

Basic Science

The Immune System and the Defense of the Body

The immune system has evolved to fight microbial invaders. As with many such fights, the invaders may be persistent, the struggle may be intense, and collateral damage may be inevitable. Animals benefit from minimizing this collateral damage; nevertheless, invaders must be repelled even at the cost of severe damage. The immune system must do “whatever it takes” to maintain the body’s integrity. Allergic responses are an essential component of these defenses, especially on the surfaces of the body, where the invaders first seek to penetrate. The skin, airways, and gastrointestinal tract are all potential invasion sites, and the local immune defenses must be armed and ready. It is these defenses that, when inappropriately activated, produce allergic diseases. Thus, a common feature of allergies is the reaction of the immune system to a perceived attack or invasion. The potential invaders do not need to be pathogens. If the system is ready, then exposure to foreign molecules (or allergens) in food, inhaled air, and on the skin might trigger the defensive cells of the body and mount aggressive defensive responses that we recognize as an allergic disease (Fig. 1.1).

Innate Immunity

Animals need to detect and eliminate microbial invaders as fast as possible. This immediate defensive response is the task of the innate immune system. The innate defenses respond rapidly, destroying invaders, while simultaneously minimizing collateral damage. Innate immune responses are triggered when cell surface pattern recognition receptors detect either microbial invasion or tissue damage. For example, cells can sense the presence of invading microbes by detecting their characteristic structural molecules. These molecules are called “pathogen-associated molecular patterns” (PAMPs). The innate immune defenses can also sense tissue damage by detecting the characteristic molecules released by damaged cells. These molecules are called “damage-associated molecular patterns” (DAMPs) or “alarmins.” Specialized sentinel cells with pattern recognition receptors can detect both PAMPs and DAMPs and, once they are detected, these sentinel cells transmit signals to attract white blood cells. The white blood cells in response converge on the invaders and destroy them during inflammation. In addition, animals make many different antimicrobial proteins, such as complement, defensins, and cytokines, that can either kill invaders directly or promote their destruction by defensive cells. Some of these antimicrobial molecules are present in normal tissues, whereas others are produced in response to the presence of PAMPs or DAMPs such as damage caused by an invading bacterium. There are multiple specialized populations of white blood cells; therefore, the body can activate different populations in different situations, thus ensuring that the forces sent to repel the invaders are optimized for the task.

The innate immune system has minimal memory capability and, therefore, each infection episode is treated similarly. The intensity and duration of innate responses, such as inflammation, remain largely unchanged regardless of how often a specific invader is encountered. These responses come at a price, such as the pain and itch of inflammation. More importantly, innate immune responses act as triggers that stimulate antigen-presenting cells to initiate adaptive immune responses, eventually leading to strong, long-term protection.

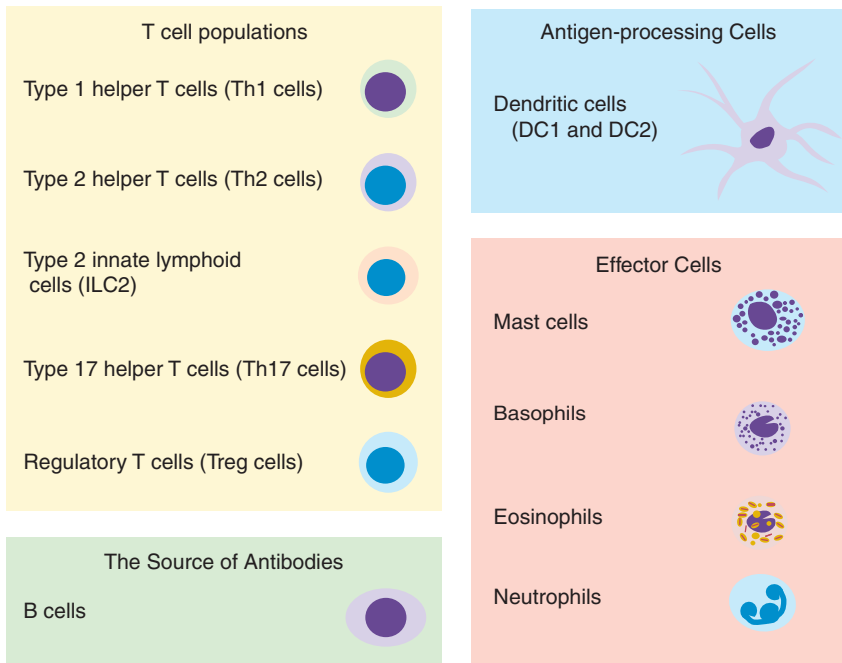


Fig. 1.1 Classification of the major cell types involved in allergic responses and a key to their identification in subsequent figures.

