Cell Mediated Immunity: Role of IL-2 Receptor, Clonal Expansion

ZCT – 210 Lecture No: 6
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FUNCTIONAL RESPONSES OF T LYMPHOCYTES

The earliest responses of antigen-stimulated T cells include:

- *Changes in the expression of various surface molecules*.
- *Secretion of cytokines and the expression of cytokine receptors*.
- *These are followed by proliferation of the antigen-specific cells*.
- *Driven in part by the secreted cytokines*, and
- *Then by differentiation of the activated cells into effector and memory cells*.

In this lecture, we will describe each of these steps, their underlying mechanisms, and their functional consequences.

Changes in Surface Molecules during T cell Activation

- After activation, there are characteristic changes in the expression of various surface molecules in T cells, which are best defined in CD4+ helper cells (Fig. 9-8).

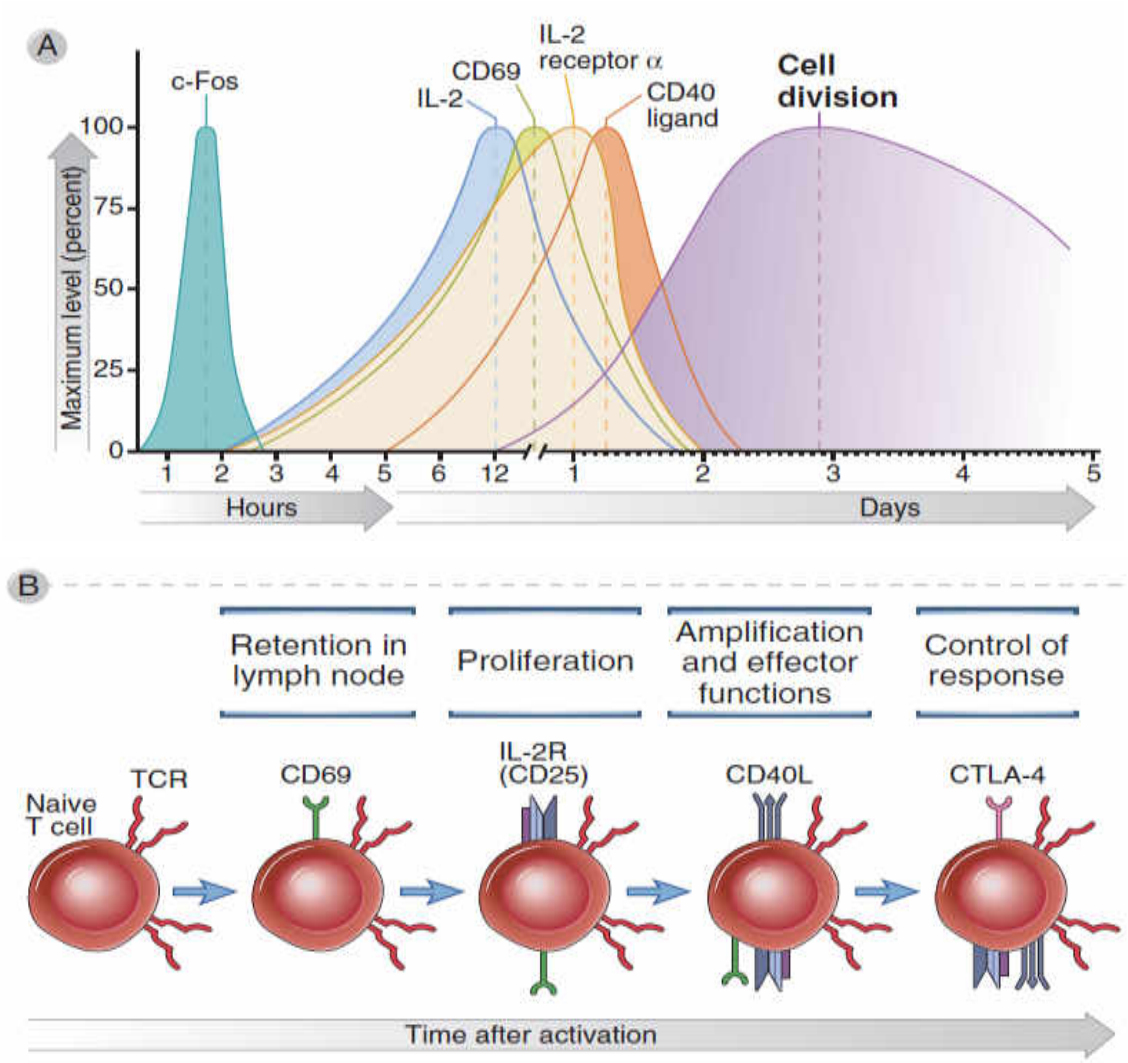


FIGURE 9–8 Changes in surface molecules after T cell activation. **A,** The approximate kinetics of expression of selected molecules after activation of T cells by antigens and costimulators are shown. The illustrative examples include a transcription factor (c-Fos), a cytokine (IL-2), and surface proteins. These proteins are typically expressed at low levels in naive T cells and are induced by activating signals. CTLA-4 (not shown) is induced 1 to 2 days after activation. The kinetics are estimates and will vary with the nature of the antigen, its dose and persistence, and the type of adjuvant. **B,** The major functions of selected surface molecules are shown and described in the text.

- Many of the molecules that are expressed in activated T cells are also involved in the functional responses of the T cells.
- Some of the functionally important molecules induced on activation are the following.
    - ***CD69.*** Within a few hours after activation, T cells increase their expression of CD69, a plasma membrane protein.

      This protein binds to and reduces surface expression of the sphingosine 1-phosphate receptor S1PR1, a receptor that mediates egress of T cells from lymphoid organs.

      The consequence of decreased S1PR1 expression is that activated T cells are retained in lymphoid organs long enough to receive the signals that initiate their proliferation and differentiation into effector and memory cells.

      After cell division, CD69 expression decreases, the activated T cells re-express high levels of S1PR1, and therefore effector and memory cells can exit the lymphoid organs.
    - ***CD25 (IL-2Rα).*** The expression of this cytokine receptor enables activated T cells to respond to the growth promoting cytokine IL-2.
    - ***CD40 ligand (CD40L, CD154).*** Within 24 to 48 hours after activation, T cells express high levels of the ligand for CD40.

      The expression of CD40L enables activated T cells to mediate their key effector functions, which are to help macrophages and B cells.

      In addition, CD40L on the T cells activates dendritic cells to become better APCs, thus providing a positive feedback loop mechanism for amplifying T cell responses.

- ***CTLA-4 (CD152).*** The expression of CTLA-4 on T cells also increases within 24 to 48 hours after activation.

  CTLA-4, a member of the CD28 family that functions as an inhibitor of T cell activation and thus as a regulator of the response.

- ***Adhesion molecules and chemokine receptors.*** After activation, T cells reduce expression of molecules that bring them to the lymphoid organs (such as L-selectin and the chemokine receptor CCR7).

  And increase the expression of molecules that are involved in their migration to peripheral sites of infection and tissue injury (such as the integrins LFA-1 and VLA-4, the ligands for E- and P-selectins, and various chemokine receptors).

  Activation also increases the expression of CD44, a receptor for the extracellular matrix molecule hyaluronan.

  Binding of CD44 to its ligand helps to retain effector T cells in the tissues at sites of infection and tissue damage.

IL-2 Secretion and IL-2 Receptor Expression

- Cytokines play critical roles in adaptive immune responses; in such responses, the major sources of cytokines are T cells, especially (but not exclusively) CD4+ helper T cells.
- The most important cytokine produced by T cells early after activation, often within 2 to 4 hours after recognition of antigen and costimulators, is interleukin-2 (IL-2).

IL-2 is a growth, survival, and differentiation factor for T lymphocytes and plays a major role in the regulation of T cell responses by virtue of its crucial role in the maintenance of regulatory T cells.

- Because of its ability to support proliferation of antigen-stimulated T cells, IL-2 was originally called T cell growth factor (TCGF).
- It acts on the same cells that produce it or on adjacent cells (i.e., it functions as an autocrine or paracrine cytokine).
- IL-2 is produced mainly by CD4+ T lymphocytes.
- Activation of T cells by antigens and costimulators stimulates transcription of the IL-2 gene and synthesis and secretion of the protein.
- IL-2 production is rapid and transient, starting within 2 to 3 hours of T cell activation, peaking at about 8 to 12 hours, and declining by 24 hours.
- CD4+ T cells secrete IL-2 into the immunologic synapse formed between the T cell and APC (see Lecture 1).
- IL-2 receptors on T cells also tend to localize to the synapse, so that the cytokine and its receptor reach sufficiently high local concentrations to initiate cellular responses.
- Secreted IL-2 is a 14 - to 17-KD glycoprotein that folds into a globular protein containing four α helices (Fig. 9-9).

- It is the prototype of the four–α-helical cytokines that interact with type I cytokine receptors (see Lecture 1).

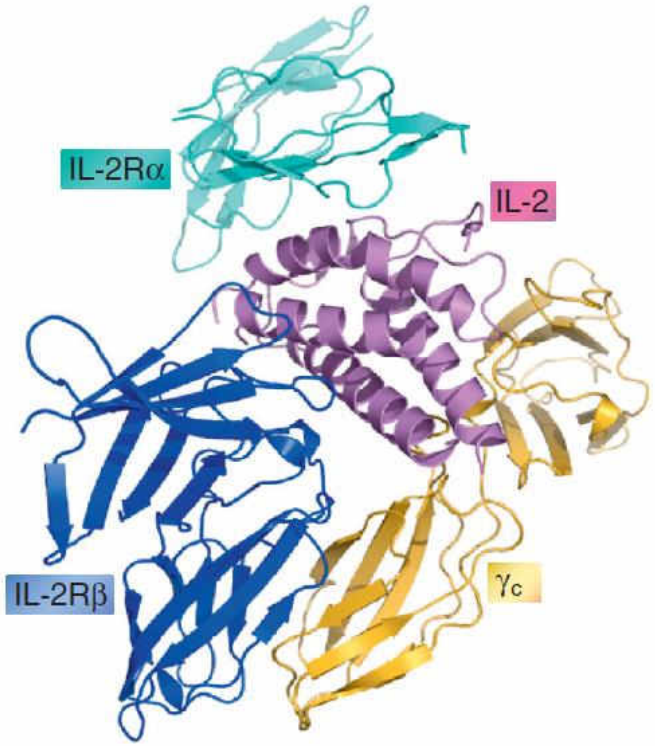


FIGURE 9–9 Structure of IL-2 and its receptor. The crystal structure of IL-2 and its trimeric receptor shows how the cytokine interacts with the three chains of to the receptor. (Reproduced from Wang X, M Rickert, and KC Garcia. Structure of the quaternary complex of interleukin-2 with its α, β and γc receptors. Science 310:1159-1163, 2005, with the permission of the publishers. Courtesy of Drs. Patrick Lupardus and K. Christopher Garcia, Stanford University School of Medicine, Palo Alto, California.)

