First line of Defense against Infection
- It includes various non-specific defense systems.
- Nonspecific defense systems
  - skin, epithelial, mucin
  - recognition of LPS + lipoteichoic acid (LTA)

Second line of Defense
- includes specific defense systems
  - e.g. B-cells → humoral immunity
  - T-cells → cell mediated immunity

However, specific and non-specific defenses are linked by cytokines/chemokines.

Darkside of immune system
Non-specific → sepsis (microbial infection of blood)
Sepsis shock (symptoms caused by microbial/antigen)
  (infection in bloodstream e.g. toxin)

Specific → autoimmune disease (e.g. multiple sclerosis, Rheumatoid arthritis, Juvenile diabetes.)
Chapter 4

A Simple squamous epithelium

B Simple cuboidal epithelium

C Stratified squamous epithelium

D Simple columnar epithelium

E Ciliated columnar epithelium

Figure 4-2 Different types of epithelial cells. (A) Simple squamous epithelium. (B) Simple cuboidal epithelium. (C) Stratified squamous epithelium (upper layers of cells are dead; typical of skin). (D) Simple columnar epithelium. (E) Ciliated columnar epithelium showing goblet cells, which secrete mucus.

that of the body interior (37°C). Accordingly, bacteria that succeed in colonizing skin must be able to adapt to the very different internal environment of the body if they manage to reach underlying tissue. Hair follicles, sebaceous glands, and sweat glands offer natural breaches in the skin that can be used by some bacteria to move past the skin surface. These natural breaches are protected by lipids that are toxic to many bacteria and by the enzyme lysozyme, which degrades the peptidoglycan cell wall of bacteria. Some pathogenic bacteria are capable of infecting hair follicles or sweat glands. This is why skin infections such as boils or furuncles are commonly centered around hair follicles.

Normal Microbiota

The defenses of the skin do not completely prevent bacterial growth, as is evident from the fact that there are bacteria capable of colonizing skin. Normally, these are innocuous gram-positive bacteria, a mixture of cocci and rods. A bacterial population that is found continuously in some body site without causing disease is called the resident microbiota of that site. The skin microbiota helps to protect against pathogenic bacteria by occupying sites that might be colonized by pathogenic bacteria. It also competes with incoming pathogens for essential nutrients. The microbiota does not completely prevent colonization of skin by potential pathogens, but colonization by pathogenic bacteria is usually transient.

Since transient colonization with pathogens can occur, and since even normally harmless skin bacteria can cause infections under certain conditions, hand washing and disinfection of hands provide a barrier to infection of oneself and other people. In hospitals, latex gloves provide yet another barrier.
Epithelia
- tightly packed (proteins cell tight junctions & desmosomes)
- polar cells - apical surface & basolateral surfaces.

- simple epithelium & stratified epithelium
  - columnar, cuboidal and squamous (flat)
- internal epithelial usually covered by a layer of mucus
  (mucosal cells)
  - barrier + sloughing off.

Skin
- stratified squamous cells
- Keratinocytes => keratin
- pH 5, 34-35°C
- Natural breaches - hair follicles and sweat glands.
  - bacteria can enter
- Skin cells in the breaches produce toxic lipids & lysozyme
- Normal microbiota - compete with pathogens
- Dendritic cells (under the skin cells)
  - process invaders and activate the specific defense
    (antigen presentation)
- Skin-associated lymphoid tissue (skin's own specific defense)
  (special lymphocytes under the skin) (SALT)
Nasal surfaces
- Goblet cells produce mucus (glycoproteins)
- continuously sloughing off in blobs
- Ciliated cells → directional
- Lysosomes
- Lactoferrin (iron-binding protein)
- defensins → toxic peptides
  - found in mouth + tongue + intestinal mucosa (crypts)
- Secretory immunoglobulin A (sIgA)
- Mucosa-associated lymphoid tissue (or gastro-intestinal-associated lymphoid tissue) (GALT)
  - macrophage, T cells + B cells
  → sIgA

Defenses (Non-specific) in Blood & Tissue.

(similar to Tc but no memory)
- Natural killer cells → attack infected cells
- Phagocytes → ingest & kill bacteria
  - neutrophil (Polymorphonuclear leukocytes, PMN)
  - monocytes, macrophages, eosinophils
- Mast cells → histamine → vasodilatation → PMN + NK + monocytes and Basophils
have associated lymphoid tissues. The best studied of these systems is the gastrointestinal-associated lymphoid tissue (GALT) found in the follicles and Peyer's patches, which are most highly concentrated in the ileum and rectum. Similar lymphoid tissues are found in the respiratory and vaginal tracts. This group of mucosal immune systems is called mucosa-associated lymphoid tissue (MALT). Skin also has a similar system, called the skin-associated lymphoid tissue (SALT). The Langerhans cells of the epidermis are the APCs of this system.

The cells of the GALT are illustrated in Figure 6–8. The M (microfold) cell takes up antigens from the lumen of the intestinal tract and passes them to GALT macrophages, which presumably act as the APCs. The process by which GALT macrophages process antigens and elicit production of cytotoxic T cells or antibodies is the same as that described in earlier sections, except that the macrophages, B cells, and T cells of the GALT home specifically to mucosal membranes and appear not to be part of the humoral immune system. Memory T and B cells stimulated by antigen processing at the GALT can migrate to other mucosal sites and vice versa. Thus, stimulation of one of the MALT sites results in general mucosal immunity. This characteristic of the MALT system is what makes oral vaccines feasible. Initially, oral vaccines stimulate the GALT, but IgA against vaccine antigens is later detectable in other sites. Thus, an oral vaccine can be used to elicit immunity to respiratory and, presumably, genital pathogens.

Currently, efforts are being made to develop vaccines administered by inhalation, so that stimulation of the bronchial MALT would produce an sIgA response at other MALT sites. These vaccines would have the advantage that they need not pass through the stomach. Vaccines that target the GALT have to be capable of surviving the low-pH and protease-rich stomach environment, a barrier that has proved problematical in some cases. Administering vaccines by rectal or vaginal suppositories is theoretically possible, but this strategy has not been actively pursued to date.

