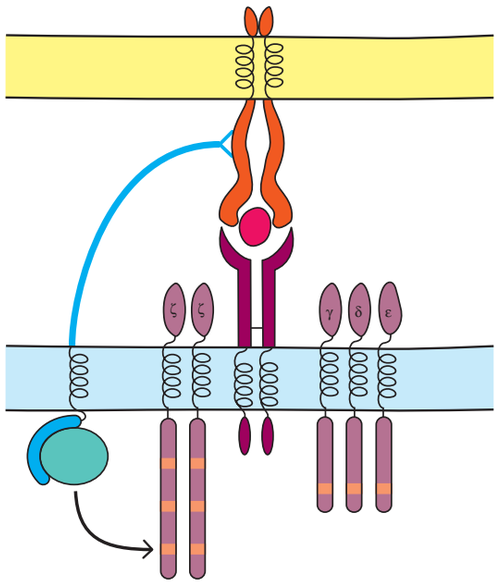
chapter 10

T-Cell Maturation, Activation, and Differentiation



Engagement of TcR by Peptide: MHC Initiates Signal Transduction

The attribute that distinguishes antigen recognition by most T cells from recognition by B cells is MHC restriction. In most cases, both the maturation of progenitor T cells in the thymus and the activation of mature T cells in the periphery are influenced by the involvement of MHC molecules. The potential antigenic diversity of the T-cell population is reduced during maturation by a selection process that allows only MHC-restricted and nonself-reactive T cells to mature. The final stages in the maturation of most T cells proceed along two different developmental pathways, which generate functionally distinct $CD4^+$ and $CD8^+$ subpopulations that exhibit class II and class I MHC restriction, respectively.

Activation of mature peripheral T cells begins with the interaction of the T-cell receptor (TCR) with an antigenic peptide displayed in the groove of an MHC molecule. Although the specificity of this interaction is governed by the TCR, its low avidity necessitates the involvement of coreceptors and other accessory membrane molecules that strengthen the TCR-antigen-MHC interaction and transduce the activating signal. Activation leads to the proliferation and differentiation of T cells into various types of effector cells and memory T cells. Because the vast majority of thymocytes and peripheral T cells express the αβ T-cell receptor rather than the γδ T-cell receptor, all references to the T-cell receptor in this chapter denote the αβ receptor unless otherwise indicated. Similarly, unless otherwise indicated, all references to T cells denote those αβ receptor-bearing T cells that undergo MHC restriction.

T-Cell Maturation and the Thymus

Progenitor T cells from the early sites of hematopoiesis begin to migrate to the thymus at about day 11 of gestation in mice and in the eighth or ninth week of gestation in humans. In a manner similar to B-cell maturation in the bone marrow, T-cell maturation involves rearrangements of the germ-line TCR genes and the expression of various membrane markers. In the thymus, developing T cells, known as **thymocytes,** proliferate and differentiate along developmental pathways that generate functionally distinct subpopulations of mature T cells.

As indicated in Chapter 2, the thymus occupies a central role in T-cell biology. Aside from being the main source of all T cells, it is where T cells diversify and then are shaped into an effective primary T-cell repertoire by an extraordinary pair of selection processes. One of these, **positive selection,** permits the survival of only those T cells whose TCRs are capable of recognizing self-MHC molecules. It is thus responsible for the creation of a self-MHC-restricted repertoire of T cells. The other, **negative selection,** eliminates T cells that react too strongly with self-MHC or with self-MHC plus self-peptides. It is an extremely important factor in generating a primary T-cell repertoire that is self-tolerant.

As shown in Figure 10-1, when T-cell precursors arrive at the thymus, they do not express such signature surface markers of T cells as the T-cell receptor, the CD3 complex, or the coreceptors CD4 and CD8. In fact, these progenitor cells have

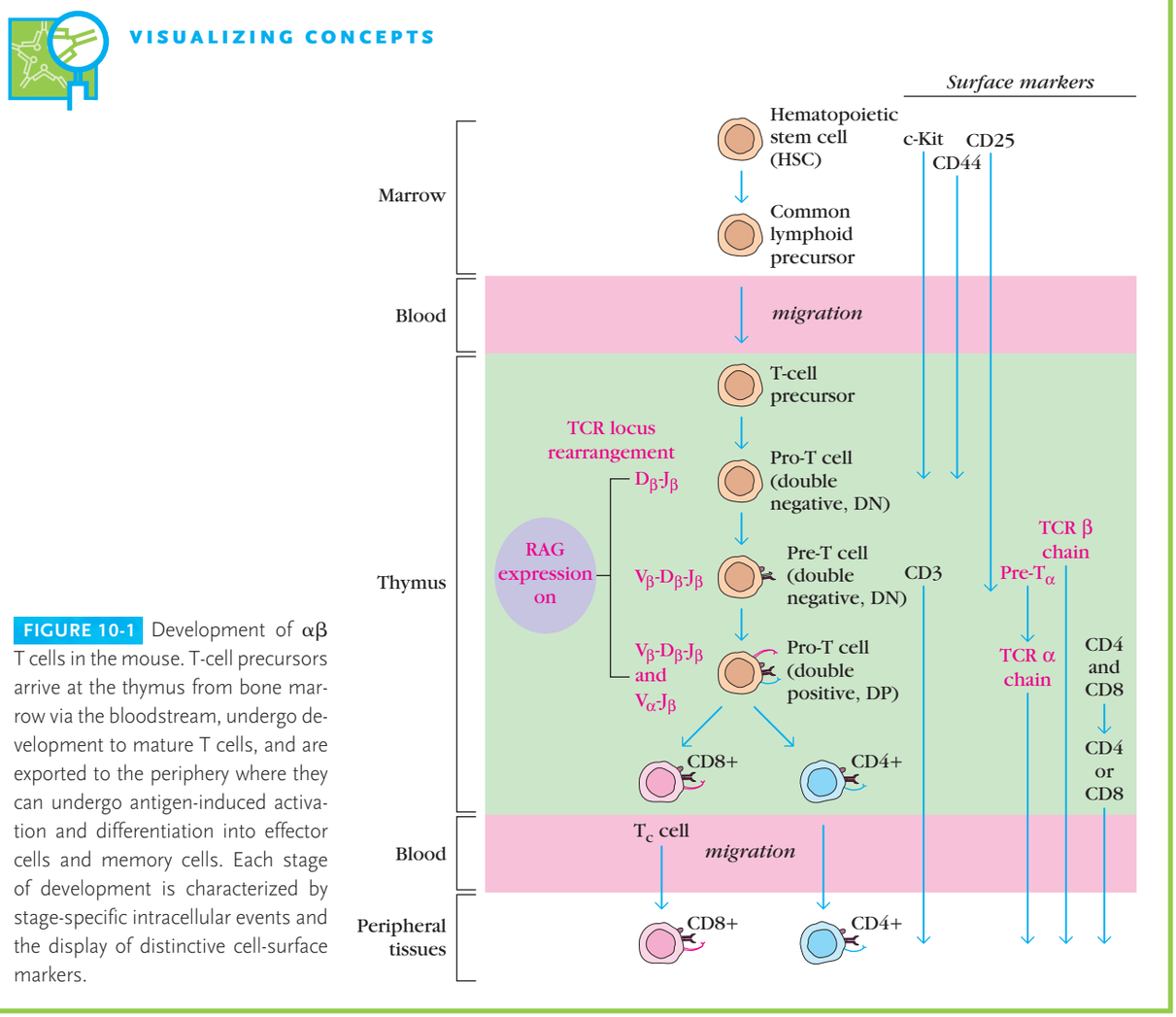


FIGURE 10-1 Development of αβ T cells in the mouse. T-cell precursors arrive at the thymus from bone marrow via the bloodstream, undergo development to mature T cells, and are exported to the periphery where they can undergo antigen-induced activation and differentiation into effector cells and memory cells. Each stage of development is characterized by stage-specific intracellular events and the display of distinctive cell-surface markers.

not yet rearranged their TCR genes and do not express proteins, such as RAG-1 and RAG-2, that are required for rearrangement. After arriving at the thymus, these T-cell precursors enter the outer cortex and slowly proliferate. During approximately three weeks of development in the thymus, the differentiating T cells progress through a series of stages that are marked by characteristic changes in their cell-surface phenotype. For example, as mentioned previously, thymocytes early in development lack detectable CD4 and CD8. Because these cells are $CD4^-CD8^-$, they are referred to as **double-negative (DN)** cells.

Even though these coreceptors are not expressed during the DN early stages, the differentiation program is progressing and is marked by changes in the expression of such cell surface molecules as c-Kit, CD44, and CD25. The initial thymocyte population displays c-Kit, the receptor for stem-cell growth factor, and CD44, an adhesion molecule involved in homing; CD25, the β-chain of the IL-2 receptor, also appears on early-stage DN cells. During this period, the cells are proliferating but the TCR genes remain unrearranged. Then the cells stop expressing c-Kit, markedly reduce CD44 expression, turn on expression of the recombinase genes *RAG-1* and *RAG-2* and begin to rearrange their TCR genes. Although it is not shown in Figure 10-1, a small percentage (<5%) of thymocytes productively rearrange the γ- and δ-chain genes and develop into double-negative $CD3^+$ γδ T cells. In mice, this thymocyte subpopulation can be detected by day 14 of gestation, reaches maximal numbers between days 17 and 18, and then declines until birth (Figure 10-2).