Several cytokines play essential roles in initiating and mediating innate responses and inflammation. These include interleukin 1 (IL-1), a cytokine produced by many different cell types. The two most important forms of IL-1 (α and β) act on type 2 helper (Th2) cells, B cells, natural killer (NK) cells, neutrophils, eosinophils, dendritic cells (DCs), fibroblasts, endothelial cells, and hepatocytes. IL-6 is also produced by many different cell types, and acts on T cells, B cells, hepatocytes, and bone marrow stromal cells. IL-8 is a proinflammatory chemokine and, similar to other chemokines, it is a relatively small (8.4 kDa) protein produced by macrophages and endothelial cells that attracts and activates neutrophils. Tumor necrosis factor- α (TNF- α) is a proinflammatory cytokine produced by macrophages, mast cells, T cells, endothelial cells, B cells, adipocytes, and fibroblasts. TNF- α is the most potent inducer of inflammation.

Adaptive Immunity

Adaptive immune responses develop when foreign antigens bind to specific receptors on lymphocytes and stimulate these cells to mount strong defensive responses. Adaptive immune responses proceed in four basic steps: Step 1, Antigen capture and processing; Step 2, Helper T cell activation; Step 3, B cell- or T cell-mediated responses that eliminate invaders, and Step 4, Generation of large populations of memory cells that respond rapidly upon subsequent exposure to an antigen (Fig. 1.2).

STEP 1: ANTIGEN CAPTURE AND PROCESSING

The initiation of any adaptive immune response requires the activation of antigen-presenting cells. These are primarily, but not exclusively, specialized DCs. Their activation is triggered by the

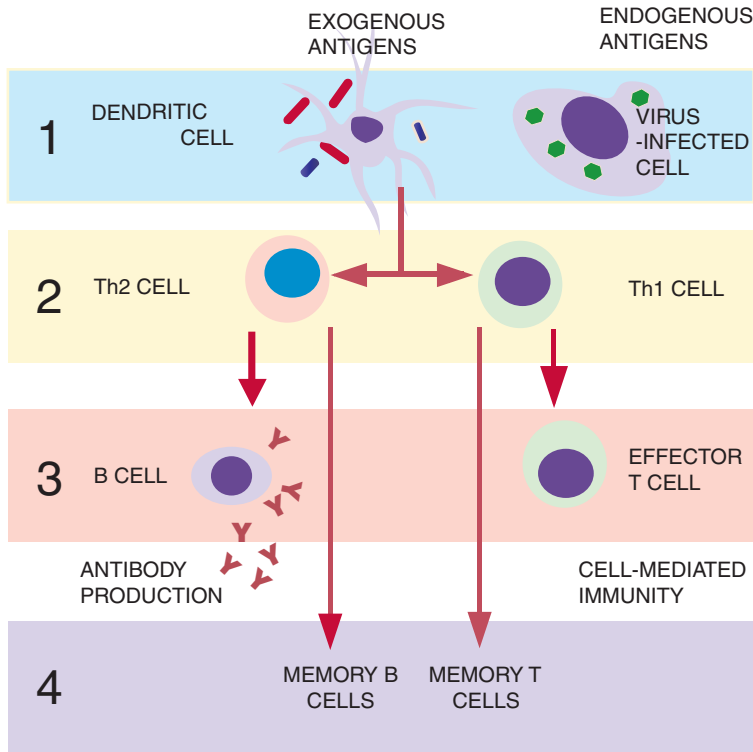


Fig. 1.2 Four steps of adaptive immunity. Step 1: appropriate processing of exogenous and endogenous antigens and differentiation of dendritic cells. Step 2: antigen presentation to the two major types of helper T cells. Step 3: either B cells or effector T cells are activated, with B cells responsible for antibody production and effector T cells responsible for killing virus-infected cells. Step 4: generation of memory T cells and B cells.

mixture of cytokines generated during the initial innate response. The activated DCs are needed to capture and process any antigens derived from the invading pathogens, especially from bacteria. DCs have a small cell body with many long cytoplasmic processes known as dendrites extending from its surface. These dendrites increase antigen capture efficiency and maximize the area of contact when they wrap themselves around lymphocytes. DCs are found throughout the body and form networks in every tissue. They are especially prominent in lymph nodes and under the skin and mucosal surfaces, which are the sites where invading microbes are most likely to be encountered.