Functional IL-2 receptors are transiently expressed on activation of naive and effector T cells; regulatory T cells always express IL-2 receptors.

- The IL-2 receptor (IL-2R) consists of three noncovalently associated proteins including IL-2Rα (CD25), IL-2/15Rβ (CD122), and γc (CD132).
- Of the three chains, only IL-2Rα is unique to the IL-2R. IL-2 binds to the α chain alone with low affinity, and this does not lead to any detectable cytoplasmic signaling or biologic response.

- IL-2/15Rβ, which is also part of the IL-15 receptor, contributes to IL-2 binding and engages JAK3-STAT5–dependent signal transduction pathways (see Lecture 1).
- The γ chain is shared with a number of cytokine receptors, including those for IL-4, IL-7, IL-9, IL-15, and IL-21, and is therefore called the common γ chain (γc).
- Even though the γc is not directly involved in binding IL-2, its association with the receptor complex is required for high-affinity IL-2 binding and for full activation of signal transduction pathways.
- The IL-2Rβγc complexes are expressed at low levels on resting T cells (and on NK cells) and bind IL-2 with a KD of approximately 10−9 M (Fig. 9-10).

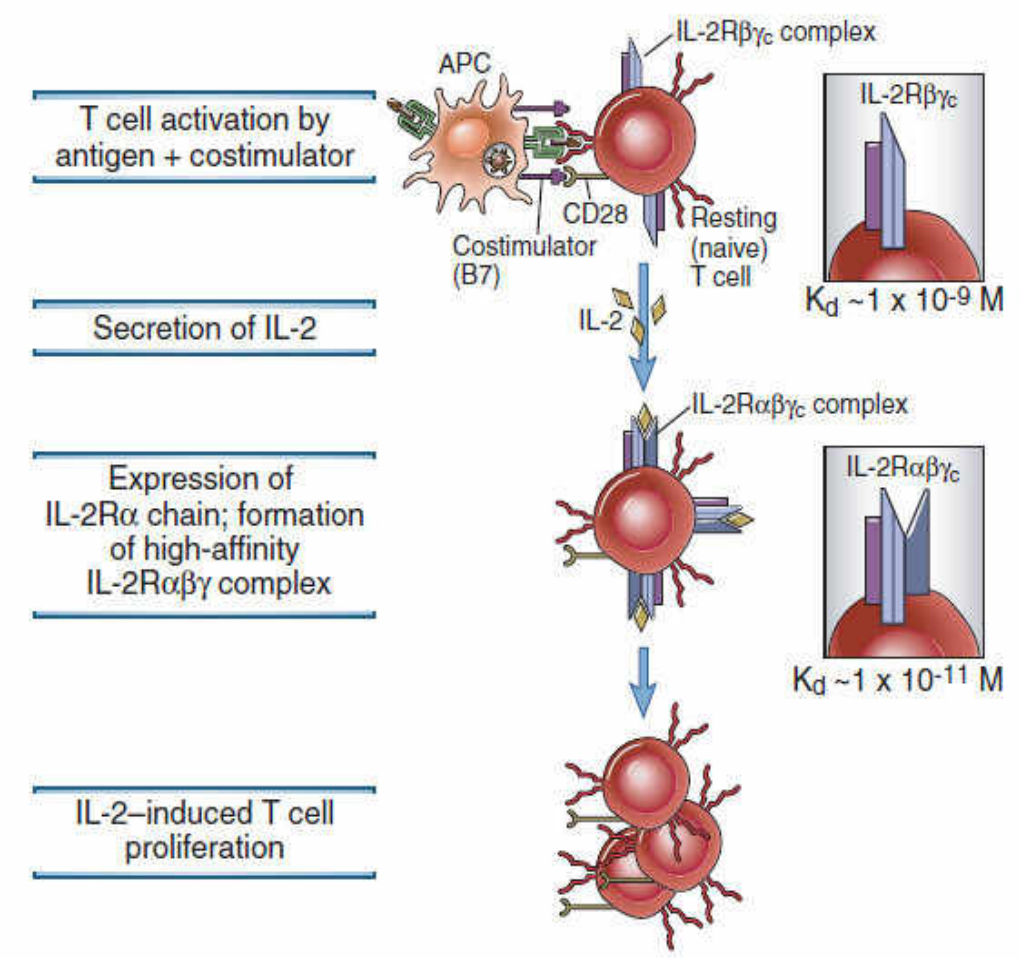


FIGURE 9–10 Regulation of IL-2 receptor expression. Resting (naive) T lymphocytes express the IL-2Rβγ complex, which has a moderate affinity for IL-2. Activation of the T cells by antigen, costimulators, and IL-2 itself leads to expression of the IL-2Rα chain and high levels of the high-affinity IL-2Rαβγ complex.

- Expression of IL-2Rα and, to a lesser extent, of IL-2Rβ is increased on activation of naive CD4+ and CD8+ T cells.
- Cells that express IL-2Rα and form IL-2Rαβγc complexes can bind IL-2 more tightly, with a KD of approximately 10−11 M, and growth stimulation of such cells occurs at a similarly low IL-2 concentration.
- IL-2, produced in response to antigen stimulation, is a stimulus for induction of IL-2Rα, providing a mechanism by which T cell responses amplify themselves.
- CD4+ regulatory T cells express the full IL-2R complex and are thus poised to respond to the cytokine.
- Chronic T cell stimulation leads to shedding of IL-2Rα, and an increased level of shed IL-2Rα in the serum is used clinically as a marker of strong antigenic stimulation (e.g., acute rejection of a transplanted organ).

Functions of IL-2

The biology of IL-2 is fascinating because it plays critical roles in both promoting and controlling T cell responses and functions (Fig. 9-11).

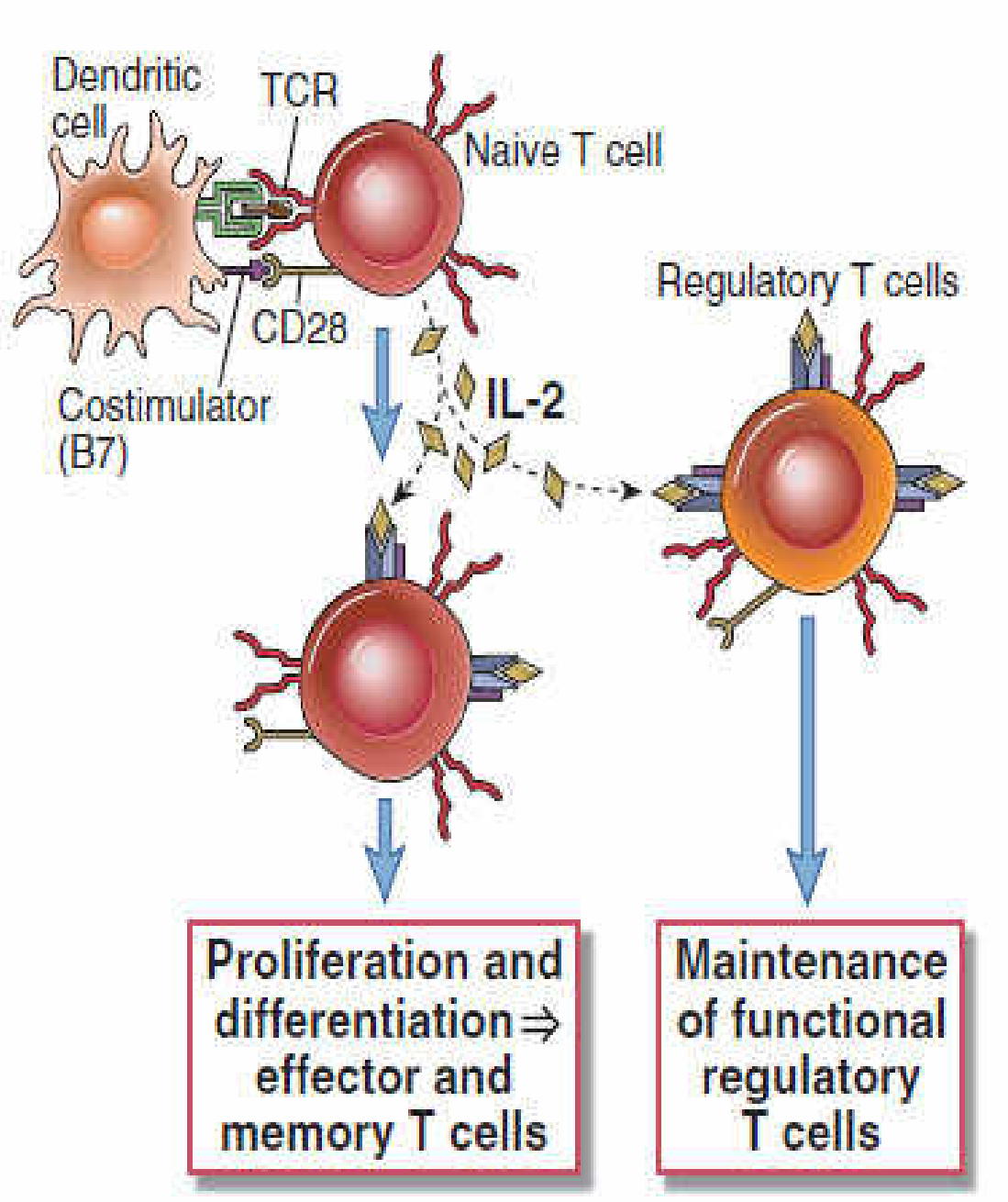


FIGURE 9–11 Biologic actions of IL-2. IL-2 stimulates the survival and proliferation of T lymphocytes, acting as an autocrine growth factor. IL-2 also maintains functional regulatory T cells and thus controls immune responses (e.g., against self-antigens).