Most double-negative thymocytes progress down the αβ developmental pathway. They stop proliferating and begin to rearrange the TCR β-chain genes, then express the β chain. Those cells of the αβ lineage that fail to productively rearrange and express β chains die. Newly synthesized β chains combine with a 33-kDa glycoprotein known as the pre-Tα chain and associate with the CD3 group to form a novel com-

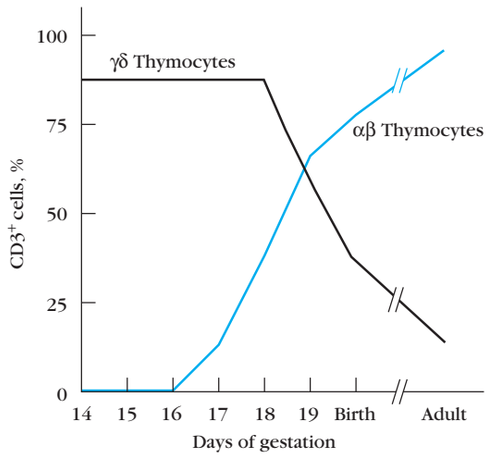


FIGURE 10-2 Time course of appearance of γδ thymocytes and αβ thymocytes during mouse fetal development. The graph shows the percentage of $CD3^+$ cells in the thymus that are double-negative ($CD4^-8^-$) and bear the γδ T-cell receptor (black) or are double-positive ($CD4^+8^+$) and bear the αβ T-cell receptor (blue).

plex called the **pre-T-cell receptor** or **pre-TCR** (Figure 10-3). Some researchers have suggested that the pre-TCR recognizes some intra-thymic ligand and transmits a signal through the CD3 complex that activates signal-transduction pathways that have several effects:

- Indicates that a cell has made a productive TCR β-chain rearrangement and signals its further proliferation and maturation.

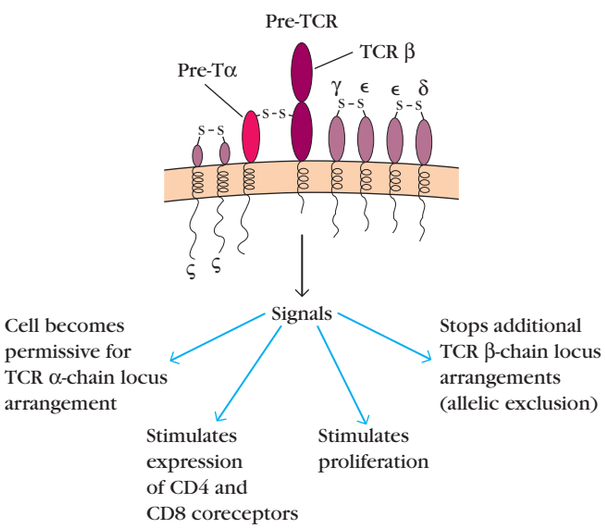


FIGURE 10-3 Structure and activity of the pre–T-cell receptor (pre-TCR). Binding of ligands yet to be identified to the pre-TCR generates intracellular signals that induce a variety of processes.

- Suppresses further rearrangement of TCR β-chain genes, resulting in allelic exclusion.
- Renders the cell permissive for rearrangement of the TCR α chain.
- Induces developmental progression to the $CD4^+8^+$ *double-positive* state.

After advancing to the **double-positive** (**DP**) stage, where both CD4 and CD8 coreceptors are expressed, the thymocytes begin to proliferate. However, during this proliferative phase, TCR α-chain gene rearrangement does not occur; both the *RAG-1* and *RAG-2* genes are transcriptionally active, but the RAG-2 protein is rapidly degraded in proliferating cells, so rearrangement of the α-chain genes cannot take place. The rearrangement of α-chain genes does not begin until the double-positive thymocytes stop proliferating and RAG-2 protein levels increase. The proliferative phase prior to the rearrangement of the α-chain increases the diversity of the T-cell repertoire by generating a clone of cells with a single TCR β-chain rearrangement. Each of the cells within this clone can then rearrange a different α-chain gene, thereby generating a much more diverse population than if the original cell had first undergone rearrangement at both the β- and α-chain loci before it proliferated. In mice, the TCR α-chain genes are not expressed until day 16 or 17 of gestation; double-positive cells expressing both CD3 and the αβ T-cell receptor begin to appear at day 17 and reach maximal levels about the time of birth (see Figure 10-2). The possession of a complete TCR enables DP thymocytes to undergo the rigors of positive and negative selection.

T-cell development is an expensive process for the host. An estimated 98% of all thymocytes do not mature—they die by apoptosis within the thymus either because they fail to make a productive TCR-gene rearrangement or because they fail to survive thymic selection. Double-positive thymocytes that express the αβ TCR-CD3 complex and survive thymic selection develop into immature **single-positive $CD4^+$** thymocytes or **single-positive $CD8^+$** thymocytes. These single-positive cells undergo additional negative selection and migrate from the cortex to the medula, where they pass from the thymus into the circulatory system.

Thymic Selection of the T-Cell Repertoire

Random gene rearrangement within TCR germ-line DNA combined with junctional diversity can generate an enormous TCR repertoire, with an estimated potential diversity exceeding 10^{15} for the αβ receptor and 10^{18} for the γδ receptor. Gene products encoded by the rearranged TCR genes have no inherent affinity for foreign antigen plus a self-MHC molecule; they theoretically should be capable of recognizing soluble antigen (either foreign or self), self-MHC molecules, or

antigen plus a nonself-MHC molecule. Nonetheless, the most distinctive property of mature T cells is that they recognize only foreign antigen combined with self-MHC molecules.

As noted, thymocytes undergo two selection processes in the thymus:

- Positive selection for thymocytes bearing receptors capable of binding self-MHC molecules, which results in **MHC restriction.** Cells that fail positive selection are eliminated within the thymus by apoptosis.
- Negative selection that eliminates thymocytes bearing high-affinity receptors for self-MHC molecules alone or self-antigen presented by self-MHC, which results in **self-tolerance.**

Both processes are necessary to generate mature T cells that are self-MHC restricted and self-tolerant. As noted already, some 98% or more of all thymocytes die by apoptosis within the thymus. The bulk of this high death rate appears to reflect a weeding out of thymocytes that fail positive selection because their receptors do not specifically recognize foreign antigen plus self-MHC molecules.

Early evidence for the role of the thymus in selection of the T-cell repertoire came from chimeric mouse experiments by R. M. Zinkernagel and his colleagues (Figure 10-4). These researchers implanted thymectomized and irradiated (A × B) F_1 mice with a B-type thymus and then reconstituted the animal's immune system with an intravenous infusion of F_1 bone-marrow cells. To be certain that the thymus graft did not contain any mature T cells, it was irradiated before being transplanted. In such an experimental system, T-cell progenitors from the (A × B) F_1 bone-marrow transplant mature within a thymus that expresses only B-haplotype MHC molecules on its stromal cells. Would these (A × B) F_1 T cells now be MHC-restricted for the haplotype of the thymus? To answer this question, the chimeric mice were infected with LCM virus and the immature T cells were then tested for their ability to kill LCM-infected target cells from the strain A or strain B mice. As shown in Figure 10-4, when T_C cells from the chimeric mice were tested on LCM virus infected target cells from strain A or strain B mice, they could only lyse LCM-infected target cells from strain B mice. These mice have the same MHC haplotype, B, as the implanted thymus. Thus, the MHC haplotype of the thymus in which T cells develop determines their MHC restriction.

Thymic stromal cells, including epithelial cells, macrophages, and dendritic cells, play essential roles in positive and negative selection. These cells express class I MHC molecules and can display high levels of class II MHC also. The interaction of immature thymocytes that express the TCR-CD3 complex with populations of thymic stromal cells results in positive and negative selection by mechanisms that are under intense investigation. First, we'll examine the details of each selection process and then study some experiments that provide insights into the operation of these processes.

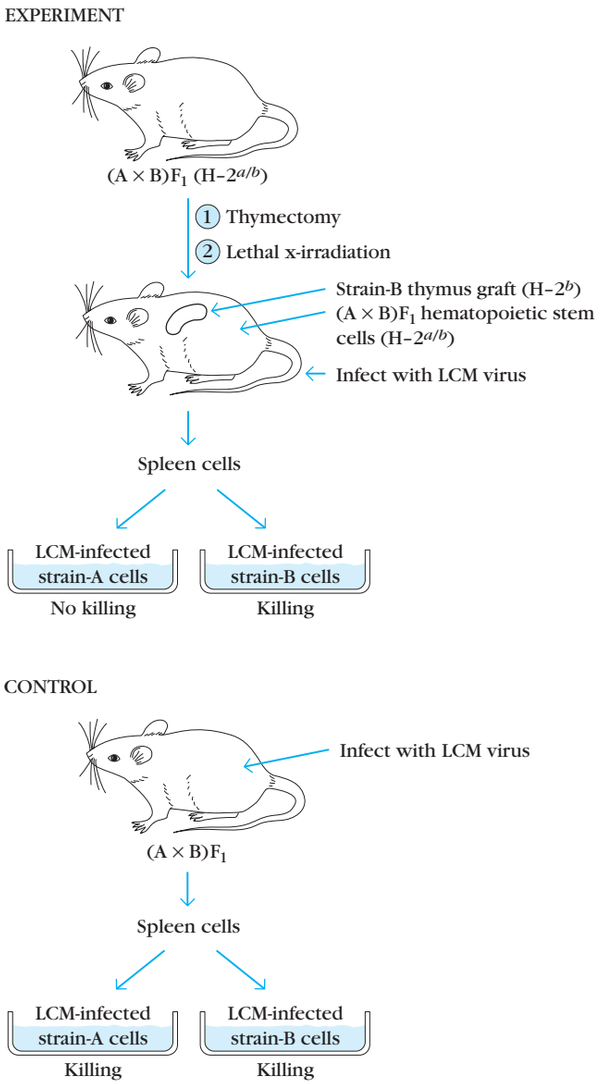


FIGURE 10-4 Experimental demonstration that the thymus selects for maturation only those T cells whose T-cell receptors recognize antigen presented on target cells with the haplotype of the thymus. Thymectomized and lethally irradiated (A × B) F_1 mice were grafted with a strain-B thymus and reconstituted with (A × B) F_1 bone-marrow cells. After infection with the LCM virus, the CTL cells were assayed for their ability to kill ^{51}Cr-labeled strain-A or strain-B target cells infected with the LCM virus. Only strain-B target cells were lysed, suggesting that the H-2^b grafted thymus had selected for maturation only those T cells that could recognize antigen combined with H-2^b MHC molecules.

