macrophage.

doctrine system. This suppression of IgG production occurs when the $Fc\gamma R$ 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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