- **IL-2 stimulates the survival, proliferation, and differentiation of antigen-activated T cells.**

  IL-2 promotes survival of cells by inducing the antiapoptotic protein Bcl-2.

  It stimulates cell cycle progression through the synthesis of cyclins and relieves a block in cell cycle progression through p27 degradation.

  In addition, IL-2 increases production of effector cytokines, such as IFN-γ and IL-4, by the T cells.

- **IL-2 is required for the survival and function of regulatory T cells** which suppress immune responses against self and other antigens.

  In fact, knockout mice lacking IL-2 or IL-2 receptors develop uncontrolled T and B cell proliferation and autoimmune disease because of a defect in regulatory T cells.

  These studies indicate other growth factors can replace IL-2 for expansion of effector T cells, but that no other cytokine can replace IL-2 for the maintenance of functional regulatory T cells.

  An interesting feature of this function of IL-2 is that regulatory T cells do not produce significant amounts of the cytokine, implying that they depend for their survival on IL-2 made by other T cells responding to foreign antigens.

- **IL-2 has also been shown to stimulate the proliferation and differentiation of NK cells and B cells in vitro**. The physiologic importance of these actions is not established.

Clonal Expansion of T Cells

T cell proliferation in response to antigen recognition is mediated primarily by a combination of signals from the antigen receptor, costimulators, and autocrine growth factors, primarily IL-2.

- The cells that recognize antigen produce IL-2 and also preferentially respond to it, ensuring that the antigen-specific T cells are the ones that proliferate the most.
- The result of this proliferation is **clonal expansion**, which generates the large number of cells required to eliminate the antigen from a small pool of naive antigen-specific lymphocytes.
- Before antigen exposure, the frequency of naive T cells specific for any antigen is 1 in 105 to 106 lymphocytes.
- After microbial antigen exposure, the frequency of all CD8+ T cells specific for that microbe may increase to about 1 in 3 to 1 in 10, representing a >50,000-fold expansion of antigen-specific CD8+ T cells, and the number of specific CD4+ cells increases to 1 in 100 to about 1 in 1000 lymphocytes (Fig. 9-12).

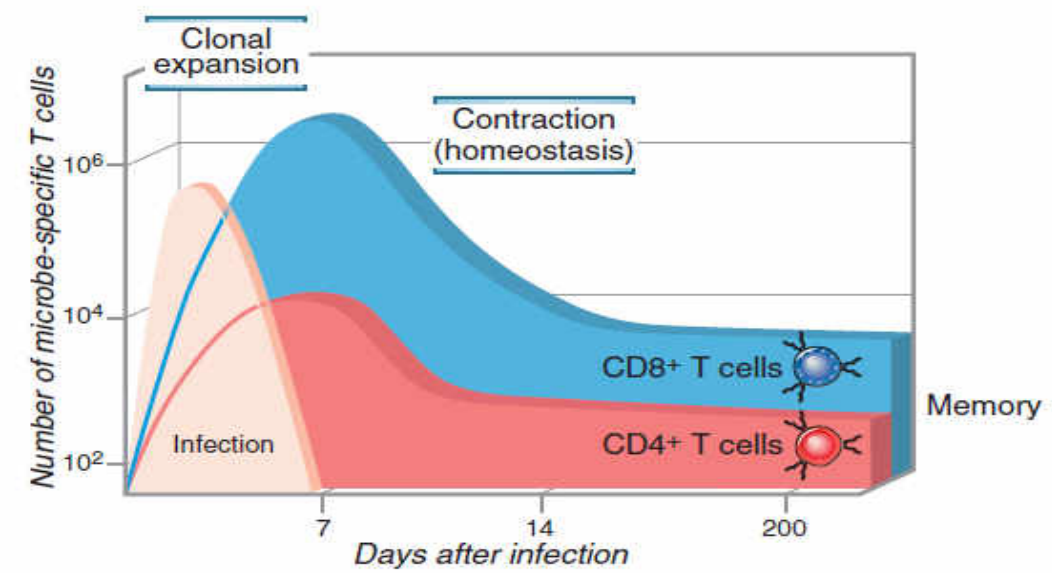


FIGURE 9–12 Clonal expansion of T cells. The numbers of CD4+ and CD8+ T cells specific for microbial antigens and the expansion and decline of the cells during immune responses are illustrated. The numbers are approximations based on studies of model microbial and other antigens in inbred mice.

- Studies in mice first showed this tremendous expansion of the antigen-specific population in some acute viral infections and, remarkably, it occurred within as little as 1 week after infection.
- Equally remarkable was the finding that during this massive antigen specific clonal expansion, "bystander" T cells not specific for the virus did not proliferate.
- The expansion of T cells specific for Epstein-Barr virus and human immunodeficiency virus (HIV) in acutely infected humans is also on this order of magnitude.
- This conclusion has been reached by analyses of antigen-specific T cell responses in humans, using either fluorescent multimers of MHC molecules loaded with particular peptides or intracellular cytokine stains of T cells stimulated with peptides derived from these viruses.
- Many of the progeny of the antigen-stimulated cells differentiate into effector cells.
- Because there are important differences in effector cells of the CD4+ and CD8+ lineages, these are described separately below.
- Effector cells are short-lived, and the numbers of antigen-specific T cells rapidly decline as the antigen is eliminated.
- After the immune response subsides, the surviving memory cells specific for the antigen number on the order of 1 in 104.

Differentiation of CD4+ T Cells into TH1, TH2, and TH17 Effector Cells

- Effector cells of the CD4+ lineage are characterized by their ability to express surface molecules and to secrete cytokines that activate other cells (B lymphocytes, macrophages, and dendritic cells).
- Whereas naive CD4+ T cells produce mostly IL-2 on activation, effector CD4+ T cells are capable of producing a large number and variety of cytokines that have diverse biologic activities.

There are three distinct subsets of CD4+ T cells, called TH1, TH2, and TH17, that function in host defense against different types of infectious pathogens and are involved in different types of tissue injury in immunologic diseases (Fig. 9-13).

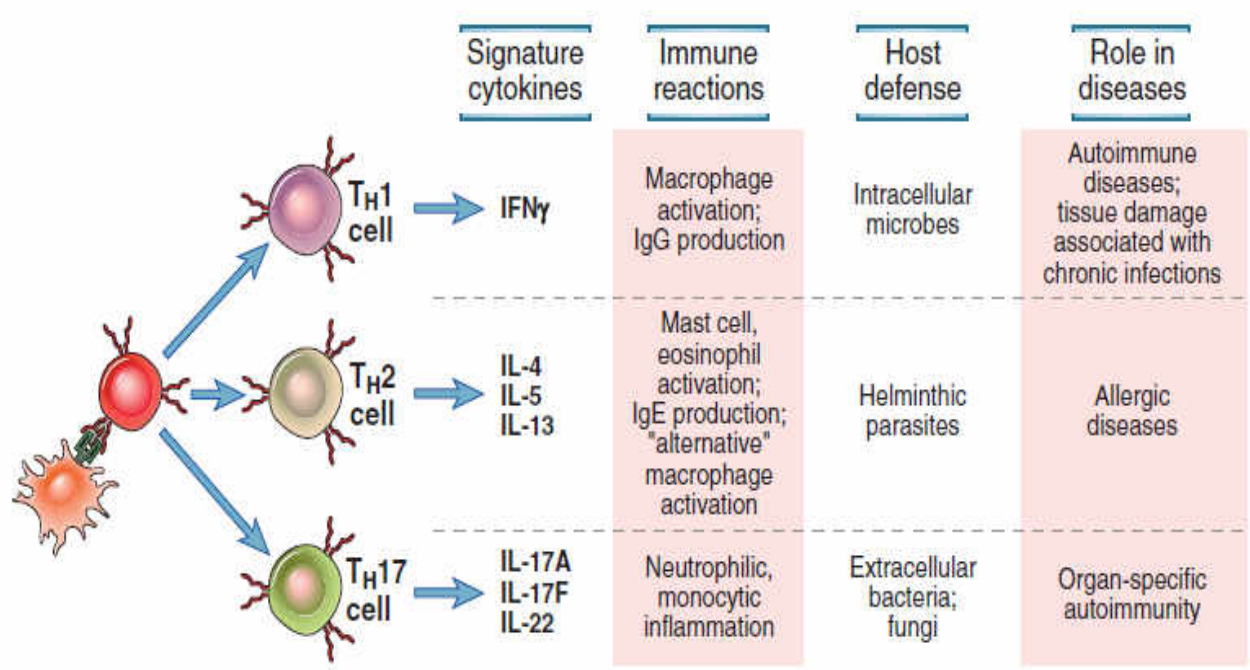


FIGURE 9–13 Properties of TH1, TH2, and TH17 subsets of CD4+ helper T cells. Naive CD4+ T cells may differentiate into distinct subsets of effector cells in response to antigen, costimulators, and cytokines. The columns to the right list the major differences between the best defined subsets.

- A fourth population, **called follicular helper T cells,** is important in antibody responses.
- Regulatory T cells are another distinct population of CD4+ T cells. Their function is to control immune reactions to self and foreign antigens.
- Although these subsets are identifiable in immune reactions (and can often be generated in cell culture), many effector CD4+ T cells produce various combinations of cytokines or only some of the cytokines characteristic of a particular subset and are not readily classifiable into separable populations.
- Whether these populations with mixed or limited cytokine patterns are intermediates in the development of the polarized effector cells or are themselves fixed populations is not known.
- It is also clear that some of these differentiated T cells may convert from one population into another by changes in activation conditions.
- The extent and significance of such **"plasticity"** are topics of active research.