Positive Selection Ensures MHC Restriction

Positive selection takes place in the cortical region of the thymus and involves the interaction of immature thymocytes with cortical epithelial cells (Figure 10-5). There is evidence that the T-cell receptors on thymocytes tend to cluster with

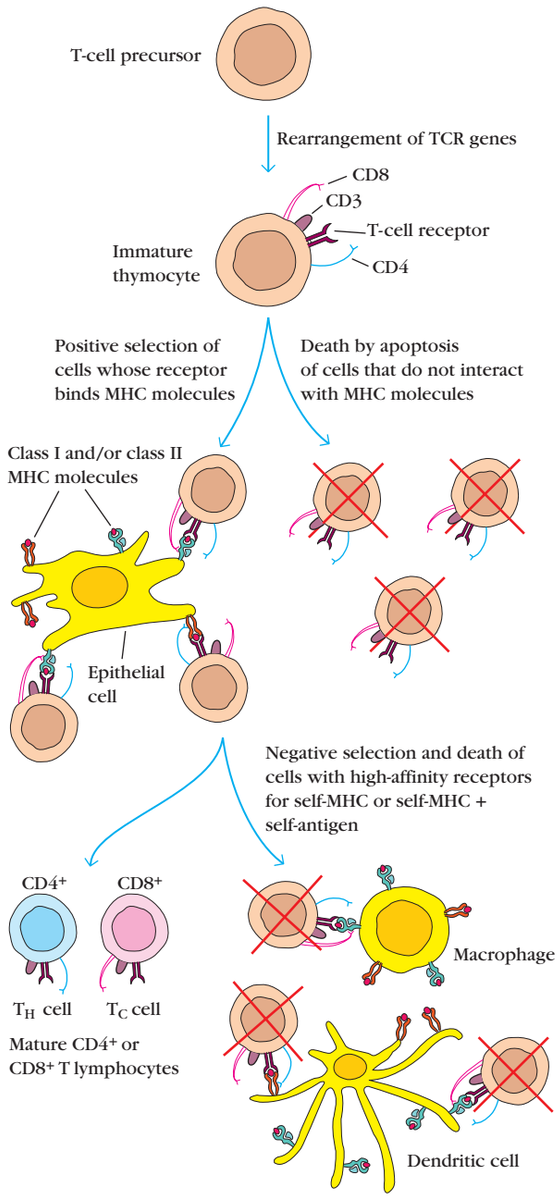


FIGURE 10-5 Positive and negative selection of thymocytes in the thymus. Thymic selection involves thymic stromal cells (epithelial cells, dendritic cells, and macrophages), and results in mature T cells that are both self-MHC restricted and self-tolerant.

MHC molecules on the cortical cells at sites of cell-cell contact. Some researchers have suggested that these interactions allow the immature thymocytes to receive a protective signal that prevents them from undergoing cell death; cells whose receptors are not able to bind MHC molecules would not interact with the thymic epithelial cells and consequently would not receive the protective signal, leading to their death by apoptosis.

During positive selection, the RAG-1, RAG-2, and TdT proteins required for gene rearrangement and modification continue to be expressed. Thus each of the immature thymocytes in a clone expressing a given β chain have an opportunity to rearrange different TCR α-chain genes, and the resulting TCRs are then selected for self-MHC recognition. Only those cells whose αβ TCR heterodimer recognizes a self-MHC molecule are selected for survival. Consequently, the presence of more than one combination of αβ TCR chains among members of the clone is important because it increases the possibility that some members will "pass" the test for positive selection. Any cell that manages to rearrange an α chain that allows the resulting αβ TCR to recognize self-MHC will be spared; all members of the clone that fail to do so will die by apoptosis within 3 to 4 days.

Negative Selection Ensures Self-Tolerance

The population of MHC-restricted thymocytes that survive positive selection comprises some cells with low-affinity receptors for self-antigen presented by self-MHC molecules and other cells with high-affinity receptors. The latter thymocytes undergo negative selection by an interaction with thymic stromal cells. During negative selection, dendritic cells and macrophages bearing class I and class II MHC molecules interact with thymocytes bearing high-affinity receptors for self-antigen plus self-MHC molecules or for self-MHC molecules alone (see Figure 10-5). However, the precise details of the process are not yet known. Cells that experience negative selection are observed to undergo death by apoptosis. Tolerance to self-antigens encountered in the thymus is thereby achieved by eliminating T cells that are reactive to these antigens.

Experiments Revealed the Essential Elements of Positive and Negative Selection

Direct evidence that binding of thymocytes to class I or class II MHC molecules is required for positive selection in the thymus came from experimental studies with knockout mice incapable of producing functional class I or class II MHC molecules (Table 10-1). Class I–deficient mice were found to have a normal distribution of double-negative, double-positive, and $CD4^+$ thymocytes, but failed to produce $CD8^+$ thymocytes. Class II–deficient mice had double-negative, double-positive, and $CD8^+$ thymocytes but lacked $CD4^+$ thymocytes. Not surprisingly, the lymph nodes of these class II–deficient mice lacked $CD4^+$ T cells. Thus, the absence of class I or II MHC molecules prevents positive selection of $CD8^+$ or $CD4^+$ T cells, respectively.

Further experiments with transgenic mice provided additional evidence that interaction with MHC molecules plays a role in positive selection. In these experiments, rearranged αβ-TCR genes derived from a $CD8^+$ T-cell clone specific for influenza antigen plus H-2^k class I MHC molecules were injected into fertilized eggs from two different mouse strains,

TABLE 10-1 Effect of class I or II MHC deficiency on thymocyte populations*

Cell type	Control mice	KNOCKOUT MICE: Class I deficient	KNOCKOUT MICE: Class II deficient
$CD4^-CD8^-$	+	+	+
$CD4^+CD8^+$	+	+	+
$CD4^+$	+	+	–
$CD8^+$	+	–	–

*Plus sign indicates normal distribution of indicated cell types in thymus. Minus sign indicates absence of cell type.

one with the H-2^k haplotype and one with the H-2^d haplotype (Figure 10-6). Since the receptor transgenes were already rearranged, other TCR-gene rearrangements were suppressed in the transgenic mice; therefore, a high percentage of the thymocytes in the transgenic mice expressed the T-cell receptor encoded by the transgene. Thymocytes expressing the TCR transgene were found to mature into $CD8^+$ T cells only in the transgenic mice with the H-2^k class I MHC haplotype (i.e., the haplotype for which the transgene receptor was restricted). In transgenic mice with a different MHC haplotype (H-2^d), immature, double-positive thymocytes expressing the transgene were present, but these thymocytes failed to mature into $CD8^+$ T cells. These findings confirmed that interaction between T-cell receptors on immature thymocytes and self-MHC molecules is required for positive selection. In the absence of self-MHC molecules, as in the H-2^d transgenic mice, positive selection and subsequent maturation do not occur.

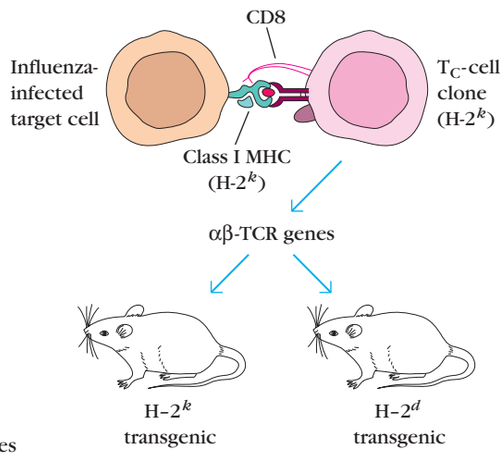
Evidence for deletion of thymocytes reactive with self-antigen plus MHC molecules comes from a number of experimental systems. In one system, thymocyte maturation was analyzed in transgenic mice bearing an αβ TCR transgene specific for the class I D^b MHC molecule plus H-Y antigen, a small protein that is encoded on the Y chromosome and is therefore a self-molecule only in male mice. In this experiment, the MHC haplotype of the transgenic mice was H-2^b, the same as the MHC restriction of the transgene-encoded receptor. Therefore any differences in the selection of thymocytes in male and female transgenics would be related to the presence or absence of H-Y antigen.

Analysis of thymocytes in the transgenic mice revealed that female mice contained thymocytes expressing the H-Y–specific TCR transgene, but male mice did not (Figure 10-7). In other words, H-Y–reactive thymocytes were self-reactive in the male mice and were eliminated. However, in the female transgenics, which did not express the H-Y antigen, these cells were not self-reactive and thus were not eliminated. When thymocytes from these male transgenic mice were cultured in vitro with antigen-presenting cells expressing the H-Y antigen, the thymocytes were observed to undergo apoptosis, providing a striking example of negative selection.

Some Central Issues in Thymic Selection Remain Unresolved

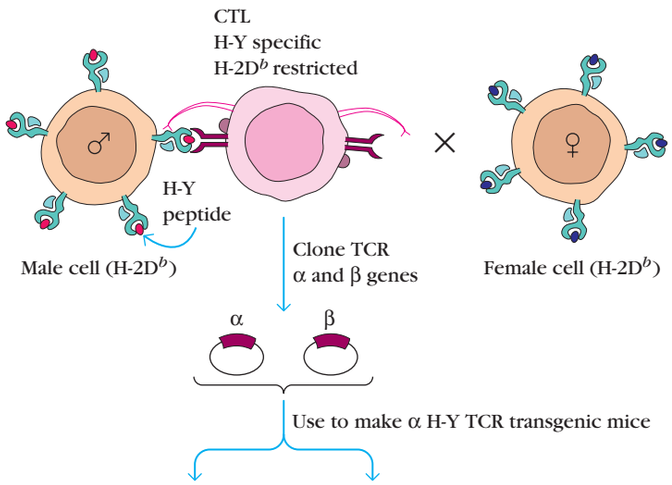
Although a great deal has been learned about the developmental processes that generate mature $CD4^+$ and $CD8^+$ T cells, some mysteries persist. Prominent among them is a seeming paradox: If positive selection allows only thymocytes reactive with self-MHC molecules to survive, and negative selection eliminates the self-MHC–reactive thymocytes, then no T cells would be allowed to mature. Since this is not the outcome of T-cell development, clearly, other factors operate to prevent these two MHC-dependent processes from eliminating the entire repertoire of MHC-restricted T cells.