Exogenous Antigens

Antigens fall into two distinct categories. The first category is typified by pathogenic bacteria that invade tissues and extracellular fluid. These invading bacteria mainly grow outside cells and are classified as “exogenous antigens.” Exogenous antigens must first be captured by DCs, and then processed and presented to helper T cells if they are to trigger an adaptive response.

When DCs encounter foreign antigens together with “danger signals” such as DAMPs from tissue damage, PAMPs from infection, and cytokines released by the inflammatory process, they mature rapidly (Fig. 1.3). Therefore, the DCs migrate toward the source of the antigen. The activated DCs capture antigens by phagocytosis. If they ingest bacteria, they can usually kill them;

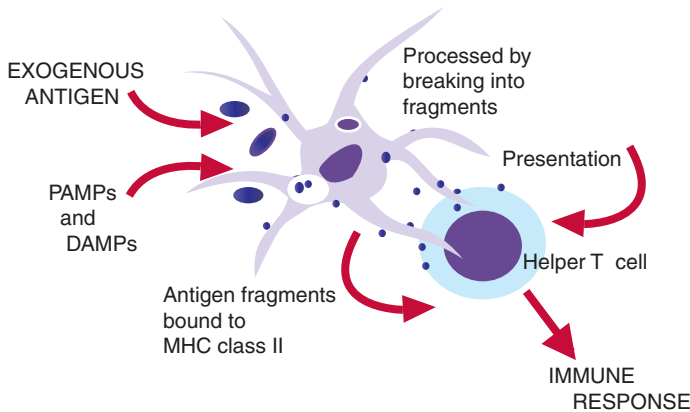


Fig. 1.3 Functions of dendritic cells (DCs). DCs capture and process exogenous antigens. Their response is stimulated by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) acting through pattern recognition receptors. Ingested antigens are broken into small peptides, which are then bound to major histocompatibility complex (MHC) molecules and the complexes carried to the DC surface, where they are recognized by helper T cells. The cytokine mixture produced by the DCs determines whether the helper T cells become type 1 helper (Th1) or type 2 helper (Th2) cells. Th2 cells are responsible for triggering allergic responses.

however, the pH within the phagosomes of DCs is less acidic than in other phagocytic cells, and the ingested antigens are not totally degraded, with some peptides remaining intact. These ingested peptides are then bound to specialized receptors called major histocompatibility complex (MHC) class II molecules. Once an antigenic peptide binds to an MHC molecule, the MHC-peptide complex is transported to the surface of the DCs. On arrival at the surface, this MHC-peptide complex is made available for inspection by any passing T cell. The DCs embrace the T cells, whereas the T cells palpate the DCs for the presence of MHC-peptide complexes. T cells express T cell antigen receptors (TCRs) on their surface. If the TCRs can bind any of these peptides, the T cells will be triggered to respond. DCs also carry their MHC-bound peptides to nearby lymph nodes, where they can be presented to many helper T cells. The processed antigen peptides will encounter and bind to the receptors on at least one T cell. Each T cell has multiple receptors of a single specificity. TCRs only bind to peptides attached to MHC molecules, and will not recognize or respond to peptides alone.

Because helper T cells must recognize MHC-peptide complexes if they are to respond to an antigen, MHC molecules effectively determine whether an animal can mount an adaptive response. MHC class II molecules can bind some, but not all, peptides created during antigen processing; therefore, they effectively select those antigen peptides that are to be presented to the T cells. The response to antigens is thus controlled largely by the MHC genes of an animal. Therefore, MHC genes can regulate immune responses, including allergies.

Endogenous Antigens

The second category of invading organism is typified by viruses that can enter cells and force them to make new viral proteins. These viral or “endogenous antigens” are processed by the cells in which they are produced. Immune responses against endogenous antigens must be aimed at detecting and destroying any cells producing abnormal or foreign proteins. Viruses take over the protein-synthesizing machinery of infected cells and use it to make new viral proteins. Therefore, T cells must be able to recognize a virus-infected cell by detecting the viral proteins expressed on

the cell surface. Living cells continually digest and recycle any proteins they produce. Therefore, these proteins are broken up into short peptides, which are then transported to a newly formed MHC class I molecule. If they fit the MHC-peptide-binding site, they will bind. Once loaded onto the MHC, the MHC-peptide complex is carried to the cell surface and displayed to any passing T cells.