When the GALT is stimulated, one outcome is production of IgA (Figure 6–8). IgA binds to the poly-Ig receptor on the basal surface of mucosal cells and is then taken up and carried in vesicles to the apical surface, where it is released. Release involves proteolytic cleavage of the receptor, and a portion of the receptor remains attached to the IgA, making it sIgA. Activation of the GALT can also lead to production of cytotoxic T cells. These cells probably remain on the basal side of the mucosa, although it is possible that during an infection some of them migrate to the apical surface, especially in areas where damage to the mucosa has occurred. GALT cytotoxic T cells are important for protection against viral infections of the gastrointestinal tract and some bacterial infections in which the bacteria multiply inside mucosal cells.

One of the many mysteries associated with the intestinal immune mucosa is the role of a type of mucosal cell called gamma-delta T cells. The majority of gamma-delta T cells are CD8+ T cells and would thus be grouped with cytotoxic T cells. However, whereas cytotoxic T cells of the humoral immune system have T-cell receptors composed of alpha and beta protein subunits, the intestinal epithelial lymphocytes (IELs) have a T-cell receptor composed of related but somewhat different protein subunits called gamma and delta. Gamma-delta T cells account for fewer than 4% of circulating CD8 cells, but they account for as many as 10 to 15% of the mucosal cells in the gastrointestinal tract; in some parts, such as the colon, the level may be as high as 40%. Recently, some light has been shed on their role. In fact, gamma-delta T cells have gone very rapidly from being cells in search of a function to cells to which too many functions are now attributed.

A particularly intriguing feature of gamma-delta T cells has been that these cells have a very limited repertoire of antigen recognition. Whereas there are thousands, perhaps millions, of types of cytotoxic and T-helper cells, each of which recognizes a different antigen, the gamma-delta T cells seem to recognize only a limited number of antigens. Also, gamma-delta T cells appear to bypass the macrophage antigen presentation
Characteristics and differentiation of the various types of leukocytes of the human body. Leukocytes can be divided into three groups: auxiliary cells (platelets, megakaryocytes, mast cells, and basophils), phagocytes (neutrophils, eosinophils, monocytes, macrophages, and dendritic cells), and lymphocytes (T and B cells and plasma cells). Arrows indicate the order of development of different cell types. A dashed arrow indicates uncertainty.

Figure 4-3 Characteristics and differentiation of the various types of leukocytes of the human body. Leukocytes can be divided into three groups: auxiliary cells (platelets, megakaryocytes, mast cells, and basophils), phagocytes (neutrophils, eosinophils, monocytes, macrophages, and dendritic cells), and lymphocytes (T and B cells and plasma cells). Arrows indicate the order of development of different cell types. A dashed arrow indicates uncertainty.

Dendritic cells, whose name comes from the fact that they are covered with spiny projections that look like the dendrites of neurons, are instrumental in initiating and stimulating the immune response. They form a link between the nonspecific and immune responses because they are activated by microbial products such as LPS. The activities of these cells will be described in more detail in chapter 6.

Another type of cell that participates in the defense against bacterial infections is the mast cell. Mast cells congregate around blood vessels. If any foreign material is detected, the mast cells release granules that contain histamine, a compound that makes blood vessels leakier (vasodilation). This helps the PMNs and NK cells, which are normally circulating in blood, to leave the bloodstream and move to the site of infection. Monocytes also circulate in blood and then differentiate into macrophages as they leave the bloodstream and migrate into tissue.

Distribution of Phagocytic and Cytotoxic Cells

Blood. PMNs, monocytes, and NK cells are produced in the bone marrow and then released into the bloodstream. PMNs are the most abundant and the most short-lived of these cells. All of these cell types are capable of doing a significant amount of damage to tissue. It is not easy to kill bacteria, which are tough little critters, and considerable firepower has to be brought to bear to destroy them. If this firepower is released into surrounding tissue, human cells become vulnerable targets. The body protects itself from these potentially
Distribution of Phagocytic cells

Blood

- PMN, monocytes + NK cells are produced in bone marrow → released into blood stream.
- They spend most of their time in blood vessels without causing damage.
- Infection triggers signal proteins (cytokines and complement factors) 
  ‣ Chemokines ⇒ transmigration

  - PMNs & NK cells pass through tumor necrosis factor (TNF), IL-1, IL-8 (interleukin), blood vessels to the tissues
  ‣ Interferon (IFN-γ) produced by leukocytes

  - Monocyte ⇒ tissue

  ‣ Monocytes mature ⇒ macrophage.

Lymph

- Lymphatic vessels network around tissue.
- To remove excess fluid buildup in infected tissue.
- Phagocytes are in the lymphatic fluid to kill bacterial infection.
- Lymphatic fluid will be cleansed at lymph nodes before returning to the bloodstream.
- Yersinia pestis is able to survive the macrophage attack and multiply in lymph nodes causing an inflammatory response in the lymph nodes → buboes → bubonic plague.

**Killing mechanism of Phagocytes**

- Fig. 4-4. Phagosome + phagolysosome
- Lysosomal proteins
- Defensins
- Proteinase
- Myeloperoxidase + NADPH oxidase → O₂⁻ (superoxide radical) → H₂O₂ + hyperchloric acid

**NK cells**
- NO (nitric oxide) + O₂ → ONOO⁻ (peroxynitrite) → HO₂⁻ + hyperchloric acid

- Perforin → causes holes on cells
- Enzymes → proteases to kill target cells.

**Collateral Damage**
- Lysozyme released
- PMN (neutrophils) die and release lysosomal granules
- NK cells kill cell-infected cells & weaken surrounding cells
- E.g. Hanta virus: pulmonary syndrome
  - Leukocytosis (increase of lymphocytes)
  - Pulmonary capillary leakage

- Salmonella enterica survives in phagosome

How?

Pathogenicity is"
can enter lymphatic vessels. Because the fluid in lymphatic vessels is returned to the bloodstream, the lymph must be cleansed before it reenters the bloodstream. This task is accomplished by lymph nodes located along the lymphatic vessels.