Experimental evidence from fetal thymic organ culture (FTOC) has been helpful in resolving this puzzle. In this system, mouse thymic lobes are excised at a gestational age of day 16 and placed in culture. At this time, the lobes consist predominantly of $CD4^-8^-$ thymocytes. Because these immature, double-negative thymocytes continue to develop in the organ culture, thymic selection can be studied under conditions that permit a range of informative experiments. Particular use has



Thymocytes in transgenics	H-2^k transgenic	H-2^d transgenic
$TCR^+/CD4^+8^+$	+	+
$TCR^+/CD8^+$	+	–

FIGURE 10-6 Effect of host haplotype on T-cell maturation in mice carrying transgenes encoding an H-2^b class I–restricted T-cell receptor specific for influenza virus. The presence of the rearranged TCR transgenes suppressed other gene rearrangements in the transgenics; therefore, most of the thymocytes in the transgenics expressed the αβ T-cell receptor encoded by the transgene. Immature double-positive thymocytes matured into $CD8^+$ T cells only in transgenics with the haplotype (H-2^k) corresponding to the MHC restriction of the TCR transgene.



	Male H-2D^b	Female H-2D^b
H-Y expression	+	–
Thymocytes		
CD4$^-$8$^-$	++	+
CD4$^+$8$^+$	+	++
CD4$^+$	+	+
CD8$^+$	–	++

FIGURE 10-7 Experimental demonstration that negative selection of thymocytes requires self-antigen plus self-MHC. In this experiment, H-2^b male and female transgenics were prepared carrying TCR transgenes specific for H-Y antigen plus the D^b molecule. This antigen is expressed only in males. FACS analysis of thymocytes from the transgenics showed that mature CD8$^+$ T cells expressing the transgene were absent in the male mice but present in the female mice, suggesting that thymocytes reactive with a self-antigen (in this case, H-Y antigen in the male mice) are deleted during thymic selection. *[Adapted from H. von Boehmer and P. Kisielow, 1990,* Science ***248:****1370.]*

been made of mice in which the peptide transporter, TAP-1, has been knocked out. In the absence of TAP-1, only low levels of MHC class I are expressed on thymic cells, and the development of CD8$^+$ thymocytes is blocked. However, when exogenous peptides are added to these organ cultures, then peptide-bearing class I MHC molecules appear on the surface of the thymic cells, and development of CD8$^+$ T cells is restored. Significantly, when a diverse peptide mixture is added, the extent of CD8$^+$ T-cell restoration is greater than when a single peptide is added. This indicates that the role of peptide is not simply to support stable MHC expression but also to be recognized itself in the selection process.

Two competing hypotheses attempt to explain the paradox of MHC-dependent positive and negative selection. The *avidity hypothesis* asserts that differences in the strength of the signals received by thymocytes undergoing positive and negative selection determine the outcome, with signal strength dictated by the avidity of the TCR-MHC-peptide interaction. The *differential-signaling hypothesis* holds that the outcomes of selection are dictated by different signals, rather than different strengths of the same signal.

The avidity hypothesis was tested with TAP-1 knockout mice transgenic for an αβ TCR that recognized an LCM virus peptide-MHC complex. These mice were used to prepare fetal thymic organ cultures (Figure 10-8). The avidity of the TCR-MHC interaction was varied by the use of different concentrations of peptide. At low peptide concentrations, few MHC molecules bound peptide and the avidity of the TCR-MHC interaction was low. As peptide concentrations were raised, the number of peptide-MHC complexes displayed increased and so did the avidity of the interaction. In this experiment, very few CD8$^+$ cells appeared when peptide was not added, but even low concentrations of the relevant peptide resulted in the appearance of significant numbers of CD8$^+$ T cells bearing the transgenic TCR receptor. Increasing the peptide concentrations to an optimum range yielded the highest number of CD8$^+$ T cells. However, at higher concentrations of peptide, the numbers of CD8$^+$ T cells produced declined steeply. The results of these experiments show that positive and negative selection can be achieved with signals generated by the same peptide-MHC combination. No signal (no peptide) fails to support positive selection. A weak signal (low peptide level) induces positive selection. However, too strong a signal (high peptide level) results in negative selection.

The differential-signaling model provides an alternative explanation for determining whether a T cell undergoes positive or negative selection. This model is a qualitative rather than a quantitative one, and it emphasizes the nature of the signal delivered by the TCR rather than its strength. At the core of this model is the observation that some MHC-peptide complexes can deliver only a weak or partly activating signal

while others can deliver a complete signal. In this model, positive selection takes place when the TCRs of developing thymocytes encounter MHC-peptide complexes that deliver weak or partial signals to their receptors, and negative selection results when the signal is complete. At this point it is not possible to decide between the avidity model and the differential-signaling model; both have experimental support. It may be that in some cases, one of these mechanisms operates to the complete exclusion of the other. It is also possible that no single mechanism accounts for all the outcomes in the cellular interactions that take place in the thymus and more than one mechanism may play a significant role. Further work is required to complete our understanding of this matter.

The differential expression of the coreceptor CD8 also can affect thymic selection. In an experiment in which CD8 expression was artificially raised to twice its normal level, the concentration of mature $CD8^+$ cells in the thymus was one-thirteenth of the concentration in control mice bearing normal levels of CD8 on their surface. Since the interaction of T cells with class I MHC molecules is strengthened by participation of CD8, perhaps the increased expression of CD8 would increase the avidity of thymocytes for class I molecules, possibly making their negative selection more likely.

Another important open question in thymic selection is how double-positive thymocytes are directed to become either $CD4^+8^-$ or $CD4^-8^+$ T cells. Selection of $CD4^+8^+$ thymocytes gives rise to class I MHC–restricted $CD8^+$ T cells and class II–restricted $CD4^+$ T cells. Two models have been proposed to explain the transformation of a double-positive precursor into one of two different single-positive lineages

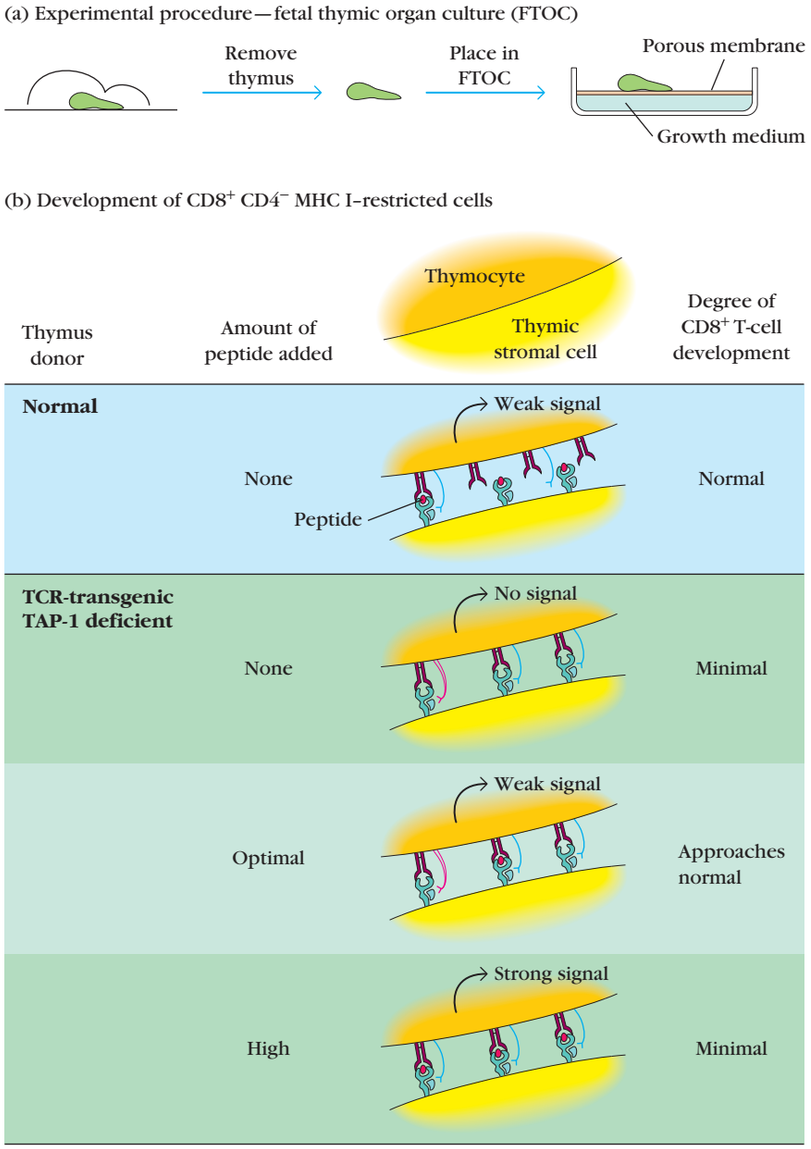


FIGURE 10-8 Role of peptides in selection. Thymuses harvested before their thymocyte populations have undergone positive and negative selection allow study of the development and selection of single positive ($CD4^+CD8^-$ and $CD4^-CD8^+$) T cells. (a) Outline of the experimental procedure for in vitro fetal thymic organ culture (FTOC). (b) The development and selection of $CD8^+CD4^-$ class I–restricted T cells depends on TCR peptide-MHC I interactions. TAP_{-1} knockout mice are unable to form peptide-MHC complexes unless peptide is added. The mice used in this study were transgenic for the α and β chains of a TCR that recognizes the added peptide bound to MHC I molecules of the TAP_{-1} knockout/TCR transgenic mice. Varying the amount of added peptide revealed that low concentrations of peptide, producing low avidity of binding, resulted in positive selection and nearly normal levels of $CD4^-CD8^+$ cells. High concentrations of peptide, producing high avidity of binding to the TCR, caused negative selection, and few $CD4^-CD8^+$ T cells appeared. *[Adapted from Ashton Rickardt et al. (1994)* Cell ***25:****651.]*