Properties of TH1, TH2, and TH17 Subsets

- Elucidation of the development, properties, and functions of subsets of effector CD4+ T cells has been one of the most impressive accomplishments of immunology research.
- It was appreciated many years ago that host responses to different infections varied greatly, as did the reactions in different immunologic diseases.
- For instance, the immune reaction to intracellular bacteria like *Mycobacterium tuberculosis* is dominated by activated macrophages, whereas the reaction to helminthic parasites consists of IgE antibody production and the activation of eosinophils.
- Along the same lines, in many chronic autoimmune diseases, tissue damage is caused by inflammation with accumulation of neutrophils, macrophages, and T cells, whereas in allergic disorders, the lesions contain abundant eosinophils along with other leukocytes.
- The realization that all these phenotypically diverse immunologic reactions are dependent on CD4+ T cells raised an obvious question: How can the same CD4+ cells elicit such different responses?
- The answer, as we now know, is that CD4+ T cells consist of subsets of effector cells that produce distinct sets of cytokines, elicit quite different reactions, and are involved in host defense against different microbes as well as in distinct types of immunologic diseases.
- The first subsets that were discovered were called TH1 and TH2 (so named because they were the first two subsets identified).
- It was subsequently found that some inflammatory diseases that were thought to be caused by TH1-mediated reactions were clearly not dependent on this

type of T cell, and this realization led to the discovery of TH17 cells (called TH17 because their characteristic cytokine is IL-17).

- In the next section, we describe the properties of these subsets and how they develop from naive T cells. We will return to their cytokine products, effector functions, and roles in cell mediated immunity in Chapter 10.

The defining characteristics of differentiated subsets of effector cells are the cytokines they produce, the transcription factors they express, and epigenetic changes in cytokine gene loci.

These characteristics of TH1, TH2, and TH17 cells are described below.

The signature cytokines produced by the major CD4+ T cell subsets are IFN-γ for TH1 cells; IL-4, IL-5, and IL-13 for TH2 cells; and IL-17 and IL-22 for TH17 cells (see Fig. 9-13).

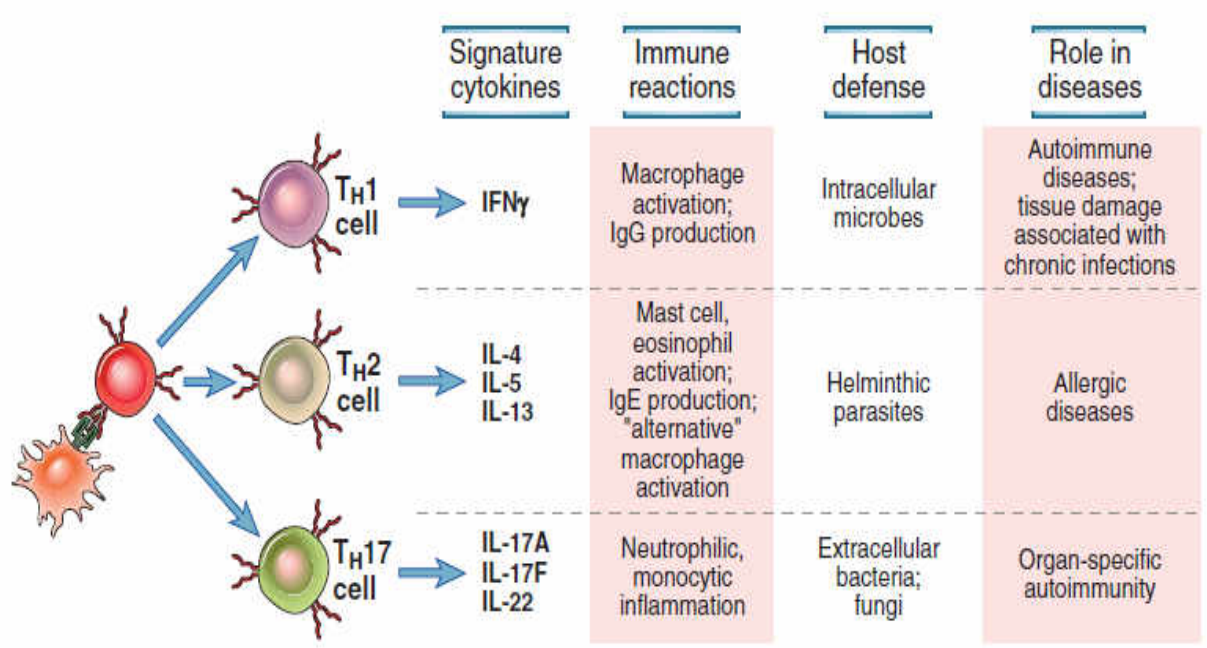


FIGURE 9–13 Properties of TH1, TH2, and TH17 subsets of CD4+ helper T cells. Naive CD4+ T cells may differentiate into distinct subsets of effector cells in response to antigen, costimulators, and cytokines. The columns to the right list the major differences between the best defined subsets.

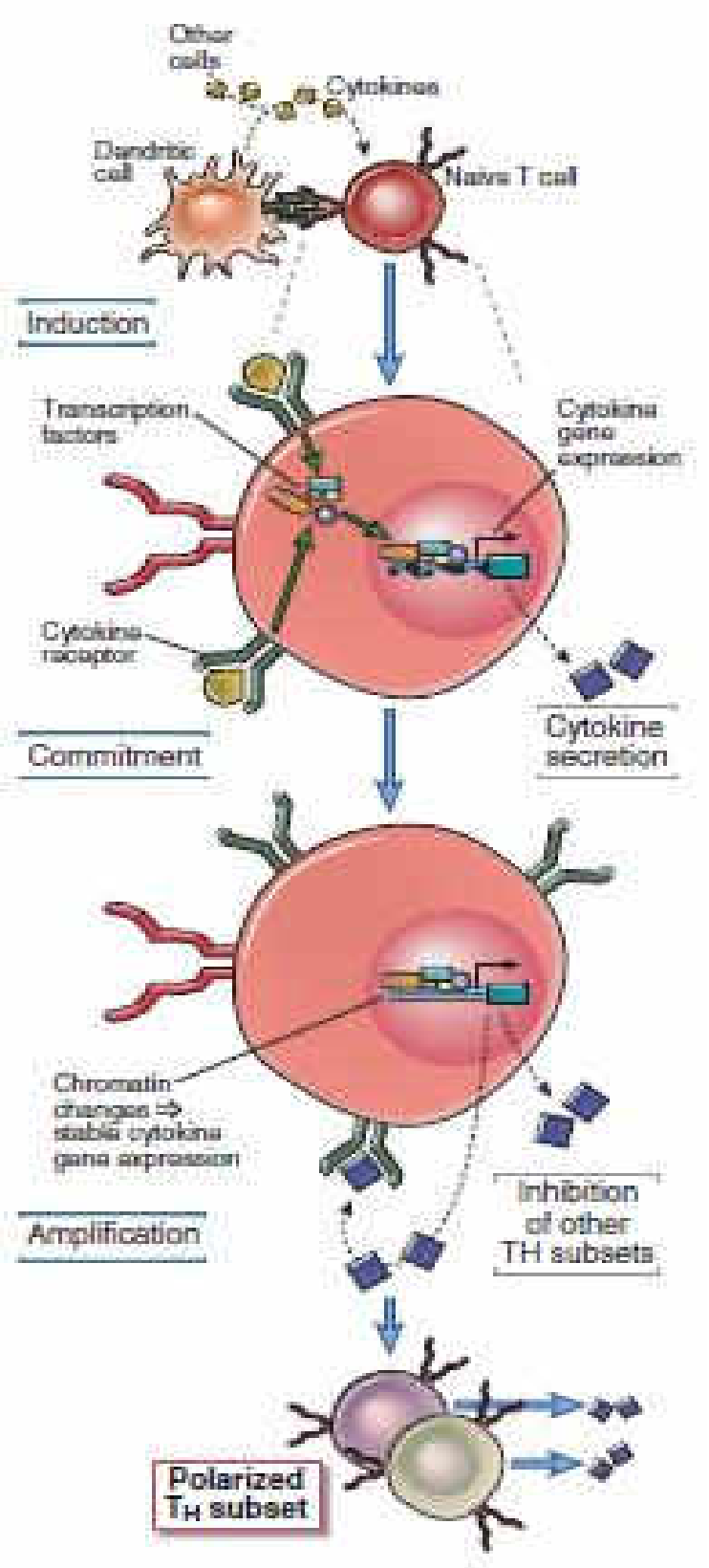
- The cytokines produced by these T cell subsets determine their effector functions and roles in diseases.
- The cytokines also participate in the development and expansion of the respective subsets (described later).
- In addition, these subsets of T cells differ in the expression of adhesion molecules and receptors for chemokines and other cytokines, which are involved in the migration of distinct subsets to different tissues (see Chapter 10).

Development of TH1, TH2, and TH17 Subsets

Differentiated TH1, TH2, and TH17 cells all develop from naive CD4+ T lymphocytes, mainly in response to cytokines present early during immune responses, and differentiation involves transcriptional activation and epigenetic modification of cytokine genes.

- The process of differentiation, which is sometimes referred to as **polarization** of T cells, can be divided into induction, stable commitment, and amplification (Fig. 9-14).
- Cytokines act on antigen-stimulated T cells to induce the transcription of cytokine genes that are characteristic of differentiation toward each subset.
- With continued activation, epigenetic changes occur so that the genes encoding that subset's cytokines are more accessible for activation, and genes that encode cytokines not produced by that subset are rendered inaccessible.
- Because of these changes, the differentiating T cell becomes progressively committed to one specific pathway.
- Cytokines produced by any given subset promote the development of this subset and inhibit differentiation toward other CD4+ subpopulations.
- Thus, positive and negative feedback loops contribute to the generation of an increasingly polarized population of effector cells.

FIGURE 9–14 Development of TH1, TH2, and TH17 subsets. Cytokines produced early in the innate or adaptive immune response to microbes promote the differentiation of naive CD4+ T cells into TH1, TH2, or TH17 cells by activating transcription factors that stimulate production of the cytokines of each subset (the early induction step). Progressive activation leads to stable changes in the expressed genes (commitment), and cytokines promote the development of each population and suppress the development of the other subsets (amplification). These principles apply to all three major subsets of CD4+ effector T cells.