Lymph nodes contain macrophages and the cells of the immune system (dendritic cells, T cells, and B cells). Bacteria that enter a lymph node are usually killed by the macrophages, but there are bacteria that are able to evade this fate. Some of the most dangerous pathogens are the ones that can survive and multiply in the lymph nodes. An example of such a pathogen is Yersinia pestis, the cause of bubonic plague. Y. pestis growing in lymph nodes creates an inflammatory response so intense that it causes the lymph nodes to become grossly distended, producing the buboes that give bubonic plague its name. A less pronounced, but still detectable, swelling of lymph nodes occurs during many types of bacterial infections and serves as a diagnostic sign of infection.

In chapter 6, you will learn that macrophages and dendritic cells of the lymph nodes also act as antigen-presenting cells and stimulatory cells that potentiate the specific defense response against bacteria. Thus, the lymph nodes not only sterilize lymph but also act as sites where the specific defense system is alerted that an infection is under way.

How Phagocytes Kill Bacteria

The steps in phagocytic killing of a bacterium are shown in Figure 4–4. The phagocyte first forms pseudopods that engulf the bacterium. After engulfment, the bacterium is encased in an endocytic vesicle called the phagosome. The phagosomal membrane contains ATPases that pump protons into the phagosome interior, reducing the internal pH to about 5. Phagocytes also carry antibacterial proteins that are as toxic to the phagocytes and surrounding tissue cells as they are to their bacterial targets. Accordingly, they are stored in an inactive form in lysosomal granules.

Fusion of a lysosomal granule with the phagosome to form the phagolysosome releases the lysosomal proteins into the phagolysosome interior. The low pH of the phagolysosome generated by the ATPases of the phagosome membrane activates lysosomal proteins. Lysosomal proteins have three main types of killing activity. Some lysosomal proteins are degradative enzymes, such as proteases and lysozyme, which destroy surface components of bacteria. Other lysosomal proteins, such as defensins, insert themselves into membranes, creating pores that cause the bacterium’s cytoplasmic components to leak into the surrounding environment. A third type of lysosomal protein, myeloperoxidase, produces reactive forms of oxygen that are toxic to many bacteria. Myeloperoxidase is only activated when it is brought into contact with an NADPH oxidase, which is located in the phagosome membrane, and when the resulting complex is exposed to the low pH of the phagolysosome interior. The reaction catalyzed by the myeloperoxidase complex is NADPH + 2O₂ → O₂⁻ (superoxide radical) + NADP⁺.

Superoxide radical is highly reactive. Its toxicity is due to the fact that it oxidizes disulfide linkages and thus inactivates essential bacterial surface proteins. It can also damage nucleic acids, because iron plus superoxide can disrupt bonds that hold the DNA bases together (Fenton reaction) to create breaks in the DNA.
Killing mechanism of phagocytes

- Bacteria engulfed by phagocytes forming phagosomes
- Phagosomes + lysosomes $\Rightarrow$ phagolysosomes
- Activated by the low pH of the phagosome, several lysosomal proteins become activated.
  1. Proteases
  2. Defensins
  3. Lysozyme
  4. Myeloperoxidase + NADPH oxidase
     $\xrightarrow{\text{L}} O_2^- \ (\text{superoxide radical})$
     $\xrightarrow{\text{L}} H_2O_2 + \text{hypochlorous acid (HOCl}^-)\$

[Salmonella enterica survive phagocytosis]

- Virulent genes of Salmonella, such as the SpI2 (pathogenicity island) encodes for virulent factors that prevent phagosome-lysosome fusion.
- Phagolysosomes also produce NO.
- NO reacts with $O_2^-$ to form peroxynitrite ion ($OONO^-$) to kill bacteria.

**Natural Killer cells**
- Perforin → causes holes on bacterial cells
- Granzymes → proteases to kill target cells.

**Collateral Damage**
- Lysozyme released
- PMNs die & release lysosomal granules
- NK cells kill infected cells & weaken surrounding cells.
- E.g. Hanta virus pulmonary syndrome
  - Leukocytosis (increase of lymphocytes)
  - Pulmonary capillary leakage.
The Innate Immune Response

Classical pathway
initiated by:
Antigen-antibody complexes

Lectin pathway
initiated by:
Binding of mannan-binding lectins to cell surfaces

Alternative pathway
initiated by:
Binding of C3b to cell surfaces (regulatory proteins protect host cell surfaces)

C3
C3b
C5a
C5b
C6
C7
C8
C9

Inflammation
C3 and C5a induce changes that contribute to local vascular permeability and attract phagocytes.

Lysis of foreign cells
Formation of a membrane attack complex, which creates pores in cell membranes, disrupting the integrity of the cell.

Opsonization
C3b binds to microbial cells, functioning as an opsonin.

mannan, a polymer of mannose typically found on microbial but not mammalian cells. When MBL binds to a surface, it can then interact with the complement component involved in initiating the classical pathway. mannose, p. 384

Alternative pathway. The alternative pathway is quite unlike the other pathways in how it is initiated; nearly any cell surface automatically triggers the pathway unless regulatory proteins specifically halt the process. This occurs because one of the complement proteins, C3b, readily binds cell surfaces. Unless regulatory proteins quickly inactivate C3b, a stabilizing protein will bind to it, allowing a subsequent cascade of reactions to occur. Host cell membranes contain molecules that bind those regulatory proteins, facilitating the inactivation of C3b before the alternative pathway is triggered. Those regulatory proteins are generally not associated with microbial surfaces, however, leading to complement activation by the alternative pathway. As we will discuss in chapter 19, some disease-causing bacteria have developed mechanisms to thwart complement activation by this pathway.

The nature of the complement system allows an exceedingly rapid and powerful response. Its activation occurs by a cascade of reactions; once a specific protein becomes activated, it functions as an enzyme, cleaving and therefore activating millions of molecules of the next protein in the cascade. In turn, each of those molecules activates multiple molecules of the next.
Cl and C9 are proteins produced by the liver (C1 to C9).
- They circulate in blood and tissues.
- Activated by proteolytic cleavage of C3 to C5.
- C3a and C5a stimulate mast cells to release their granules (histamine) causing inflammation.