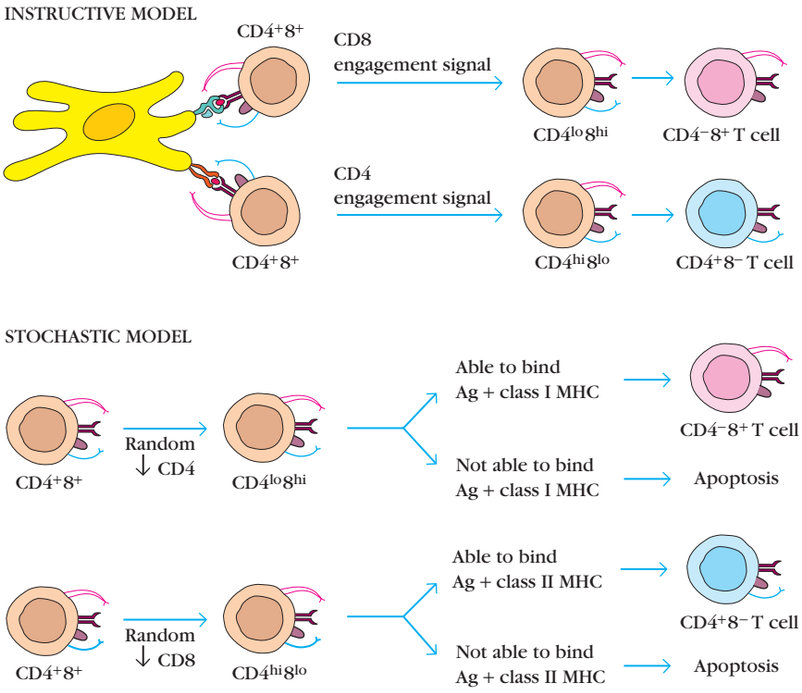


FIGURE 10-9 Proposed models for the role of the CD4 and CD8 coreceptors in thymic selection of double positive thymocytes leading to single positive T cells. According to the instructive model, interaction of one coreceptor with MHC molecules on stromal cells results in down-regulation of the other coreceptor. According to the stochastic model, down-regulation of CD4 or CD8 is a random process.

(Figure 10-9). The *instructional model* postulates that the multiple interactions between the TCR, $CD8^+$ or $CD4^+$ coreceptors, and class I or class II MHC molecules instruct the cells to differentiate into either $CD8^+$ or $CD4^+$ single-positive cells, respectively. This model would predict that a class I MHC–specific TCR together with the CD8 coreceptor would generate a signal that is different from the signal induced by a class II MHC–specific TCR together with the CD4 coreceptor. The *stochastic model* suggests that CD4 or CD8 expression is switched off randomly with no relation to the specificity of the TCR. Only those thymocytes whose TCR and remaining coreceptor recognize the same class of MHC molecule will mature. At present, it is not possible to choose one model over the other.

T_H-Cell Activation

The central event in the generation of both humoral and cell-mediated immune responses is the activation and clonal expansion of T_H cells. Activation of T_C cells, which is generally similar to T_H-cell activation, is described in Chapter 14. T_H-cell activation is initiated by interaction of the TCR-CD3 complex with a processed antigenic peptide bound to a class II MHC molecule on the surface of an antigen-presenting cell. This interaction and the resulting activating signals also involve various accessory membrane molecules on the T_H cell and the antigen-presenting cell. Interaction of a T_H cell with antigen initiates a cascade of biochemical events that induces the resting T_H cell to enter the cell cycle, proliferating and differentiating into memory cells or effector cells. Many of the gene products that appear upon interaction with antigen can be grouped into one of three categories depending on how early they can be detected after antigen recognition (Table 10-2):

- *Immediate genes,* expressed within half an hour of antigen recognition, encode a number of transcription factors, including c-Fos, c-Myc, c-Jun, NFAT, and NF-κB
- *Early genes,* expressed within 1–2 h of antigen recognition, encode IL-2, IL-2R (IL-2 receptor), IL-3, IL-6, IFN-γ, and numerous other proteins
- *Late genes,* expressed more than 2 days after antigen recognition, encode various adhesion molecules

These profound changes are the result of signal-transduction pathways that are activated by the encounter between the TCR and MHC-peptide complexes. An overview of some of the basic strategies of cellular signaling will be useful background for appreciating the specific signaling pathways used by T cells.

Signal-Transduction Pathways Have Several Features in Common

The detection and interpretation of signals from the environment is an indispensable feature of all cells, including those of the immune system. Although there are an enormous number of different signal-transduction pathways, some common themes are typical of these crucial integrative processes:

TABLE 10-2 Time course of gene expression by T_H cells following interaction with antigen

Gene product	Function	Time mRNA expression begins	Location	Ratio of activated to nonactivated cells
		IMMEDIATE		
c-Fos	Protooncogene; nuclear-binding protein	15 min	Nucleus	> 100
c-Jun	Cellular oncogene; transcription factor	15–20 min	Nucleus	?
NFAT	Transcription factor	20 min	Nucleus	50
c-Myc	Cellular oncogene	30 min	Nucleus	20
NF-κB	Transcription factor	30 min	Nucleus	> 10
		EARLY		
IFN-γ	Cytokine	30 min	Secreted	> 100
IL-2	Cytokine	45 min	Secreted	> 1000
Insulin receptor	Hormone receptor	1 h	Cell membrane	3
IL-3	Cytokine	1–2 h	Secreted	> 100
TGF-β	Cytokine	< 2 h	Secreted	> 10
IL-2 receptor (p55)	Cytokine receptor	2 h	Cell membrane	> 50
TNF-β	Cytokine	1–3 h	Secreted	> 100
Cyclin	Cell-cycle protein	4–6 h	Cytoplasmic	> 10
IL-4	Cytokine	< 6 h	Secreted	> 100
IL-5	Cytokine	< 6 h	Secreted	> 100
IL-6	Cytokine	< 6 h	Secreted	> 100
c-Myb	Protooncogene	16 h	Nucleus	100
GM-CSF	Cytokine	20 h	Secreted	?
		LATE		
HLA-DR	Class II MHC molecule	3–5 days	Cell membrane	10
VLA-4	Adhesion molecule	4 days	Cell membrane	> 100
VLA-1, VLA-2, VLA-3, VLA-5	Adhesion molecules	7–14 days	Cell membrane	> 100, ?, ?, ?

SOURCE: Adapted from G. Crabtree, *Science* **243:**357.

- *Signal transduction begins with the interaction between a signal and its receptor.* Signals that cannot penetrate the cell membrane bind to receptors on the surface of the cell membrane. This group includes water-soluble signaling molecules and membrane-bound ligands (MHC-peptide complexes, for example). Hydrophobic signals, such as steroids, that can diffuse through the cell membrane are bound by intracellular receptors.
- *Signals are often transduced through* ***G proteins,*** membrane-linked macromolecules whose activities are controlled by binding of the guanosine nucleotides GTP and GDP, which act as molecular switches. Bound GTP turns on the signaling capacities of the G protein; hydrolysis of GTP or exchange for GDP turns off the signal by returning the G protein to an inactive form. There are two major categories of G proteins. *Small G proteins* consist of a single polypeptide chain of about 21 kDa. An important small G protein, known as Ras, is a key participant in the activation of an important proliferation-inducing signal-transduction cascade triggered by binding of ligands to their receptor tyrosine kinases. *Large G proteins* are composed of α, β, and γ subunits and are critically involved in many processes, including vision, olfaction, glucose metabolism, and phenomena of immunological interest such as leukocyte chemotaxis.

- *Signal reception often leads to the generation within the cell of a "second messenger,"* a molecule or ion that can diffuse to other sites in the cell and evoke changes. Examples are cyclic nucleotides (cAMP, cGMP), calcium ion (Ca^{2+}), and membrane phospholipid derivatives such as diacylglycerol (DAG) and inositol triphosphate (IP_3).
- *Protein kinases and protein phosphatases are activated or inhibited.* Kinases catalyze the phosphorylation of target residues (tyrosine, serine, or threonine) of key elements in many signal-transduction pathways. Phosphatases catalyze dephosphorylation, reversing the effect of kinases. These enzymes play essential roles in many signal-transduction pathways of immunological interest.
- *Many signal transduction pathways involve the signal-induced assembly of some components of the pathway.* Molecules known as *adaptor proteins* bind specifically and simultaneously to two or more different molecules with signaling roles, bringing them together and promoting their combined activity.
- *Signals are amplified by enzyme cascades.* Each enzyme in the cascade catalyzes the activation of many copies of the next enzyme in the sequence, greatly amplifying the signal at each step and offering many opportunities to modulate the intensity of a signal along the way.
- *The default setting for signal-transduction pathways is OFF.* In the absence of an appropriately presented signal, transmission through the pathway does not take place.

Multiple Signaling Pathways Are Initiated by TCR Engagement

The events that link antigen recognition by the T-cell receptor to gene activation echo many of the themes just reviewed. The key element in the initiation of T-cell activation is the recognition by the TCR of MHC-peptide complexes on antigen-presenting cells.