STEP 2: HELPER T CELL ACTIVATION

Helper T cells are found in follicles and germinal centers within lymph nodes. Each helper T cell is covered by approximately 30,000 identical foreign TCRs. The T cells ignore normal cellular proteins because they lack the receptors to bind them. However, any foreign peptides will bind to some TCRs. Once the TCRs bind sufficient antigenic peptides correctly, the T cell will be driven to respond by turning on the genes for certain cytokines and cell surface molecules and dividing.

There are two other antigen-responsive lymphocyte populations, called B cells and cytotoxic T cells. These cannot respond properly to foreign antigens unless they first receive their instructions from the helper T cells.

The binding of an MHC-peptide complex to a TCR is insufficient by itself to trigger a helper T cell response. Therefore, when DCs present their processed antigen load to helper T cells, they must transmit additional signals. The first, as described above, is delivered when TCRs bind the antigen peptides attached to the MHC molecules. The second signal provides the T cells with additional critical co-stimulation via prolonged strong adhesion between the cells and is mediated by cell surface adhesion molecules. The third set of signals determine how naïve helper T cells will develop and the precise form the resulting immune response will take. These signals are provided by the mixture of cytokines secreted by the DCs. For example, some antigens trigger DCs to secrete IL-12, which acts on undifferentiated helper T cells causing them to develop into type 1 helper (Th1) cells and trigger a type 1 immune response. Other antigens are processed so that they cause DCs to secrete a cytokine mixture containing IL-4 and IL-6. These cytokines act on undifferentiated helper T cells to cause them to differentiate into Th2 cells, stimulating type 2 responses, especially allergies (Fig. 1.4). DCs that stimulate type 1 responses are called DC1 cells. Those that stimulate type 2 responses are called DC2 cells. (A third population of helper T cells called type 17 helper [Th17] cells are discussed in Chapter 2.)

STEP 3: B AND T CELL RESPONSES

The division of the adaptive immune system into two major branches is based on the need to recognize two distinctly different categories of foreign invaders—“exogenous” and “endogenous.” Antibodies produced by a type 2 response will bind to bacteria, free virus particles, and parasites and promote their destruction. Antibodies also provide the first line of defense against organisms that may survive within cells. However, once these organisms succeed in entering cells, then antibodies are ineffective and a type 1 response mediated by cytotoxic T cells is needed to kill the infected cell, release cytokines that inhibit microbial growth, or prevent pathogen survival within the infected cells.

B Cell Responses

Helper T cells are essential for B cell activation and are required to produce high-affinity antibodies and immune memory. B cells can also capture and process antigens, present them to helper T cells, and then receive co-stimulation from the same T cells. B cells thus play two roles: they respond to antigens by making antibodies and act as antigen-presenting cells. Helper T cells stimulate these B cell responses using secreted cytokines and via interacting cell receptors.