Other cells
Cytokines
Dendritic cell
Naive T cell
Induction
Transcription factors
Cytokine gene expression
Cytokine receptor
Cytokine secretion
Commitment
Chromatin changes ⇒ stable cytokine gene expression
Inhibition of other TH subsets
Amplification
Polarized TH subset

- There are several important general features of T cell subset differentiation.

The cytokines that drive the development of CD4+ T cell subsets are produced by APCs (primarily dendritic cells and macrophages) and other immune cells (such as NK cells and basophils or mast cells) present at the site of the immune response.

- Dendritic cells that encounter microbes and display microbial antigens are activated to produce cytokines (as well as costimulators, described earlier) as part of innate immune responses to the microbes (see Chapter 4). Different microbes may stimulate dendritic cells to produce distinct sets of cytokines, perhaps because the microbes are recognized by different microbial sensors in the cells. Other cells of innate immunity, such as NK cells and mast cells, also produce cytokines that influence the pattern of T cell subset development.

Stimuli other than cytokines may also influence the pattern of helper T cell differentiation.

- Some studies indicate that different subsets of dendritic cells selectively promote either TH1 or TH2 differentiation; the same principle may be true for TH17 cells. In addition, the genetic makeup of the host is an important determinant of the pattern of T cell differentiation. Inbred mice of some strains develop TH2 responses to the same microbes that stimulate TH1 differentiation in most other strains. Strains of mice that develop TH2-dominant responses are susceptible to infections by intracellular microbes (see Chapter 15).

The distinct cytokine profiles of differentiated cell populations are controlled by particular transcription factors that activate cytokine gene transcription and by chromatin modifications affecting cytokine gene loci.

- The transcription factors are themselves activated or induced by cytokines as well as by antigen receptor stimuli. Each subset expresses its own

characteristic set of transcription factors. As the subsets become increasingly polarized, the gene loci encoding that subset's signature cytokines undergo histone modifications (changes in methylation and acetylation) and consequent chromatin remodeling events, so that these loci are "accessible" and in an "open" chromatin configuration, whereas the loci for other cytokines (those not produced by that subset) are in an inaccessible chromatin state. These epigenetic changes ensure that each subset can produce only its characteristic collection of cytokines. It is likely that epigenetic changes in cytokine gene loci correlate with stable phenotypes, and before these changes are established, the subsets may be plastic and convertible.

Each subset of differentiated effector cells produces cytokines that promote its own development and may suppress the development of the other subsets.

- This feature of T cell subset development provides a powerful amplification mechanism. For instance, IFN-γ secreted by TH1 cells promotes further TH1 differentiation and inhibits the generation of TH2 and TH17 cells. Similarly, IL-4 produced by TH2 cells promotes TH2 differentiation, and IL-21 produced by TH17 cells enhances TH17 differentiation. Thus, each subset amplifies itself and may inhibit the other subsets. For this reason, once an immune response develops along one effector pathway, it becomes increasingly polarized in that direction, and the most extreme polarization is seen in chronic infections or in chronic exposure to environmental antigens, when the immune stimulation is prolonged.

Differentiation of each subset is induced by the types of microbes which that subset is best able to combat.

- For instance, the development of TH1 cells from antigen-stimulated T cells is driven by intracellular microbes, against which the principal defense is TH1 mediated. Conversely, the immune system responds to helminthic parasites

by the development of TH2 cells, and the cytokines produced by these cells are critical for combating helminths. Similarly, TH17 responses are induced by some bacteria and fungi and are most effective at defending against these microbes. The generation and effector functions of these differentiated T cells are an excellent illustration of the concept of *specialization of adaptive immunity,* which refers to the ability of the immune system to respond to different microbes in ways that are optimal for combating those microbes. With this background, we proceed to a description of the signals for and mechanisms of development of each subset.

TH1 Differentiation

TH1 differentiation is driven mainly by the cytokines IL-12 and IFN-γ and occurs in response to microbes that activate dendritic cells, macrophages, and NK cells (Fig. 9-15).

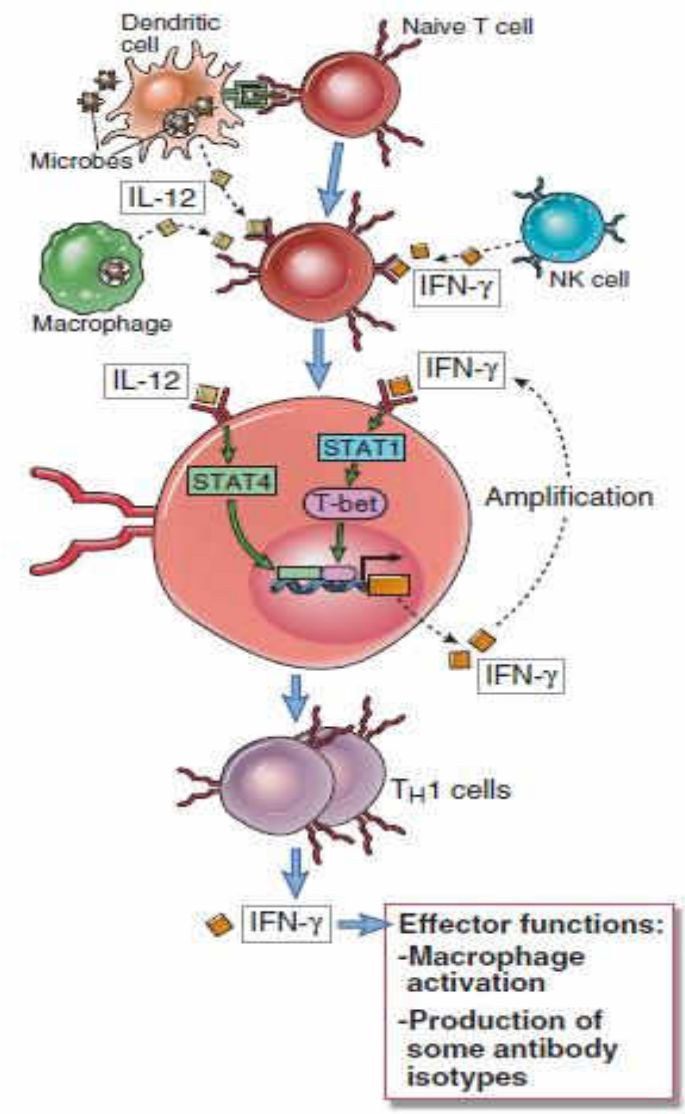


FIGURE 9–15 Development of TH1 cells. IL-12 produced by dendritic cells and macrophages in response to microbes, including intracellular microbes, and IFN-γ produced by NK cells (all part of the early innate immune response to the microbes) activate the transcription factors T-bet, STAT1, and STAT4, which stimulate the differentiation of naive CD4+ T cells to the TH1 subset. IFN-γ produced by the TH1 cells amplifies this response and inhibits the development of TH2 and TH17 cells.

- The differentiation of antigen-activated CD4+ T cells to TH1 effectors is stimulated by many intracellular bacteria, such as *Listeria* and mycobacteria, and by some parasites, such as *Leishmania,* all of which infect dendritic cells and macrophages.
- It is also stimulated by viruses and by protein antigens administered with strong adjuvants.
- A common feature of these infections and immunization conditions is that they elicit innate immune reactions that are associated with the production of certain cytokines, including IL-12, IL-18, and type I interferons.
- All these cytokines promote TH1 development; of these, IL-12 is probably the most potent.
- Knockout mice lacking IL-12 are extremely susceptible to infections with intracellular microbes.
- IL-18 synergizes with IL-12, and type I interferons may be important for TH1 differentiation in response to viral infections, especially in humans.
- Other microbes stimulate NK cells to produce IFN-γ, which is itself a strong TH1-inducing cytokine and also acts on dendritic cells and macrophages to induce more IL-12 secretion.
- Once TH1 cells have developed, they secrete IFN-γ, which promotes more TH1 differentiation and thus strongly amplifies the reaction.
- In addition, IFN-γ inhibits the differentiation of naive CD4+ T cells to the TH2 and TH17 subsets, thus promoting the polarization of the immune response in one direction.
- T cells may further enhance cytokine production by dendritic cells and macrophages, by virtue of CD40 ligand (CD40L) on activated T cells engaging CD40 on the APCs and stimulating IL-12 secretion.

IFN-γ and IL-12 stimulate TH1 differentiation by activating the transcription factors T-bet, STAT1, and STAT4 (see Fig. 9-15).

- T-bet, a member of the T-box family of transcription factors, is considered to be the master regulator of TH1 differentiation.
- T-bet expression is induced in naive CD4+ T cells in response to antigen and IFN-γ.
- IFN-γ activates the transcription factor STAT1, which in turn stimulates expression of T-bet.
- T-bet then promotes IFN-γ production through a combination of direct transcriptional activation of the IFN-γ gene and by inducing chromatin remodeling of the IFN-γ locus.
- The ability of IFN-γ to stimulate T-bet expression and the ability of T-bet to enhance IFN-γ transcription set up a positive amplification loop that drives differentiation of T cells toward the TH1 phenotype.
- IL-12 contributes to TH1 commitment by binding to receptors on antigen-stimulated CD4+ T cells and activating the transcription factor STAT4, which further enhances IFN-γ production.
- Mice deficient in IL-12, IL-12 receptor, T-bet, or STAT4 cannot mount effective TH1 responses to infections, and humans with genetic deficiencies in the IL-12R signaling pathway have impaired responses to infections with several kinds of intracellular bacteria.

TH2 Differentiation

TH2 differentiation is stimulated by the cytokine IL-4 and occurs in response to helminths and allergens (Fig. 9-16).