As described in Chapter 9, the TCR consists of a mostly extracellular ligand-binding unit, a predominantly intracellular signaling unit, the CD3 complex, and the homodimer of ζ (zeta) chains. Experiments with knockout mice have shown that all of these components are essential for signal transduction. Two phases can be recognized in the antigen-mediated induction of T-cell responses:

- *Initiation.* The engagement of MHC-peptide by the TCR leads to clustering with CD4 or CD8 coreceptors as these coreceptors bind to invariant regions of the MHC molecule (Figure 10-10). Lck, a protein tyrosine kinase associated with the cytoplasmic tails of the coreceptors, is brought close to the cytoplasmic tails of the TCR complex and phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAMs, described in Chapter 9). The phosphorylated tyrosines in the ITAMs of the zeta chain provide docking sites to which a protein tyrosine kinase called ZAP-70 attaches (step 2 in Figure 10-10) and becomes active. ZAP-70 then catalyzes the phosphorylation of a number of membrane-associated adaptor molecules (step 3), which act as anchor points for the recruitment of several intracellular signal transduction pathways. One set of pathways involves a form of the enzyme phospholipase C (PLC), which anchors to an adaptor molecule, is activated by phosphorylation and cleaves a membrane phospholipid to generate second messengers. Another set activates small G proteins.
- *Generation of multiple intracellular signals.* Many signaling pathways are activated as a consequence of the steps that occur in the initiation phase, as shown to the right in Figure 10-10, and described below.

We shall consider several of the signaling pathways recruited by T-cell activation, but the overall process is quite complex and many of the details will not be presented here. The review articles suggested at the end of this chapter provide extensive coverage of this very active research area.

Phospholipase Cγ (PLCγ): PLCγ is activated by phosphorylation and gains access to its substrate by binding to a membrane-associated adaptor protein (Figure 10-11a). PLCγ hydrolyzes a phospholipid component of the membrane to generate inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 causes a rapid release of Ca^{2+} from the endoplasmic reticulum and opens Ca^{2+} channels in the cell membrane (Figure 10-11b). DAG activates protein kinase C, a multifunctional kinase that phosphorylates many different targets (Figure 10-11c).

Ca^{2+}: Calcium ion is involved in an unusually broad range of processes, including vision, muscle contraction, and many others. It is an essential element in many T-cell responses, including a pathway that leads to the movement of a major transcription factor, NFAT, from the cytoplasm into the nucleus (Figure 10-11b). In the nucleus, NFAT supports the transcription of genes required for the expression of the T-cell growth-promoting cytokines IL-2, IL-4, and others.

Protein kinase C (PKC): This enzyme, which affects many pathways, causes the release of an inhibitory molecule from the transcription factor NF-κB, allowing NF-κB to enter the nucleus, where it promotes the expression of genes required for T-cell activation (Figure 10-11c). NF-κB is essential for a variety of T-cell responses and provides survival signals that protect T cells from apoptotic death.

The Ras/MAP kinase pathway: Ras is a pivotal component of a signal-transduction pathway that is found in many cell types and is evolutionarily conserved across a spectrum of eukaryotes from yeasts to humans. Ras is a small G protein whose activation by GTP initiates a cascade of protein kinases known as the mitogen-activated protein kinase (MAP kinase) pathway. As shown in Figure 10-12, phosphorylation of the end product of this cascade, MAP kinase (also called

VISUALIZING CONCEPTS

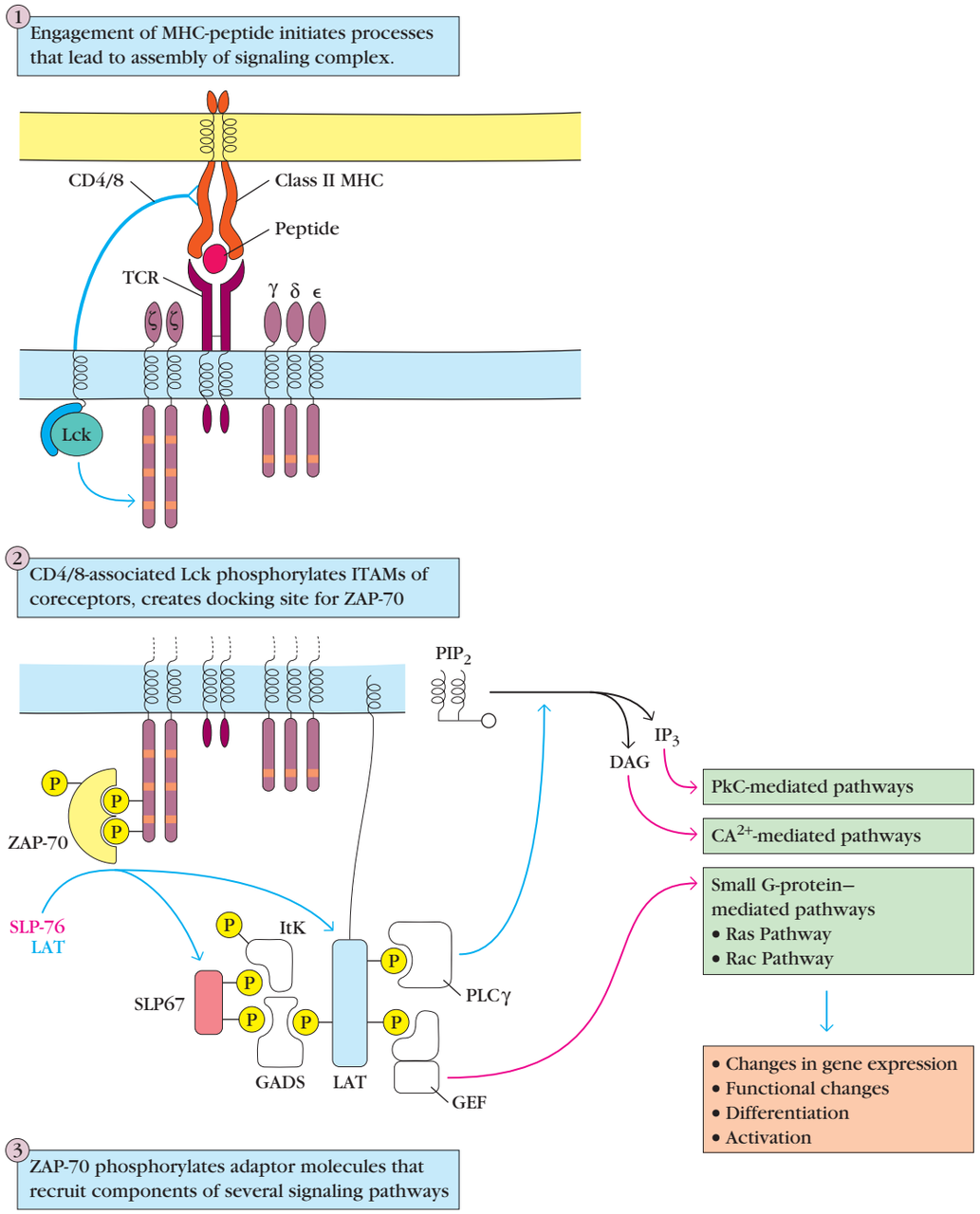


FIGURE 10-10 Overview of TCR-mediated signaling. TCR engagement by peptide-MHC complexes initiates the assembly of a signaling complex. An early step is the Lck-mediated phosphorylation of ITAMs on the zeta (ζ) chains of the TCR complex, creating docking sites to which the protein kinase ZAP-70 attaches and becomes activated by phosphorylation. A series of ZAP-70-catalyzed protein phosphorylations enable the generation of a variety of signals. (Abbreviations: DAG = diacylglycerol; GADS = Grb2-like adaptor downstream of Shc; GEF = guanine nucleotide exchange factor; ITAM = immunoreceptor tyrosine-based activation motif; Itk = inducible T cell kinase; IP3 = inositol 1,4,5 triphosphate; LAT = linker of activated T cells; PIP_2 = phosphoinositol biphosphage; PLCγ = phospholipase C gamma; Lck = lymphocyte kinase; SLP-76 = SH2-containing leukocyte-specific protein of 76 kDa; ZAP-70 = zeta associated protein of 70 kDa.)