- C5a -> chemotaxis (pol) -> attracts PMNs and monocytes.
- Does not bind to human cells.
- C3b binds to bacterial surface -> opsonization.
- Binds to C3 convertases to form C5 convertase.
- Opsonization process that phagocytes take up bacteria.
- Activated by C3b or Fc of an antibody.

- C5b binds to EF C6, C7, C8, and C9 -> membrane attack complex (MAC).
- Attach to LPS of gram-negative bacteria and forms holes on bacterial membrane.
PMNs produced in bone marrow

Blood vessel

Endothelial cells

Selectins on endothelial cells mediate loose attachment

IL-8 induces proteins on PMN surface to give tight binding

PECAM aids transmigration

IL-1, TNF-α, IL-8, IFN-γ activate PMNs

C5a, bacterial peptides attract PMNs to site where bacteria are growing

C3b opsonizes bacteria

Figure 5-6 Roles of various cytokines in directing the exit of PMNs from the bloodstream at particular sites. Initially, new proteins are expressed on the surfaces of PMNs and endothelial cells, permitting a loose reversible binding. This gives PMNs a rolling motility as they flow through the blood vessel. Other cytokines cause changes in the cell surfaces that result in tighter binding. The PMNs stop moving, flatten against the vessel wall, force themselves across the endothelial wall, and then move chemotactically along a C5a gradient. PAF, platelet-activating factor; PECAM, platelet-endothelial cell adhesion molecule.

agulation cascade components on nerve endings in the inflamed region.

If the phagocytes are successful in eliminating the invading bacterium, a second set of cytokines begins to predominate. These cytokines (e.g., IL-4, IL-10, and IL-13) downregulate production of TNF-α and reduce the killing activities of phagocytes, thus allowing the phagocyte defense system to return to its normal, relatively inactive, level.

Other Activities of Cytokines

Some of the other roles of the proinflammatory cytokines are also illustrated in Figure 5-4 and explain common symptoms of infectious diseases other than localized infection: fever, somnolence, malaise, anorexia, chills, decrease in blood iron levels, and weight loss. Cytokines IL-1 and TNF-α interact with the hypothalamus and adrenal gland to produce fever and somno-
1. Classical pathway (specific immune system ⇒ related to tissue by antibodies)

2. Alternative pathway (non-specific immune system ⇒ not involved with antibodies)
   - Streptococcus pneumoniae produces capsule that prefers to bind to serum factor H than C.

3. Collectin pathway (non-specific, mannose binding lectin)

**Collectin-mannose pathway**

- Bact. (Bacteria) interacts with collectin in our immune system, forming C2 and C4.
- C2b and C2a bind C4b and C4a to form C2b-C4b-mannose complex with collectin.
- C3 convertase (C3a and C3b) is formed, leading to C5 convertase (C5a and C5b).
- C5a and C5b form membrane attack complex (MAC) by interacting with C7, C8, C9.
**Figure 5-3 Activation of complement.** (A) Classical pathway. Two IgG molecules or one IgM molecule attached to the surface of a bacterium bind complement component C1, causing an autoproteolytic event that activates it. C1, C4b, and C2b bind to one another and to the bacterium's surface to form C3 convertase. Addition of C3b produces C5 convertase, which triggers assembly of the membrane attack complex (MAC). The mannose-binding lectins or collectins activate the classical pathway similarly to antibodies, except that they interact with C4 and C2 rather than C1. After that point, both are the same. (B) Alternative pathway. C3-H₂O, an activated form of C3 that resembles C3b in conformation, is normally produced at low levels. If it binds a host cell surface, which preferentially binds serum factor H, H binds to C3b produced by the C3-H₂O complex and targets it for destruction by serum protein I. If C3-H₂O binds to the surface of a bacterium, it can form a complex with Bb (C3 convertase). Addition of more C3b produces C5 convertase, which triggers assembly of the membrane attack complex (MAC).

C₃-H₂O binds to B on the bacterium's surface, another serum protein, D, cleaves B to Ba and Bb. The resulting C₃-H₂O/Bb complex produces C₃b, which can then bind Bb to form a C₃/C₅ convertase. The initial C₃bBb complex is the C₃ convertase, which cleaves C₃ to form more C₃a and C₃b. The newly generated C₃b binds to the same bacterial surface, and when this bound C₃b comes in contact with the C₃bBb complex already on the surface, this new complex becomes the C₅ convertase. Some bacteria produce a polysaccharide surface coating, called a capsule, which preferentially binds serum protein H, rather than B. The effect of this is to eliminate C₃b as it is deposited on the surface and to prevent effective opsonization of the bacterial surface.

In both the classical and alternative pathways, it is important to keep the accelerated production of C₃a, C₃b, C₅a, and C₅b under control. To this end, most C₃b molecules on the bacterium's surface are proteolytically cleaved to produce iC₃b. iC₃b is an effective opsonin but cannot help to form a C₃ or C₅ convertase complex.

**Role of Cytokines and Chemokines in Directing the Phagocyte Response**

Cytokines are glycoproteins produced by a variety of cells, including monocytes, macrophages, NK cells, endothelial cells, lymphocytes, and fibroblasts. Chemo-
trigger the phosphorylation cascade that transmits the signal. How CD14 transmits its signals is just beginning to be understood, and the answer seems to be that it interacts with other cell surface proteins that actually mediate the signal transduction.