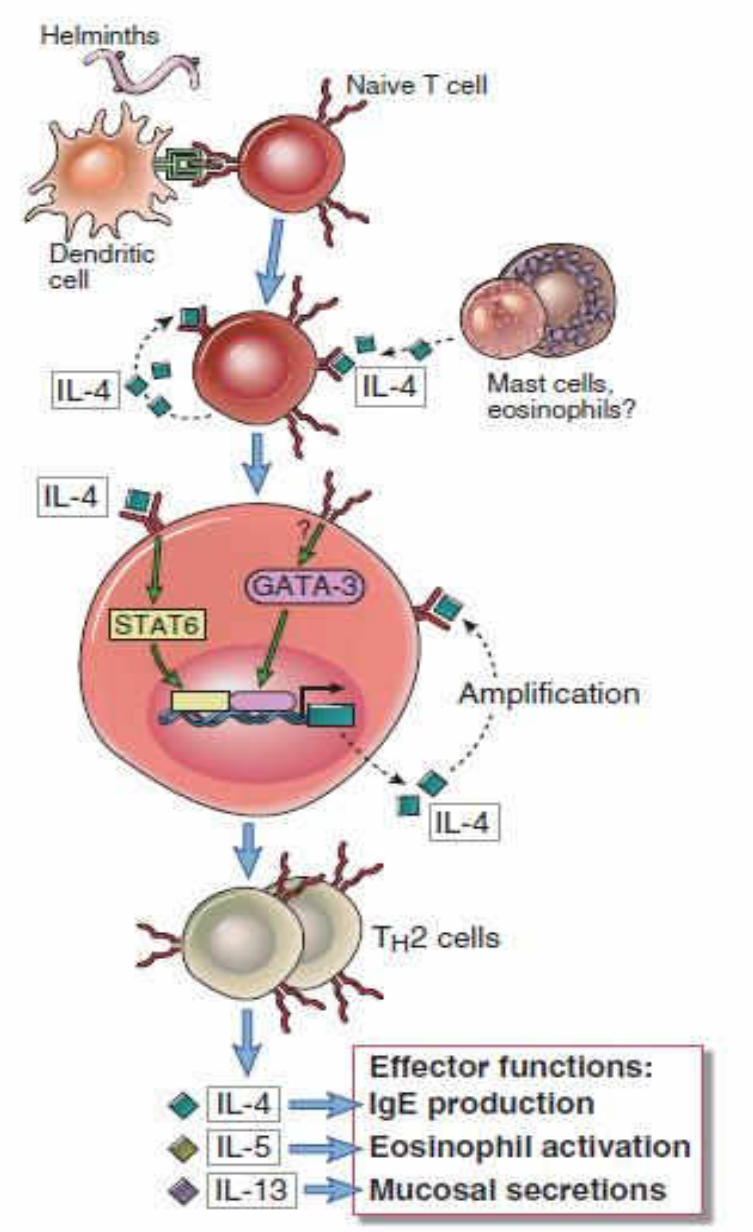


FIGURE 9–16 Development of TH2 cells. IL-4 produced by activated T cells themselves or by mast cells and eosinophils, especially in response to helminths, activates the transcription factors GATA-3 and STAT6, which stimulate the differentiation of naive CD4+ T cells to the TH2 subset. IL-4 produced by the TH2 cells amplifies this response and inhibits the development of TH1 and TH17 cells.

- Helminths and allergens cause chronic T cell stimulation, often without the strong innate immune responses that are required for TH1 differentiation.
- Thus, TH2 cells may develop in response to microbes and antigens that cause persistent or repeated T cell stimulation without much inflammation or the production of pro-inflammatory cytokines that drive TH1 and TH17 responses.
- The differentiation of antigen-stimulated T cells to the TH2 subset is dependent on IL-4, which raises an interesting question:

Because differentiated TH2 cells are the major source of IL-4 during immune responses to protein antigens, where does the IL-4 come from before TH2 cells develop?

- In some situations, such as helminthic infections, IL-4 produced by mast cells and, possibly, other cell populations, such as basophils recruited into lymphoid organs and eosinophils, may contribute to TH2 development.
- Another possibility is that antigen-stimulated CD4+ T cells secrete small amounts of IL-4 from their initial activation.
- If the antigen is persistent and present at high concentrations, the local concentration of IL-4 gradually increases.
- If the antigen also does not trigger inflammation with attendant IL-12 production, the result is increasing differentiation of T cells to the TH2 subset.
- Once TH2 cells have developed, the IL-4 they produce serves to amplify the reaction and inhibits the development of TH1 and TH17 cells.

IL-4 stimulates TH2 development by activating the transcription factor STAT6, and STAT6, together with TCR signals, induces expression of GATA-3 (see Fig. 9-16).

- GATA-3 is a transcription factor that acts as a master regulator of TH2 differentiation, enhancing expression of the TH2 cytokine genes IL-4, IL-5, and IL-13, which are located in the same genetic locus.
- GATA-3 works by directly interacting with the promoters of these genes and also by causing chromatin remodeling, which opens up the locus for accessibility to other transcription factors.
- This is similar to the way in which T-bet influences IFN-γ expression. GATA-3 functions to stably commit differentiating cells toward the TH2 phenotype, enhancing its own expression through a positive feedback loop.
- Furthermore, GATA-3 blocks TH1 differentiation by inhibiting expression of the signaling chain of the IL-12 receptor.
- Knockout mice lacking IL-4, STAT6, or GATA-3 are deficient in TH2 responses.

TH17 Differentiation

The development of TH17 cells is stimulated by proinflammatory cytokines produced in response to bacteria and fungi (Fig. 9-17).

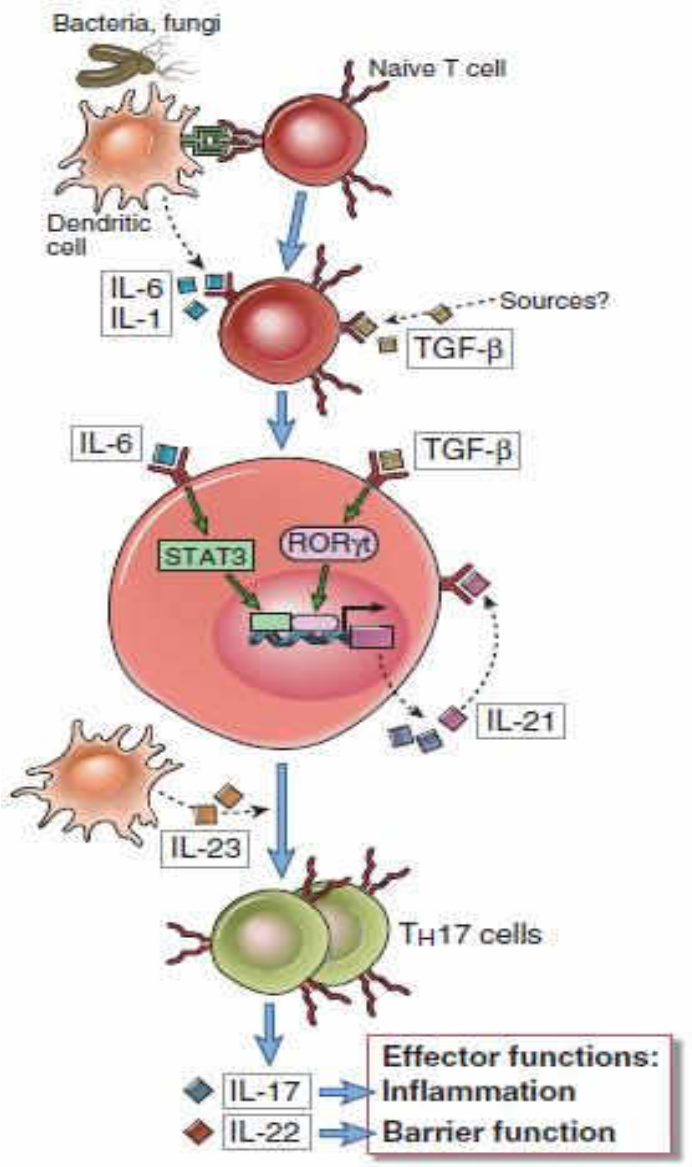


FIGURE 9–17 Development of TH17 cells. IL-1 and IL-6 produced by APCs and transforming growth factor-β (TGF-β) produced by various cells activate the transcription factors RORγt and STAT3, which stimulate the differentiation of naive CD4+ T cells to the TH17 subset. IL-23, which is also produced by APCs, especially in response to fungi, stabilizes the TH17 cells. TGF-β may promote TH17 responses indirectly by suppressing TH1 and TH2 cells, both of which inhibit TH17 differentiation (not shown in the figure). IL-21 produced by the TH17 cells amplifies this response.

- Various bacteria and fungi act on dendritic cells and stimulate the production of cytokines including IL-6, IL-1, and IL-23.
- Engagement of the lectin like receptor Dectin-1 on dendritic cells by fungal products is a signal for the production of these cytokines.
- The combination of cytokines that drive TH17 cell development may be produced not only in response to particular microbes, such as fungi, but also when cells infected with various bacteria and fungi undergo apoptosis and are ingested by dendritic cells.
- IL-23 may be more important for the proliferation and maintenance of TH17 cells than for their induction.
- TH17 differentiation is inhibited by IFN-γ and IL-4; therefore, strong TH1 and TH2 responses tend to suppress TH17 development.
- A surprising aspect of TH17 differentiation is that TGF-β, which is produced by many cell types and is an anti-inflammatory cytokine (see Chapter 14), promotes the development of proinflammatory TH17 cells when other mediators of inflammation, such as IL-6 or IL-1, are present.
- Some experimental results indicate that TGF-β does not directly stimulate TH17 development but is a potent suppressor of TH1 and TH2 differentiation and thus removes the inhibitory effect of these two subsets and allows the TH17 response to develop under the influence of IL-6 or IL-1.
- According to this idea, the action of TGF-β in promoting TH17 responses is indirect.
- TH17 cells produce IL-21, which may further enhance their development, providing an amplification mechanism.

The development of TH17 cells is dependent on the transcription factors RORγt and STAT3 (see Fig. 9-17).