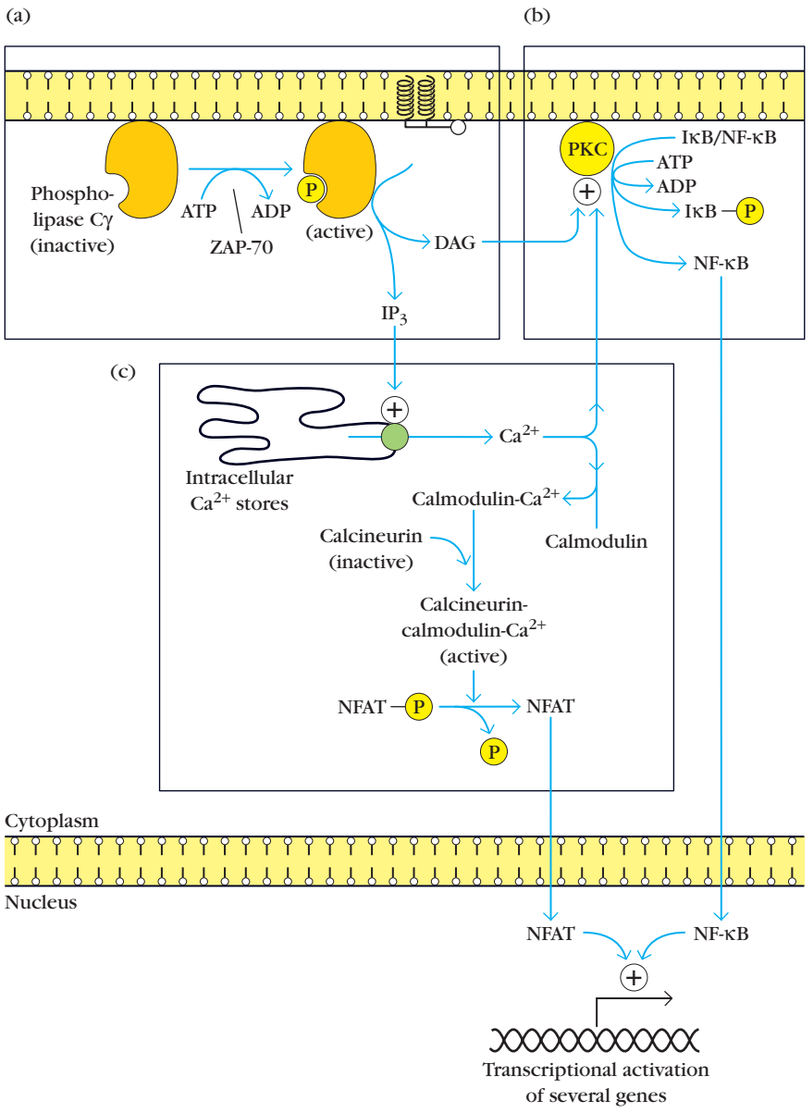


FIGURE 10-11 Signal-transduction pathways associated with T-cell activation. (a) Phospholipase Cγ (PLC) is activated by phosphorylation. Active PLC hydrolyzes a phospholipid component of the plasma membrane to generate the second messengers, DAG and IP_3. (b) Protein kinase C (PKC) is activated by DAG and Ca^{2+}. Among the numerous effects of PKC is phosphorylation of IkB, a cytoplasmic protein that binds the transcription factor NF-κB and prevents it from entering the nucleus. Phosphorylation of IkB releases NF-κB, which then translocates into the nucleus. (c) Ca^{2+}-dependent activation of calcineurin. Calcineurin is a Ca^{2+}/calmodulin dependent phosphatase. IP_3 mediates the release of Ca^{2+} from the endoplasmic reticulum. Ca^{2+} binds the protein calmodulin, which then associates with and activates the Ca^{2+}/calmodulin-dependent phosphatase calcineurin. Active calcineurin removes a phosphate group from NFAT, which allows this transcription factor to translocate into the nucleus.

ERK), allows it to activate Elk, a transcription factor necessary for the expression of Fos. Phosphorylation of Fos by MAP kinase allows it to associate with Jun to form AP-1, which is an essential transcription factor for T-cell activation.

Co-Stimulatory Signals Are Required for Full T-Cell Activation

T-cell activation requires the dynamic interaction of multiple membrane molecules described above, but this interaction, by itself, is not sufficient to fully activate naive T cells. Naive T cells require more than one signal for activation and subsequent proliferation into effector cells:

- *Signal 1,* the initial signal, is generated by interaction of an antigenic peptide with the TCR-CD3 complex.
- A subsequent antigen-nonspecific co-stimulatory signal, *signal 2,* is provided primarily by interactions between CD28 on the T cell and members of the B7 family on the APC.

There are two related forms of B7, B7-1 and B7-2 (Figure 10-13). These molecules are members of the immunoglobulin superfamily and have a similar organization of extracellular domains but markedly different cytosolic domains. Both B7 molecules are constitutively expressed on dendritic cells and induced on activated macrophages and activated B cells. The ligands for B7 are CD28 and CTLA-4 (also known as CD152), both of which are expressed on the T-cell membrane as disulfide-linked homodimers; like B7, they are members of the immunoglobulin superfamily (Figure 10-13). Although CD28 and CTLA-4 are structurally similar glycoproteins, they act antagonistically. Signaling through

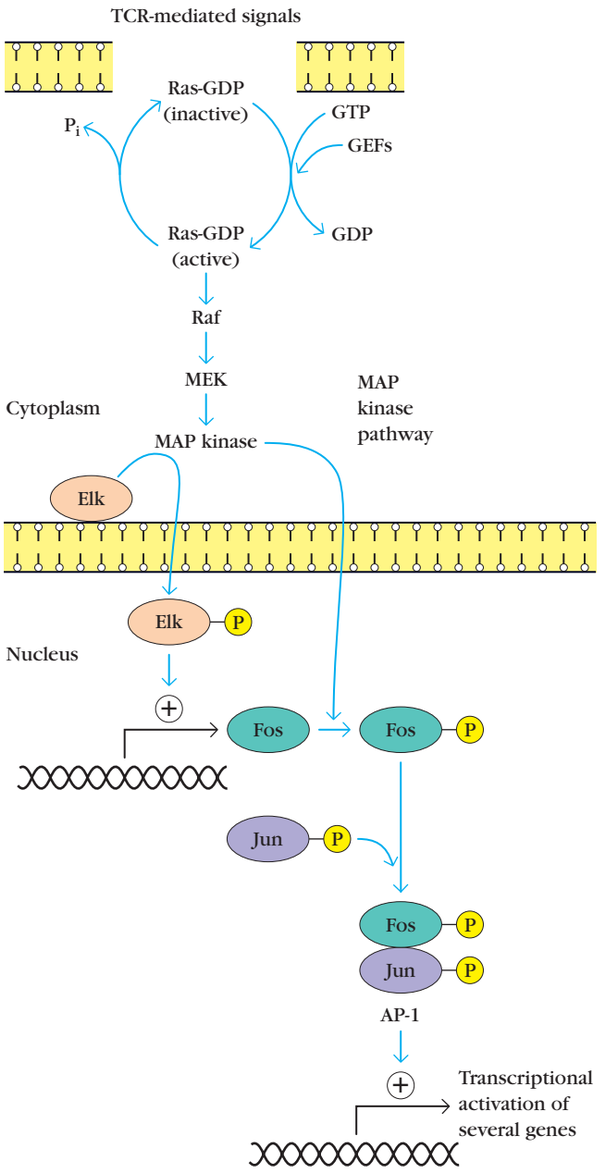


FIGURE 10-12 Activation of the small G protein, Ras. Signals from the T-cell receptor result in activation of Ras via the action of specific guanine nucleotide exchange factors (GEFs) that catalyze the exchange of GDP for GTP. Active Ras causes a cascade of reactions that result in the increased production of the transcription factor Fos. Following their phosphorylation, Fos and Jun dimerize to yield the transcription factor AP-1. Note that all these pathways have important effects other than the specific examples shown in the figure.

CD28 delivers a positive co-stimulatory signal to the T cell; signaling through CTLA-4 is inhibitory and down-regulates the activation of the T cell. CD28 is expressed by both resting and activated T cells, but CTLA-4 is virtually undetectable on resting cells. Typically, engagement of the TCR causes the induction of CTLA-4 expression, and CTLA-4 is readily detectable within 24 hours of stimulation, with maximal expression within 2 or 3 days post-stimulation. Even though the peak surface levels of CTLA-4 are lower than those of CD28, it still competes favorably for B7 molecules because it has a significantly higher avidity for these molecules than CD28 does. Interestingly, the level of CTLA-4 expression is increased by CD28-generated co-stimulatory signals. This provides regulatory braking via CTLA-4 in proportion to the acceleration received from CD28. Some of the importance of CTLA-4 in the regulation of lymphocyte activation and proliferation is revealed by experiments with CTLA-4 knockout mice. T cells in these mice proliferate massively, which leads to lymphadenopathy (greatly enlarged lymph nodes), splenomegaly (enlarged spleen), and death at 3 to 4 weeks after birth. Clearly, the production of inhibitory signals by engagement of CTLA-4 is important in lymphocyte homeostasis.

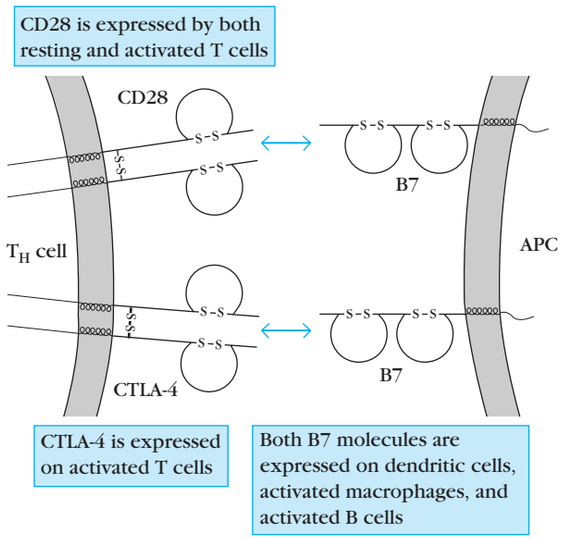


FIGURE 10-13 T_H-cell activation requires a co-stimulatory signal provided by antigen-presenting cells (APCs). Interaction of B7 family members on APCs with CD28 delivers the co-stimulatory signal. Engagement of the closely related CTLA-4 molecule with B7 produces an inhibitory signal. All of these molecules contain at least one immunoglobulin-liké domain and thus belong to the immunoglobulin superfamily. *[Adapted from P. S. Linsley and J. A. Ledbetter, 1993,* Annu. Rev. Immunol. ***11:****191.]*

CTLA-4 can effectively block CD28 co-stimulation by competitive inhibition at the B7 binding site, an ability that holds promise for clinical use in autoimmune diseases and transplantation. As shown in Figure 10-14, an ingeniously engineered chimeric molecule has been designed to explore the therapeutic potential of CTLA-4. The Fc portion of human IgG has been fused to the B7-binding domain of CTLA-4 to produce a chimeric molecule called CTLA-4Ig. The human Fc region endows the molecule with a longer half-life in the body and the presence of B7 binding domains

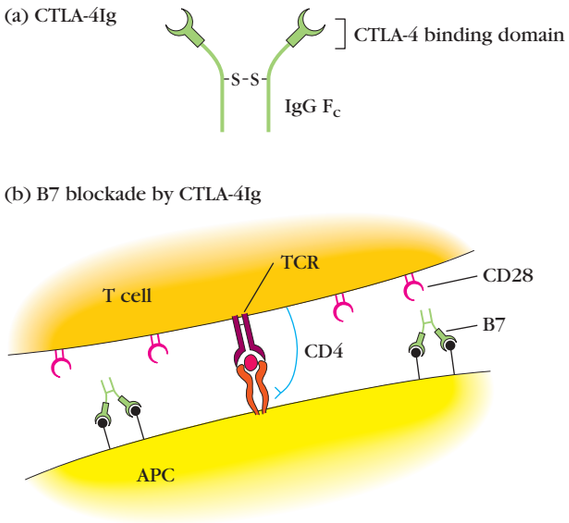


FIGURE 10-14 CTLA-4Ig, a chimeric suppressor of co-stimulation. (a) CTLA-4Ig, a genetically engineered molecule in which the Fc portion of human IgG is joined to the B7-binding domain of CTLA-4. (b) CTLA-4Ig blocks costimulation by binding to B7 on antigen presenting cells and preventing the binding of CD28, a major co-stimulatory molecule of T cells.

allows it to bind to B7. A promising clinical trial of CTLA-4 has been conducted in patients with psoriasis vulgaris, a T-cell–mediated autoimmune inflammatory skin disease. During the course of this trial, forty-three patients received four doses of CTLA-4Ig, and 46% of this group experienced a 50% or greater sustained improvement in their skin condition. Further studies of the utility of CTLA-4Ig are also being pursued in other areas.