Before proceeding to the proteins that might serve the signal transduction function, let us first consider a protein that is involved in the interaction between CD14 and LPS, LPS binding protein. LPS bound to LPS binding protein interacts more effectively with CD14 than does unbound LPS. Presumably, the complex formed by LPS, LPS binding protein, and CD14 is more effective at initiating a signal than is LPS binding to CD14. A long-standing mystery is how cells such as endothelial cells and fibroblasts, which do not normally have CD14 on their surfaces, nonetheless respond to LPS by producing cytokines. The answer may lie in the fact that macrophages cleave the GPI-CD14 bond to release CD14 in a soluble form. This soluble form of CD14 appears to enable other cell types, which do not normally produce CD14, to respond to LPS. How CD14, aided by LPS binding protein, allows endothelial cells, for example, to react to LPS is unknown. Until recently, little was known about how lipoteichoic acid (LTA), a surface lipid-carbohydrate molecule found in gram-positive bacteria, and peptidoglycan fragments from both gram-positive and gram-negative bacteria caused cytokine release. Apparently, LTA and peptidoglycan fragments also bind to CD14 and through this interaction elicit cytokine production. It is not yet clear whether there are specialized LTA binding proteins, analogous to LPS binding protein.

Still another class of receptors for LPS and possibly for other bacterial molecules such as LTA and pepti-
Cytokynes - mediate inflammatory response to microbial antigens.

- glycoproteins produced by: monocytes, macrophages, NK cells, lymphocytes, endothelial cells, fibroblasts

- triggered by LPS or lipoteichoic acid + peptidoglycan fragments (LTA) →

**UP-REGULATE**

- Bacterial lgs, LPS → LPS binds to LPS binding protein complex

  → bind to COX on a macrophage

- TNF-α, IL-1, IL-3, IL-8

  - stimulates the macrophage to secret cytokines
  - TNF is tumor necrosis factor
  - IL = interleukin.
TNF-α, IL-1, and IL-8 activate oxidative burst of PMNs.

IFN-γ stimulates macrophages to increase their killing ability.

A combination of complement activation and cytokine release inflames.

Down-regulate

Once the invading bacterium is eliminated

IL-4, IL-10, and IL-13 produced

These decrease production of TNF-α and other inflammation-causing cytokines

Immune and system back to normal.
kines are small peptides that have many of the same functions, especially attracting phagocytes and activating them, much as complement components C5a and C3a do. Cytokines are larger than chemokines and play a central role in regulating the activities of the cells of the nonspecific and specific defense systems. Figure 5-4 gives an overview of the nonspecific responses to infection and shows how many of these responses rely on cytokines. Just as complement is activated by bacterial surfaces, cytokine release is triggered by interaction between cytokine-producing cells and molecules on the surfaces of the invading bacterium. In the case of gram-negative bacteria, the outer membrane LPS that activates complement is also the molecule that stimulates cytokine production. Although the surface molecules of other types of bacteria that activate complement and stimulate cytokine release have not been nearly as well studied, it appears likely that the same surface molecules on these bacteria both activate complement and stimulate cytokine production.

The process by which bacterial surface molecules trigger cytokine release is best understood in the case of LPS. LPS is released from the bacterial surface by the lysis of bacteria. LPS binds to CD14, a protein receptor on macrophages and other cytokine-producing cells (Figure 5-5). Binding of LPS to CD14 triggers a signal transduction pathway that culminates in cytokine production and release by the stimulated cell. CD14 is a somewhat unusual signal transducer because it is anchored in the macrophage membrane by glycosylphosphatidylinositol (GPI) rather than being part of a membrane-anchored protein complex, as most better-known signal-transducing receptors are. Usually, signal-transducing proteins have a membrane-spanning tail that is exposed in the cytoplasm and interacts with various signal-transducing enzymes to

**Figure 5-4** Overview of nonspecific responses to infection and the effects of cytokines on these responses. Cytokines include TNF-α, IL-1, and IL-6.
2nd line of defense
Antibodies and Cytotoxic T cells, and Helper T cells

Antibodies
- produced by B-lymphocytes/cells
- B-cells mature in bone marrow

\[ \text{antigen binding sites} \]
\[ \text{light chain \( (2 \times) \)} \]
\[ \text{heavy chain \( (2 \times) \)} \]
\[ 150 \text{ kDa} \]

- Binding to antigens
- Phagocytic binding
- Complement binding
- Clonal

- IgG (immunoglobulin G), Ig E, Ig D \( \Rightarrow \) monomer
- Ig A \( \Rightarrow \) dimer
- Ig M \( \Rightarrow \) pentamer

Epitope - Antibody-binding site on an antigen (4-16 aa).
- One antigen can have many epitopes.
Roles of Serum Antibodies (IgG & IgM)

<table>
<thead>
<tr>
<th>Role</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutralization of Toxins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neutralize microbes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(prevent binding to cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opsonization</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PMN receptors</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>Macrophage receptors</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Complement activation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cross placenta</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
IgG (immunoglobulin G)

most common antibody type.

IgG1 to IgG4
- IgG1 and IgG3 → opsonizing antibodies
- IgG1, IgG2 + IgG3 → activate complement proteins
- IgG also mediate attachment of cytotoxic NK cells, phagocytes
  - Antibody-dependent cell-mediated cytotoxicity (ADCC)

IgG's are the only antibodies that cross the placenta to protect infants during the first 6 months.

IgM
- low affinity to antigens
- but most effective to activate complement
- neutralization of microbes and toxin (can bind to lots of cells)

IgE
- binds to mast cells → release histamine granules
  - vasoactivity (dilation)
  - cause hypersensitivity
IgA
- Secretory IgA (sIgA)
- neutralize microbes and toxins
- sIgA does not activate complement.

IgG vs. IgM
Avidity
- affinity + valence
- Affinity = strength of interaction between an antigen-binding site of an antibody and an epitope on an antigen
- Valence = no. of antigen-binding sites available for epitope

Valle of IgG = 2
Valle of IgM = 10

Cytotoxic T cells
- similar killing mechanism as NK cells
- Cytotoxic T cells are specific to specific bacteria, antigens
- they develop specific receptors for a certain pathogen
- Cytotoxic T cells attack host cells if they are infected by bacteria or viruses
- it has memory (= memory cells)
- Cytotoxic T cells produce perforin and granulysin; death
  - perforin: pores holes in host cells, causing cell death
  - facilitating granulysin to enter the cells to kill the invading agents
Antigen Presentation

- Class I Major Histocompatibility Complex (MHC) protein.
- Activate Tc cells

Once the Tc cell binds to the presented antigen, Tc will be activated to:
1) replicate
2) secret perforin and granulysin
3) attract more phagocytes by secreting IFN-γ
4) form memory Tc cells.
Class II MHC (only in macrophages & B cells)

- Activate Th cells

Stimulate Th cell to become Th1 cell, causing inflammation.