- TGF-β and the inflammatory cytokines, mainly IL-6 and IL-1, work cooperatively to induce the production of RORγt, a transcription factor that is a member of the retinoic acid receptor family.
- RORγt is a T cell–restricted protein encoded by the *RORC* gene, so sometimes the protein may be referred to as RORc.
- The inflammatory cytokines, notably IL-6, activate the transcription factor STAT3, which functions with RORγt to drive the TH17 response.
- Mutations in the gene encoding STAT3 are the cause of a rare human immune deficiency disease called Job's syndrome because patients present with multiple bacterial and fungal abscesses of the skin, resembling the biblical punishments visited on Job. These patients have defective TH17 responses.
- TH17 cells appear to be especially abundant in mucosal tissues, particularly of the gastrointestinal tract, suggesting that the tissue environment influences the generation of this subset, perhaps by providing high local concentrations of TGF-β and other cytokines.
- This observation also suggests that TH17 cells may be especially important in combating intestinal infections and in the development of intestinal inflammation.
- The development of TH17 cells in the gastrointestinal tract is also dependent on the local microbial population.
- The functions of differentiated effector cells of the CD4+ lineage are mediated by surface molecules, primarily CD40 ligand, and by secreted cytokines.
- We will describe the cytokines produced by differentiated CD4+ effector cells and their functions in Chapter 10.

Differentiation of CD8+ T Cells into Cytotoxic T Lymphocytes

The activation of naive CD8+ T cells requires antigen recognition and second signals, but the nature of the second signals may be different from those for CD4+ cells. We have previously described the role of dendritic cells in presenting antigens to and costimulating naive CD8+ cells.

The full activation of naive CD8+ T cells and their differentiation into functional CTLs and memory cells may require the participation of CD4+ helper cells.

- In other words, helper T cells can provide second signals for CD8+ T cells. The requirement for helper cells may vary according to the type of antigen exposure.
- In the setting of a strong innate immune response to a microbe, if APCs are directly infected by the microbe, or if cross-presentation of microbial antigens is efficient, CD4+ T cell help may not be required.
- CD4+ helper T cells may be required for CD8+ T cell responses to latent viral infections, organ transplants, and tumors, all of which tend to elicit relatively weak innate immune reactions.
- The varying importance of CD4+ T cells in the development of CTL responses is illustrated by studies with mice that lack helper T cells.
- In these mice, some viral infections fail to generate effective CTLs or CD8+ memory cells and are not eradicated, whereas other viruses do stimulate effective CTL responses.
- A lack of CD4+ T cell helper function is the accepted explanation for the defects in CTL generation seen in individuals infected with HIV, which infects and eliminates only CD4+ T cells. Helper T cells may promote CD8+ T cell activation by several mechanisms (Fig. 9-18).

- Helper T cells may secrete cytokines that stimulate the differentiation of CD8+ T cells.
- Antigen-stimulated helper T cells express CD40 ligand (CD40L), which binds to CD40 on APCs and activates ("licenses") these APCs to make them more efficient at stimulating the differentiation of CD8+ T cells.

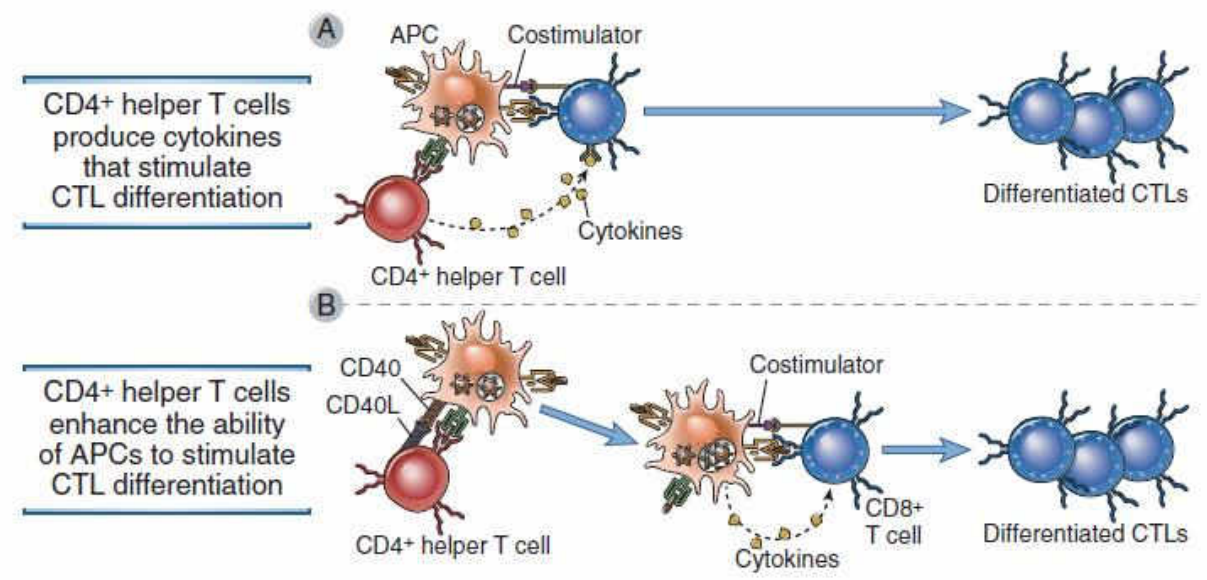


FIGURE 9–18 Role of helper T cells in the differentiation of CD8+ T lymphocytes. CD4+ helper T cells promote the development of CD8+ CTLs by secreting cytokines that act directly on the CD8+ cells **(A)** or by activating APCs to become more effective at stimulating the differentiation
of the CD8+ T cells **(B)**.

Differentiation of CD8+ T cells into effector CTLs involves acquisition of the machinery to perform target cell killing.

- The most specific feature of CTL differentiation is the development of membrane-bound cytoplasmic granules that contain proteins, including perforin and granzymes, whose function is to kill other cells (described in Chapter 10 Abbas Immunology).
- In addition, differentiated CTLs are capable of secreting cytokines, mostly IFN-γ, that function to activate phagocytes.

- The molecular events in CTL differentiation involve transcription of genes encoding these effector molecules.
- Two transcription factors that are required for this program of new gene expression are T-bet (which we discussed in relationship to TH1 differentiation earlier) and eomesodermin, which is structurally related to T-bet.

Development of Memory T Cells

T cell–mediated immune responses to an antigen usually result in the generation of memory T cells specific for that antigen, which may persist for years, even a lifetime.

- Thus, memory cells provide optimal defense against pathogens that are prevalent in the environment and may be repeatedly encountered.
- The success of vaccination is attributed in large part to the ability to generate memory cells on initial antigen exposure.
- Edward Jenner's classic experiment of successful vaccination of a child against smallpox is a demonstration of a memory response.
- Despite the importance of this historic observation, many fundamental questions about the generation of memory cells have still not been answered.
- As expected, the size of the memory pool is proportional to the size of the naive antigen-specific population.
- Memory cells may develop from effector cells along a linear pathway, or effector and memory populations follow divergent differentiation and are two alternative fates of lymphocytes activated by antigen and other stimuli (Fig. 9-19).
- The mechanisms that determine whether an individual antigen-stimulated T cell will become a short-lived effector cell or enter the long-lived memory cell pool are not established.
- The signals that drive the development of memory cells are also not established.
- One possibility is that memory cells contain transcription factors that are different from those present in effector cells, and the nature of the transcription factors influences the choice of differentiation pathway.

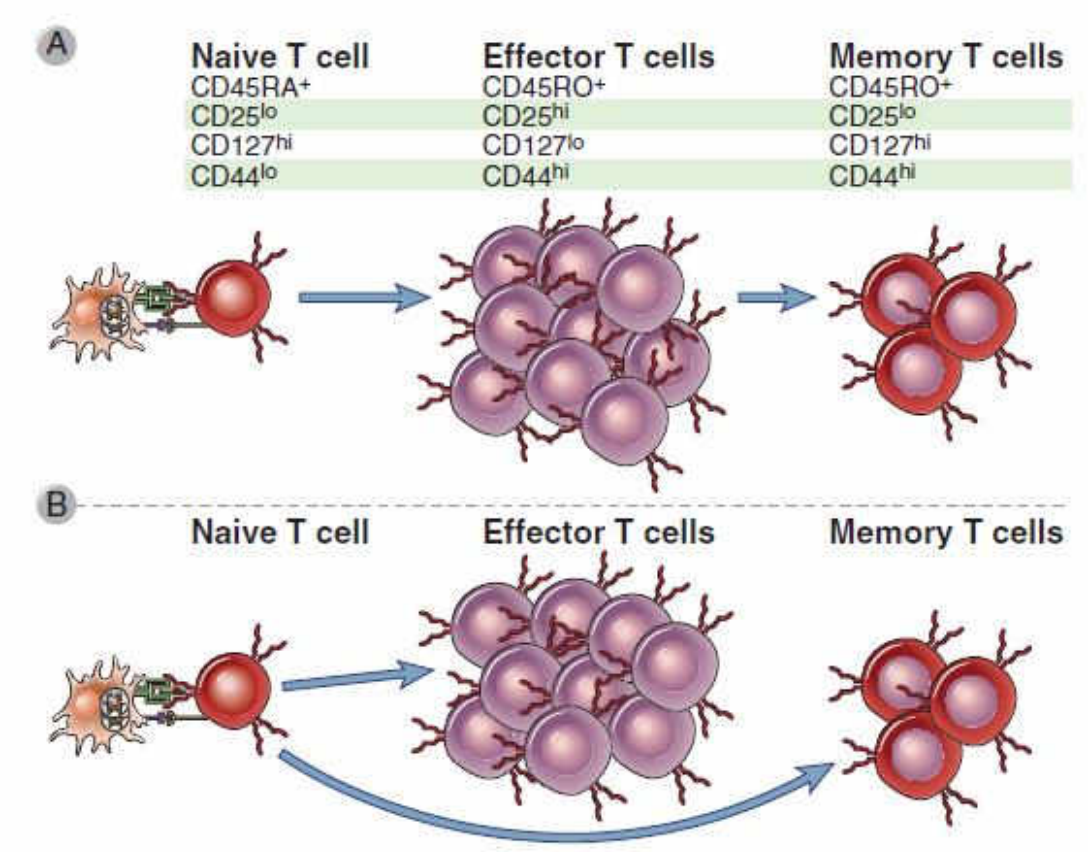


FIGURE 9–19 Development of memory T cells. In response to antigen and costimulation, naive T cells differentiate into effector and memory cells. Some of the phenotypic markers of these cell populations are shown in A. **A,** According to the linear model of memory T cell differentiation, most effector cells die and some survivors develop into the memory population. **B,** According to the branched differentiation model, effector and memory cells are alternative fates of activated T cells.