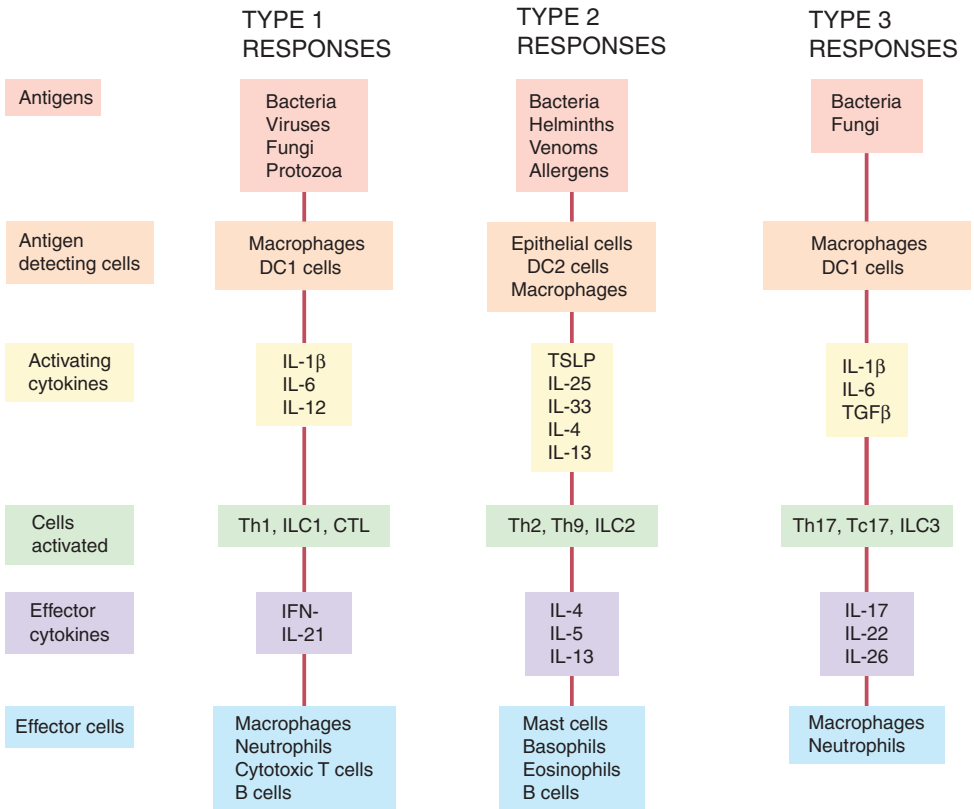


Fig. 1.4 Comparison of the three major types of adaptive immune responses. The development of each type is determined by the effects of the cytokine mixtures the responding T cells are exposed to. These results are determined by antigen-processing dendritic cells. Once the helper T cells have differentiated, they control further development by the mixture of cytokines they secrete. Type 3 responses are proinflammatory responses triggered by helper T cells that secrete interleukin-17, and are discussed in Chapter 2.

B cells are activated by helper T cells (mainly Th2 cells) within lymph nodes. When a B cell encounters an antigen that binds its receptors (B cell receptors [BCRs]), it will, with appropriate co-stimulation, respond by upregulating its BCR genes, thereby increasing the production and secretion of these receptors into body fluids, where they are called antibodies. Each B cell is covered with approximately 200,000 to 500,000 identical antigen receptors. Antibodies are simply BCRs released into body fluids, all belonging to the family of proteins called immunoglobulins (Igs).

Although the binding of an antigen to a BCR is an essential first step, this is usually insufficient to activate B cells. The complete activation of a B cell requires co-stimulatory signals from helper T cells and their cytokines. When helper T cells “help” B cells, they start the process that leads to B cell division and the development into antibody-secreting cells. The “help” also triggers somatic mutation within B cell IgG genes and results in a progressive increase in antibody-binding affinity.

When appropriately stimulated by helper T cells and co-stimulated by cytokines, B cells divide. This division is asymmetric so that one daughter cell receives a lot of antigens while the other daughter cell receives very little or none. The cell that receives a lot of antigen then

differentiates into an antibody-producing plasma cell. The cell that receives less antigen continues the cycle of dividing and mutating and eventually becomes a memory cell. The cells destined to become plasma cells develop a rough endoplasmic reticulum, increase their antibody synthesis rate, and secrete large quantities of immunoglobulins.

A key feature of B cell responses is the progressive increase in the affinity of antibodies for their antigens over time. These increases in antibody affinity occur within germinal centers in lymph nodes and the spleen. B cells stimulated by antigens migrate to these germinal centers where they proliferate. B cells divide every 6–8 h so that within a few days a single B cell may develop into a clone of several thousand cells. During this phase of rapid B cell division, the BCR variable region genes (encoding the antigen-binding sites on the BCR) mutate, on average, once per division. This repeated random mutation ensures that progeny B cells have BCRs that differ from the parent cell. Once these cells have been clonally expanded, they are presented with antigens by DCs. Because of these mutations, some B cells bind the antigen with greater affinity, and others bind it with less. A selection process then occurs. If a mutation has resulted in greater receptor affinity for the antigen, this stimulates more B cell proliferation. If the affinity decreases, then B cell stimulation is also reduced. Thus, cycles of somatic mutation and selection lead to a rapid improvement in antigen binding—a process called affinity maturation. The high-affinity, antigen-selected B cells eventually leave the germinal center to form either plasma cells or memory B cells. In contrast, those B cells that have reduced antigen binding will die. Thus, the antibodies produced by B cells progressively increase their affinity for antigens and hence their effectiveness as the response proceeds.