Th1 cells secret:
1) cytokines to activate and attract phagocytes
2) form memory Th1 cells

\[ \text{stimulate } \text{Th cell to become } \text{Th1 cell, } \begin{aligned} \underline{\text{proliferation}} \\ \text{causing inflammation. of } \text{Th1} \end{aligned} \]

Th1 cells secrete:
1) cytokines to activate and attract phagocytes
2) form memory Th1 cells

\[ \text{stimulate Th cell to become Th2 cell.} \]

Th2 secrete certain cytokines (e.g. IL-4) to convert immature B cells to mature and differentiate to plasma cells that produce large amt. of antibodies.

1) form memory Th2 cells
2) proliferation of Th2
Antigen presentation

- Antigen has to be presented to activate T cells (T cytotoxic and T helper cells).

- Antigen-presenting cells: macrophages, dendritic cells (MHC-II)

  $\rightarrow Th_1 \rightarrow Th_{12}$ and B cells.

- Antigen presentation involves the major histocompatibility complex (MHC)

  $\odot \rightarrow MHC-I \rightarrow$ any nucleated cells

  $Th \rightarrow MHC-II$

- Intracellular pathogens $\rightarrow$ likely to elicit an MHC-I

- Soluble antigens (lysed bacteria) $\rightarrow$ MHC-II
Links to Nonspecific Defenses

For the human body to have the nonspecific defenses acting independently of the specific defenses would be inefficient, especially since the two types of defense can act synergistically. Not surprisingly, there are a lot of links between the two systems. One set of links is cytokines and chemokines. PMNs contribute to the Th1/Th2 decision-making process through cytokine production. Complement comes up in connection with both C3b-mediated opsonization and interaction with antibodies to kill bacteria directly. Macrophages can act as APCs or phagocytic cells. Th1 cells activate macrophages to greater killing power.

T-Independent Antigens

The APC-T-cell pathway responds to protein antigens but not necessarily to nonprotein antigens, such as polysaccharides and lipids. Because T cells are not involved in responding to antigens of this type, such antigens are called T-independent antigens. Polysaccharides and lipids can elicit an immune response in children and adults, but not in infants under the age of 2 years. Thus, the ability to respond to T-independent antigens develops after birth. This is an important consideration, because it means that vaccines consisting of T-independent antigens are not effective until an infant has become old enough to respond to these antigens.

T-independent antigens provoke an antibody response but not a cell-mediated response because they interact directly with B cells. Unactivated B cells display the antibody they produce on their surfaces. Polysaccharides and lipids are characterized by repetitive epitopes. If these epitopes bind to antibodies exposed on the surface of a B cell, they cause cross-linking of the antibodies. Cross-linking of surface antibodies stimulates the B cell to increase production of more antibodies and to release them (Figure 6-7). The T-independent response is particularly important for protection against bacterial pathogens that can avoid phagocytosis by covering themselves with a polysaccharide layer (capsule). Such bacteria are not effectively opsonized by C3b. They are ingested and killed by phagocytes only if antibodies that bind to capsular antigens are elicited and act as opsonins.

Although the T-independent response provides protection against capsule-producing bacteria, it has some important drawbacks. First, the antibody response elicited by T-independent antigens is not as strong as the T-cell-dependent response. Nor is it as long lasting, because no memory cells are developed. Second, the main antibodies elicited by T-independent antigens are IgM and IgG2. IgG2 does not opsonize, and IgM does so less effectively than IgG1 and IgG3. Third, as already mentioned, young infants do not mount a T-independent response. Unfortunately, infants are one of the highest risk groups for contracting serious infections due to capsule-producing bacteria (e.g., pneumonia and meningitis). A strategy for improving the immune response

Figure 6-5 Model for development and roles of Th1 and Th2 cells in humans. Antibody nomenclature for mice is somewhat different.
turn will stimulate production of antibodies or activation of macrophages. Display on MHC-I allows the APC to activate and stimulate the proliferation of cytotoxic T cells. Display of an epitope on MHC-II leads to activation and proliferation of T-helper cells, which aid in the production of antibodies or produce IFN-γ, which activates macrophages and cytotoxic T cells.

How the APC decides whether to display an epitope on MHC-I or MHC-II has received a lot of attention, because an understanding of the properties that lead to each type of presentation is critical for the design of vaccines. Although this area is still quite controversial, some basic rules are beginning to emerge. Pathogens, such as viruses and bacteria that enter the cytoplasm or nucleus of an APC (intracellular pathogens), are most likely to elicit an MHC-I display of their antigens (Figure 6–4). Even particulate antigens that do not escape the phagocytic vesicle can elicit an MHC-I-linked display. By contrast, soluble antigens, such as peptides or proteins from lysed bacteria, are displayed mainly on MHC-II. Thus, to use peptides to elicit a cytotoxic T-cell response, it is desirable to present the peptides in particulate form, e.g., bound up in a complex consisting of inert materials which will encourage their processing via the MHC-I pathway.