Properties of Memory T Cells

Memory T cells have several characteristics that are responsible for their survival and rapid activation.

The defining properties of memory cells are their ability to survive in a quiescent state after antigen is eliminated and to mount larger and more rapid responses to antigens than do naive cells.

- Whereas naive T cells live for weeks or months and are replaced by mature cells that develop in the thymus, memory T cells may survive for months or years.
- Thus, as humans age in an environment in which they are constantly exposed to and responding to infectious agents, the proportion of memory cells induced by these microbes compared with naive cells progressively increases.
- In individuals older than 50 years or so, half or more of circulating T cells may be memory cells.
- The rapid response of memory cells to antigen challenge has been documented in many studies done in humans and experimental animals.
- For example, in studies in mice, naive T cells respond to antigen in vivo in 5 to 7 days, and memory cells respond within 1 to 3 days.

The number of memory T cells specific for any antigen is greater than the number of naive cells specific for the same antigen.

- As we know, proliferation leads to a large clonal expansion in all immune responses and differentiation into effector cells, most of which die after the antigen is eliminated.
- The surviving cells of the expanded clone are memory cells, and they are typically 10- to 100-fold more numerous than the pool of naive cells before antigen encounter.

- The increased clone size is a major reason that antigen challenge in a previously immunized individual induces a more robust response than the first immunization in a naive individual.

Memory cells express increased levels of antiapoptotic proteins, which may be responsible for their prolonged survival.

- These proteins include Bcl-2 and Bcl-XL, which promote mitochondrial stability and block apoptosis induced by a deficiency of survival signals (see Fig. 14-7, Chapter 14 Abbas Immunology). The presence of these proteins allows memory cells to survive even after antigen is eliminated and innate immune responses have subsided, and the normal signals for T cell survival and proliferation are no longer present.

Memory cells undergo slow proliferation, and this ability to self-renew may contribute to the long life span of the memory pool.

The cycling of these cells may be driven by cytokines. Because of the capacity for self-renewal, memory cells have been likened to stem cells.

The maintenance of memory cells is dependent on cytokines but does not require antigen recognition.

- The most important cytokine for the maintenance of memory CD4+ and CD8+ T cells is IL-7, which also plays a key role in early lymphocyte development and in the survival of naive T cells.
- Predictably, high expression of the IL-7 receptor (CD127) is characteristic of memory T cells.
- Memory CD8+ T cells also depend on the related cytokine IL-15 for their survival.

- IL-7 and IL-15 induce the expression of anti-apoptotic proteins and stimulate low-level proliferation, both of which maintain populations of memory T cells during long periods.
- The independence of memory cells from antigen recognition has been best demonstrated by experiments in mice in which antigen receptors are genetically deleted after mature lymphocytes have developed.
- In these mice, the number of naive lymphocytes drops rapidly but memory cells are maintained.

The gene loci for cytokines and other effector molecules may be fixed in an accessible configuration in memory cells.

- There is some evidence that the chromatin surrounding cytokine genes in CD4+ memory T cells and genes encoding molecules such as perforin in memory CD8+ T cells are in an accessible configuration, perhaps because of changes in methylation and acetylation of histones.
- As a result, these genes may be poised to respond rapidly to antigen challenge, accounting for the rapid effector responses of memory cells.
- Memory T cells are also less dependent on costimulation than are naive cells, allowing memory cells to respond to antigens presented by a wide range of APCs in peripheral tissues; in contrast, naive T cells are dependent on antigen presentation by mature dendritic cells in lymphoid organs.
- The relative costimulation independence of memory T cells may also be related to the accessible state of the gene loci that encode molecules involved in T cell responses.
- The most reliable phenotypic markers for memory T cells appear to be the surface expression of the IL-7 receptor and a protein of unknown function

called CD27 and the absence of markers of naive and recently activated T cells (Fig. 9-19).

- In humans, most naive T cells express the 200-kD isoform of a surface molecule called CD45 that contains a segment encoded by an exon designated A.
- This CD45 isoform can be recognized by antibodies specific for the A-encoded segment and is therefore called CD45RA (for "restricted A").
- In contrast, most memory T cells express a 180-kD isoform of CD45 in which the A exon RNA has been spliced out; this isoform is called CD45RO.
- However, this way of distinguishing naive from memory T cells is not perfect, and interconversion between CD45RA+ and CD45RO+ populations has been documented.

Both CD4+ and CD8+ memory T cells are heterogeneous and can be subdivided into subsets based on their homing properties and functions.

- **Central memory** T cells express the chemokine receptor CCR7 and L-selectin and home mainly to lymph nodes. They have a limited capacity to perform effector functions when they encounter antigen, but they undergo brisk proliferative responses and generate many effector cells on antigen challenge.
- **Effector memory** T cells, on the other hand, do not express CCR7 or L-selectin and home to peripheral sites, especially mucosal tissues. On antigenic stimulation, effector memory T cells produce effector cytokines such as IFN-γ or rapidly become cytotoxic, but they do not proliferate much. This effector subset, therefore, is poised for a rapid response to a repeated exposure to a microbe, but complete eradication of the infection may also require large numbers of effectors generated from the central memory T cells. It is unclear

if all memory T cells can be classified into central and effector memory cells. Memory T cells are also heterogeneous in terms of cytokine profiles. For example, some CD4+ memory T cells may be derived from precursors before commitment to the TH1, TH2, or TH17 phenotype, and when activated by re-exposure to antigen and cytokines, they can differentiate into any of these subsets. Other memory T cells may be derived from fully differentiated TH1, TH2, or TH17 effectors and retain their respective cytokine profiles on reactivation. Memory CD8+ T cells may also exist that maintain some of the phenotypic characteristics of differentiated CTLs.

DECLINE OF T CELL RESPONSES

Elimination of antigen leads to contraction of the T cell response, and this decline is responsible for maintaining homeostasis in the immune system.

There are several reasons that the response declines.

- As the antigen is eliminated and the innate immune response associated with antigen exposure abates, the signals that normally keep activated lymphocytes alive and proliferating are no longer active.
- As mentioned earlier, costimulation and growth factors like IL-2 stimulate expression of the anti-apoptotic proteins Bcl-2 and Bcl-XL in the activated lymphocytes, and these proteins keep cells viable.
- As the level of costimulation and the amount of available IL-2 decrease, the levels of anti-apoptotic proteins in the cells drop.
- At the same time, growth factor deprivation activates sensors of cellular stress (such as the BH3-only protein Bim), which trigger the mitochondrial pathway of apoptosis and are no longer opposed by the anti-apoptotic proteins.
- The net result of these changes is that most of the cells that were produced by activation die and the generation of newly activated cells declines, so the pool of antigen-activated lymphocytes contracts.
- There has been much interest in the possibility that various regulatory mechanisms play a role in the normal contraction of immune responses.
- Such mechanisms might include the inhibitory receptors CTLA-4 and PD-1, apoptosis induced by death receptors of the TNF receptor superfamily (such as TNFRI and Fas), and regulatory T cells.
- However, it is still not established that these control mechanisms are essential for the normal decline of most immune responses.

Suggestive Questions:

1. What are the 5 cardinal features of responses of antigen-stimulated T cells?
2. Write short note on the following:
   a. CD69
   b. *CD25 (IL-2Rα).*
   c. *CD40 ligand (CD40L, CD154).*
   d. *CTLA-4 (CD152).*
   e. *Adhesion molecules and chemokine receptors.*
3. Diagrammatically describe the changes that occur on the surface molecule during T cell activation.
4. Write a short note on IL-2 or T cell Growth Factor (TCGF).
5. Diagrammatically explain the regulation of IL-2 Receptor expression.
6. Discuss the various biological actions of IL-2.
7. Write a short note on clonal expansion of T cells.
8. Discuss in brief the role of IL-2 in clonal expansion of T cells.
9. Write short note on the Epigenetic basis of changes occurring in cytokine gene loci in relation to characteristics of differentiated subsets of effector cells.
10. Explain the properties of TH1, TH2 and TH17 subsets of CD4+ Helper cells.
11. Justify the statement that "Stimuli other than cytokines may also influence the pattern of helper T cell differentiation.
12. Comment on the statement that "The distinct cytokine profiles of differentiated cell populations are controlled by particular transcription factors that activate cytokine gene transcription and by chromatin modifications affecting cytokine gene loci."
13. Comment on the statement that "Each subset of differentiated effector cells produces cytokines that promote its own development and may suppress the development of the other subsets"

14. Comment on the statement that “Differentiation of each subset is induced by the types of microbes which that subset is best able to combat.”
15. Comment on the statement that “TH1 differentiation is driven mainly by response to microbes that activate dendritic cells, macrophages, and NK cells”.
16. Explain the role of IFN-γ and IL-12 in TH1 differentiation.
17. Comment on the statement that “TH2 differentiation is stimulated by the cytokine IL-4 and occurs in response to helminths and allergens”.
18. Explain the role of IL-4 in TH2 development.
19. Comment on the statement that “The development of TH17 cells is stimulated by proinflammatory cytokines produced in response to bacteria and fungi”
20. Explain the role of the transcription factors RORγt and STAT3 on the development of TH17 cells.
21. Justify the role of CD4+ Helper cells in full activation of naive CD8+ T cells and their differentiation into functional CTLs and memory cells.
22. Justify the statement that “T cell–mediated immune responses to an antigen usually result in the generation of memory T cells specific for that antigen”
23. Diagrammatically show the Linear Differentiation Model and Branched Differentiation Model for T cells.
24. Point out the various properties of Memory T cells.
25. Comment on the statement that “The number of memory T cells specific for any antigen is greater than the number of naive cells specific for the same antigen.”
26. Comment on the statement that “Memory cells express increased levels of antiapoptotic proteins, which may be responsible for their prolonged survival.”

27. Comment on the statement that “Memory cells undergo slow proliferation, and this ability to self-renew may contribute to the long life span of the memory pool.”
28. Comment on the statement that “The maintenance of memory cells is dependent on cytokines but does not require antigen recognition.”
29. Differentiate between Central Memory and Effector Memory.
30. Comment on the statement “Elimination of antigen leads to contraction of the T cell response”.