Effector T Cell Responses

Intracellular organisms are eliminated by two processes. Either infected cells are killed rapidly by cytotoxic T cells so that the invader has no time to grow, or infected macrophages develop the ability to destroy the intracellular organisms. In general, cells infected with viruses that enter the cell cytosol or nucleus are killed by T cells, whereas organisms such as bacteria or parasites that reside within endosomes are destroyed by T cell-activated macrophages.

If endogenous antigens presented by MHC class I molecules can bind to the antigen receptors of a T cell, the T cell will respond. For example, when a virus infects a cell, some viral proteins will be expressed on the cell surface. Circulating T cells might have TCRs that bind these processed viral peptides. The T cells that respond to these endogenous antigens carry the cell surface protein called CD8, which is used to bind to the MHC class I molecules on the virus-infected cells. Once the two cells are tightly bound, their receptors interact, signals are exchanged, and the T cells kill the infected cells.

Cytotoxic T cells must be highly sensitive to the presence of viruses so that they can kill all the virus-infected cells as fast as possible. Within a few minutes of binding to a target cell, the T cell cytoplasmic granules fuse with the T cell membrane so that their toxic contents are injected into the target cell. Soon after coming into contact with a cytotoxic T cell, its target cell starts to undergo apoptosis and is dead in less than 10 min. Cytotoxic T cells can disengage and then move on to kill other target cells. Several cytotoxic cells may also join in killing a single target.

Macrophages are also activated by Th1 cell-derived gamma interferon. Once activated, these macrophages secrete proteases, cytokines such as interferons, vasoactive molecules, and complement components. Activated macrophages move more rapidly in response to chemotactic stimuli, and contain increased amounts of lysosomal enzymes and respiratory burst metabolites, and they are more avidly phagocytic than normal cells. They produce greatly increased amounts of nitric oxide synthase and kill intracellular organisms or tumor cells by generating high nitric oxide levels.

STEP 4: MEMORY CELL GENERATION

In addition to mounting an immediate defensive response, both B and T cells generate populations of memory cells. These memory cells can respond more rapidly and effectively to antigens when they re-encounter them. These memory cells confer immediate protection and generate secondary immune responses.

Memory B Cells

Primary antibody responses do not persist because the responding B and plasma cells are short-lived and die within months. However, if all these cells died, immunological memory could not develop. Some B cells must persist as memory cells, which survive within the lymph nodes and bone marrow, where they proliferate and form germinal centers. These cells persist under the influence of survival and rescue signals. If a second antigen dose is given to a primed animal, it will encounter large numbers of responsive memory B cells. Therefore, secondary immune responses are much greater than primary immune responses. Immunoglobulin class switching also occurs so that immunoglobulin G (IgG) or IgE antibodies are produced in preference to the IgM characteristic of the primary response. In addition, memory B cells secrete antibodies with a much higher affinity for antigens than primary plasma cells because of somatic mutation and affinity maturation.

Memory T Cells

Naïve CD8⁺ T cells are long-lived cells that continuously recirculate among tissues, the bloodstream, and lymphoid organs. Once they encounter antigens, they must multiply rapidly to keep pace with the growth of the invading pathogens. The number of responding T cells may increase more than 1000-fold within a few days. They reach a peak 5–7 days after infection when pathogen-specific, cytotoxic T cells can make up 50% to 70% of the total CD8⁺ T cell population. In contrast to the prolonged B cell responses, however, the effector phase of T cell responses is very brief, and cytotoxicity occurs only in the presence of antigens, which is logical. Excessive sustained cytotoxic activity by T cells or the overproduction of cytokines can cause severe tissue damage.

As with other lymphocytes, the asymmetric division of an effector T cell generates two daughter cells with different fates. The dividing T cell is polarized because one pole of the cell contains the antigen-binding structures. The other pole contains molecules excluded from the contact site. Thus, when the cell divides, it forms two distinctly different daughter cells. The daughter cell adjacent to the contact site is the precursor of the effector T cells. The daughter cell formed at the opposite pole is the precursor of the memory T cells. The proximal cell has increased expression of effector molecules. The distal cell has increased lipid metabolism and expression of anti-apoptotic molecules and lives much longer. Once an infection has been eliminated, up to 95% of effector T cells undergo apoptosis within 1 to 2 weeks. However, memory T cells persist for months or years, lurking in the tissues and lymphoid organs and remaining functionally silent until they re-encounter antigens. When that happens, they respond very rapidly.

Suggested Reading

Tizard IR. *Veterinary Immunology: An Introduction*. 10th ed. Elsevier; 2018.