Interaction between APCs and T Cells: the T-Cell-Dependent Response

T cells have specialized protein complexes on their surfaces called T-cell receptors. The genes encoding T-cell-receptor proteins undergo extensive rearrangements during development of the T cells. The result is a pool of T cells with surface receptors that recognize a variety of epitopes. When an APC displays a particular MHC-I-peptide or MHC-II-peptide complex on its surface, only a few of the vast pool of available T cells will have a T-cell receptor capable of recognizing that MHC-peptide combination. These T cells bind the MHC-peptide complex via their T-cell receptors. Cytotoxic T cells have a protein on their surfaces, CD8, which helps the T-cell receptor respond to peptides displayed on MHC-I. CD8 binds to MHC-I and stabilizes the interaction between the MHC-I-peptide complex and the T-cell receptor. T-helper cells have a different surface protein, CD4, which helps their T-cell receptors respond to peptides displayed on MHC-II; CD4 stabilizes the interaction between the MHC-II-peptide complex and T-cell receptor. Other proteins on the surface of the APC and the T cell, called costimulatory molecules (e.g., CD54, CD11a/CD18, CD58, CD2), must also interact to make the binding between APC and T cell tight enough to stimulate the APC to release cytokines (e.g., IL-1, TNF-α). The cytokines stimulate the T cell to proliferate and become activated. The contacts between different surface proteins of the APC and the T cell help ensure that specific binding of the correct T-cell receptor to an MHC-peptide complex will result in T-cell activation. Once activated, T cells begin to proliferate. Most of the resulting T cells are involved in combating the invading microbes. A few of the T cells, however, become mem-

![Cellular Immunity](cell-mediated immunity)

**Figure 6–4** Characteristics of antigen (Ag) determine whether the antigen is presented on MHC-I (to trigger the cytotoxic T-cell [TC] response) or on MHC-II (to trigger the T-helper cell response).
Several functionally distinct subsets of T cells have been identified. Two major subpopulations are distinguished from each other by the presence of specific cell surface proteins called CD4 or CD8. A single mature T cell has only one of these proteins (Figure 20.9). The CD4 population is subdivided into two functional subsets called TH1, or T helper 1 cells, and TH2, or T helper 2 cells. TH1 cells participate in cell-mediated immunity and are responsible for recruiting and activating nonspecific effector cells such as phagocytes. They are thus often called T inflammatory cells. TH2 cells stimulate B lymphocytes to produce large amounts of antibody. In most cases, little if any antibody is made by B cells without TH2 cooperation (see Section 20.11). The second major T cell population, CD8 cells, have only a single functional T cell set, the T cytotoxic (Tc) cells, also known as CTLs or cytotoxic T lymphocytes. The Tc cells kill antigen-bearing cells directly and specifically through interaction between a cell surface antigen on the target cell and the antigen-specific T cell receptor. In addition to functional differences and surface proteins, T cell subsets are differentiated from one another by their unique pattern of secreted cytokines, a group of molecules that influences the metabolic and functional activities of leukocytes (see Section 20.8). Table 20.2 compares functional properties, surface antigens, and cytokine production of B and T lymphocytes.

**20.4 Concept Check**

Lymphocytes are antigen-specific leukocytes. B lymphocytes have immunoglobulin antigen receptors on their surface, whereas T cells have antigen-specific T cell receptors. T cells are divided into a number of subsets based on their surface proteins and functional characteristics.

- What are the chief differences between B cells and T cells?
- Differentiate between TH1 cells and Tc cells. Are their surface proteins different? Are their secreted cytokines different?

### 20.5 Immunoglobulins (Antibodies)

The next three sections of this chapter discuss the antigen-specific molecules of the immune system. We begin with immunoglobulins because we understand their structure and function in detail. They serve as the model for an antigen-specific receptor molecule. Immunoglobulins (antibodies) are protein molecules that are able to combine with antigenic determinants. They are found in the serum and in other body fluids such as gastric secretions and milk. Serum containing antigen-specific antibody is often called antiserum. Immunoglobulins (Igs) can be separated into five major classes on the basis of their physical, chemical, and immunological properties: IgG, IgA, IgM, IgD, and IgE (Table 20.3). Immunoglobulin class IgG has been further divided into four immunologically distinct subclasses called IgG1, IgG2, IgG3, and IgG4. These subclasses are genetically, structurally, and functionally different from one another. On initial immunization, the first immunoglobulin to appear is IgM, a pentameric immunoglobulin with a molecular weight of about 970,000; IgG appears later. In most individuals about 80% of the serum immunoglobulins are IgG proteins, and these have therefore been studied extensively.

#### Immunoglobulin Structure

Immunoglobulin G is the most common circulating antibody, and thus we will discuss its structure in detail. Immunoglobulin G has a molecular weight of...
Production of antibodies by B cells (Humoral Immunity)

1. T-cell dependent pathway.
   - involves TH2 cells (T-helper 2 cells)
   - Fig. 6.6

2. T-Independent pathway (Fig. 6.7)
   - It does not involve TH2 cells
   - Activated by T-independent antigens (e.g. mostly polysaccharides and lipids).
   - This pathway is important for protection against Streptococcus pneumoniae, Haemophilus influenzae b and etc. Why?
   - Disadvantages:
     1. produces mostly IgM & IgG2
        ⇒ low affinity (IgM) and low opsonization (IgG2)
     2. no memory cells produces
     3. Antibody response is not as strong as the T-cell dependent response
     4. infants < 2 years old don't have this pathway.
16.5 Clonal Selection and Expansion of Lymphocytes

Immature B cells: As these develop, a functionally limitless assortment of B cell receptors is randomly generated.

Naive B cells: Each cell is programmed to recognize a specific epitope on an antigen; B-cell receptors guide that recognition.

Activated B cells: These cells are able to proliferate because their B-cell receptors are bound to antigen X and the cells have received required accessory signals from T_H cells.

Plasma cells (effector B cells): These descendants of activated B cells secrete large quantities of antibody molecules that bind to antigen X.

Memory B cells: These long-lived descendants of activated B cells recognize antigen X.

FIGURE 16.7 Clonal Selection and Expansion During the Antibody Response

In a means of clarifying discussions of lymphocyte characteristics, descriptive terms are sometimes used:

- **Immature lymphocytes** are those that have not fully developed their antigen specific receptor.
- **Naive lymphocytes** have an antigen receptor, but have not yet encountered the antigen to which they are programmed to respond.

- **Activated lymphocytes** are able to proliferate; they have bound antigen by means of their antigen receptor and have received any required accessory signals from another cell, confirming the danger of the antigen.
- **Effector lymphocytes** are descendants of activated lymphocytes that have become armed with the ability to produce specific cytokines or other substances. This endows the cell with specific protective attributes, or **effector functions**. Plasma
Antibodies and Cytotoxic T Cells

From the image, it appears to discuss the immune response to epitopes, the role of B cells and their interaction with T cells, the production of antibodies, and the concept of T-independent and T-dependent responses.

The text mentions the binding of epitopes to antibodies on the surface of B cells, the uptake of epitopes by B cells, and the subsequent proliferation and activation of these cells. It also discusses the production of antibodies, with some becoming memory cells.

The text further explains the role of APCs (antigen-presenting cells) in linking epitopes to MHC-II, and the subsequent activation of Th cells. It describes the production of IgG2 and IgM antibodies, and the difference between T-dependent and T-independent responses.

The text also touches on the role of T-independent antigens and extending this response to infants through conjugate vaccines, which link a portion of the polysaccharide capsule to a protein. Such vaccines are called conjugate vaccines and are processed by APCs, noted for their muscle and for their high intelligence, and elicit a T-dependent response that ultimately results in the production of antibodies that recognize the polysaccharide antigen.

Figure 6-7 shows the T-independent production of antibodies, highlighting the interaction between B cells, epitope-MHC-II complexes, and T-cell receptors.

The text concludes by discussing mucosal immunity, an important immune response to infectious diseases, and questions about the accuracy of the T-independent response, particularly in infants and the protection against S. pneumoniae.

This indicates that there may be some sort of memory response to polysaccharide antigens, and that the account of the T-independent response remains correct, although infants are not protected by polysaccharide vaccines. The text also mentions the development of a conjugate vaccine that protects against S. pneumoniae, which includes only 7 of the nearly 100 serotypes of S. pneumoniae.
- Polysaccharide vaccines will not be useful for infants.

- Conjugated vaccines can circumvent this problem.
  - e.g. Hib - Haemophilus influenzae type b. (Infant meningitis)
  - PCV - Pneumococcal conjugate (Otitis media meningitis, pneumonia)

**Mucosal Immunity**

- GALT (gastrointestinal Associated lymphoid tissue)
  - e.g. ileum & rectum

- MALT (e.g. respiratory and vaginal tracts)
have associated lymphoid tissues. The best studied of these systems is the gastrointestinal-associated lymphoid tissue (GALT) found in the follicles and Peyer's patches, which are most highly concentrated in the ileum and rectum. Similar lymphoid tissues are found in the respiratory and vaginal tracts. This group of mucosal immune systems is called mucosa-associated lymphoid tissue (MALT). Skin also has a similar system, called the skin-associated lymphoid tissue (SALT). The Langerhans cells of the epidermis are the APCs of this system.

The cells of the GALT are illustrated in Figure 6-8. The M (microfold) cell takes up antigens from the lumen of the intestinal tract and passes them to GALT macrophages, which presumably act as the APCs. The process by which GALT macrophages process antigens and elicit production of cytotoxic T cells or antibodies is the same as that described in earlier sections, except that the macrophages, B cells, and T cells of the GALT home specifically to mucosal membranes and appear not to be part of the humoral immune system. Memory T and B cells stimulated by antigen processing at the GALT can migrate to other mucosal sites and vice versa. Thus, stimulation of one of the MALT sites results in general mucosal immunity. This characteristic of the MALT system is what makes oral vaccines feasible. Initially, oral vaccines stimulate the GALT, but sIgA against vaccine antigens is later detectable in other sites. Thus, an oral vaccine can be used to elicit immunity to respiratory and, presumably, genital pathogens.

Currently, efforts are being made to develop vaccines administered by inhalation, so that stimulation of the bronchial MALT would produce an sIgA response at other MALT sites. These vaccines would have the advantage that they need not pass through the stomach. Vaccines that target the GALT have to be capable of surviving the low-pH and protease-rich stomach environment, a barrier that has proved problematic in some cases. Administering vaccines by rectal or vaginal suppositories is theoretically possible, but this strategy has not been actively pursued to date.

When the GALT is stimulated, one outcome is production of IgA (Figure 6-8). IgA binds to the poly-Ig receptor on the basal surface of mucosal cells and is then taken up and carried in vesicles to the apical surface, where it is released. Release involves proteolytic cleavage of the receptor, and a portion of the receptor remains attached to the IgA, making it sIgA. Activation of the GALT can also lead to production of cytotoxic T cells. These cells probably remain on the basal side of the mucosa, although it is possible that during an infection some of them migrate to the apical surface, especially in areas where damage to the mucosa has occurred. GALT cytotoxic T cells are important for protection against viral infections of the gastrointestinal tract and some bacterial infections in which the bacteria multiply inside mucosal cells.

One of the many mysteries associated with the intestinal immune mucosa is the role of a type of mucosal cell called gamma-delta T cells. The majority of gamma-delta T cells are CD8+ T cells and would thus be grouped with cytotoxic T cells. However, whereas cytotoxic T cells of the humoral immune system have T-cell receptors composed of alpha and beta protein subunits, the intestinal epithelial lymphocytes (IELs) have a T-cell receptor composed of related but somewhat different protein subunits called gamma and delta. Gamma-delta T cells account for fewer than 4% of circulating CD8 cells, but they account for as many as 10 to 15% of the mucosal cells in the gastrointestinal tract; in some parts, such as the colon, the level may be as high as 40%. Recently, some light has been shed on their role. In fact, gamma-delta T cells have gone very rapidly from being cells in search of a function to cells to which too many functions are now attributed.

A particularly intriguing feature of gamma-delta T cells has been that these cells have a very limited repertoire of antigen recognition. Whereas there are thousands, perhaps millions, of types of cytotoxic and T-helper cells, each of which recognizes a different antigen, the gamma-delta T cells seem to recognize only a limited number of antigens. Also, gamma-delta T cells appear to bypass the macrophage antigen presentation
why does not antibody recognize self-compounds?

Lymphocytes

Receptors

Self antigens

Binding of self antigens

Clonal deletion of lymphocytes that have receptors for self