Clonal Anergy Ensues If a Co-Stimulatory Signal Is Absent

T_H-cell recognition of an antigenic peptide–MHC complex sometimes results in a state of nonresponsiveness called **clonal anergy,** marked by the inability of cells to proliferate in response to a peptide-MHC complex. Whether clonal expansion or clonal anergy ensues is determined by the presence or absence of a co-stimulatory signal (signal 2), such as that produced by interaction of CD28 on T_H cells with B7 on antigen-presenting cells. Experiments with cultured cells show that, if a resting T_H cell receives the TCR-mediated signal (signal 1) in the absence of a suitable co-stimulatory signal, then the T_H cell will become anergic. Specifically, if resting T_H cells are incubated with glutaraldehyde-fixed APCs, which do not express B7 (Figure 10-15a), the fixed APCs are able to present peptides together with class II MHC molecules, thereby providing signal 1, but they are unable to provide the necessary co-stimulatory signal 2. In the absence of a co-stimulatory signal, there is minimal production of cytokines, especially of IL-2. Anergy can also be induced by incubating T_H cells with normal APCs in the presence of the Fab portion of anti-CD28, which blocks the interaction of CD28 with B7 (Figure 10-15b).

Two different control experiments demonstrate that fixed APCs bearing appropriate peptide-MHC complexes can deliver an effective signal mediated by T-cell receptors. In one experiment, T cells are incubated both with fixed APCs bearing peptide-MHC complexes recognized by the TCR of the T cells and with normal APCs, which express B7 (Figure 10-15d). The fixed APCs engage the TCRs of the T cells, and the B7 molecules on the surface of the normal APCs crosslink the CD28 of the T cell. These T cells thus receive both signals and undergo activation. The addition of bivalent anti-CD28 to mixtures of fixed APCs and T cells also provides effective co-stimulation by crosslinking CD28 (Figure 10-15e). Well-controlled systems for studying anergy in vitro have stimulated considerable interest in this phenomenon. However, more work is needed to develop good animal systems for establishing anergy and studying its role in vivo.

Superantigens Induce T-Cell Activation by Binding the TCR and MHC II Simultaneously

Superantigens are viral or bacterial proteins that bind simultaneously to the V_β domain of a T-cell receptor and to the α chain of a class II MHC molecule. Both exogenous and endogenous superantigens have been identified. Crosslinkage of a T-cell receptor and class II MHC molecule by either type of superantigen produces an activating signal that induces T-cell activation and proliferation (Figure 10-16).

Exogenous superantigens are soluble proteins secreted by bacteria. Among them are a variety of **exotoxins** secreted by gram-positive bacteria, such as staphylococcal enterotoxins, toxic-shock-syndrome toxin, and exfoliative-dermatitis toxin. Each of these exogenous superantigens binds particular V_β sequences in T-cell receptors (Table 10-3) and crosslinks the TCR to a class II MHC molecule.

Endogenous superantigens are cell-membrane proteins encoded by certain viruses that infect mammalian cells. One group, encoded by mouse mammary tumor virus (MTV), can integrate into the DNA of certain inbred mouse strains; after integration, retroviral proteins are expressed on the membrane of the infected cells. These viral proteins, called **minor lymphocyte stimulating (Mls)** determinants, bind particular V_β sequences in T-cell receptors and crosslink the TCR to a class II MHC molecule. Four Mls superantigens, originating in different MTV strains, have been identified.

Because superantigens bind outside of the TCR antigen-binding cleft, any T cell expressing a particular V_β sequence will be activated by a corresponding superantigen. Hence, the activation is polyclonal and can affect a significant percentage (5% is not unusual) of the total T_H population. The massive activations that follow crosslinkage by a superantigen results in overproduction of T_H-cell cytokines, leading to systemic toxicity. The food poisoning induced by staphy-

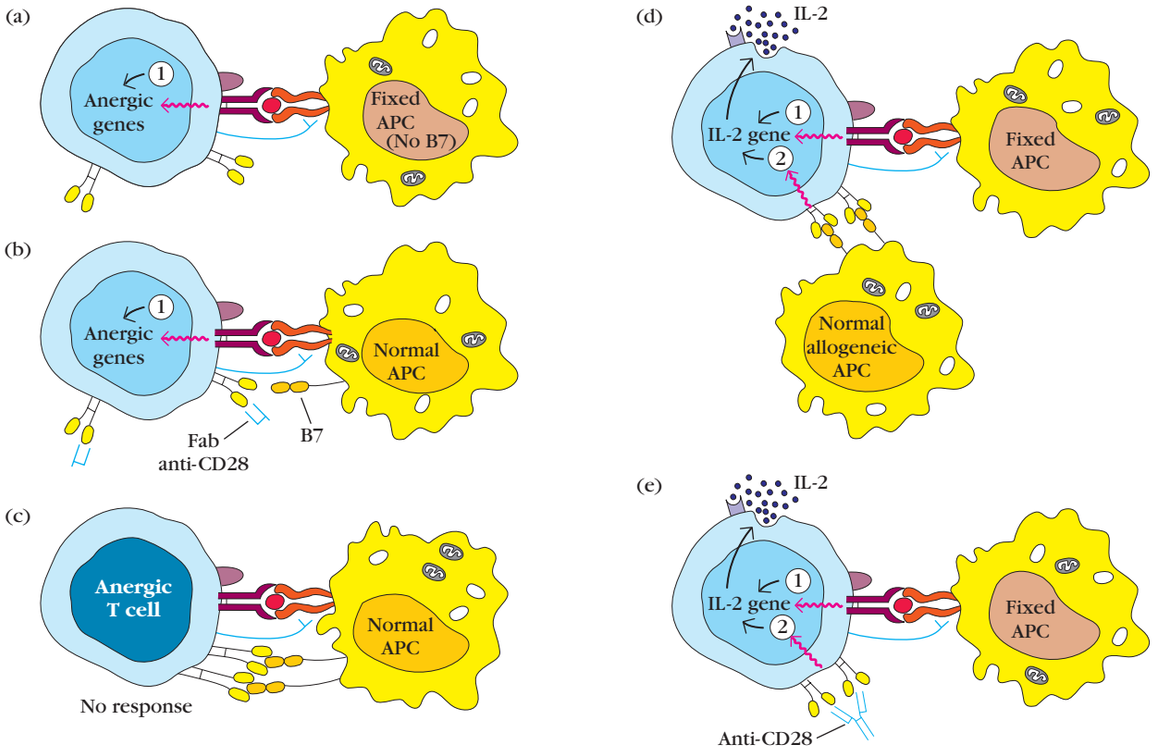


FIGURE 10-15 Experimental demonstration of clonal anergy versus clonal expansion. (a,b) Only signal 1 is generated when resting T_H cells are incubated with glutaraldehyde-fixed antigen-presenting cells (APCs) or with normal APCs in the presence of the Fab portion of anti-CD28. (c) The resulting anergic T cells cannot respond to normal APCs. (d,e) In the presence of normal allogeneic APCs or anti-CD28, both of which produce the co-stimulatory signal 2, T cells are activated by fixed APCs.

lococcal enterotoxins and the toxic shock induced by toxic-shock-syndrome toxin are two examples of the consequences of cytokine overproduction induced by superantigens.

Superantigens can also influence T-cell maturation in the thymus. A superantigen present in the thymus during thymic processing will induce the negative selection of all thymocytes bearing a TCR V_β domain corresponding to the superantigen specificity. Such massive deletion can be caused by exogeneous or endogenous superantigens and is characterized by the absence of all T cells whose receptors possess V_β domains targeted by the superantigen.

T-Cell Differentiation

$CD4^+$ and $CD8^+$ T cells leave the thymus and enter the circulation as resting cells in the G_0 stage of the cell cycle. There are about twice as many $CD4^+$ T cells as $CD8^+$ T cells in the periphery. T cells that have not yet encountered antigen (naive T cells) are characterized by condensed chromatin, very little cytoplasm, and little transcriptional activity. Naive T cells continually recirculate between the blood and lymph systems. During recirculation, naive T cells reside in secondary lymphoid tissues such as lymph nodes. If a naive cell does not encounter antigen in a lymph node, it exits through the efferent lymphatics, ultimately draining into the thoracic duct and rejoining the blood. It is estimated that each naive T cell recirculates from the blood to the lymph nodes and back again every 12–24 hours. Because only about 1 in 10^5 naive T cells is specific for any given antigen, this large-scale recirculation increases the chances that a naive T cell will encounter appropriate antigen.

Activated T Cells Generate Effector and Memory T Cells

If a naive T cell recognizes an antigen-MHC complex on an appropriate antigen-presenting cell or target cell, it will be activated, initiating a *primary response*. About 48 hours after activation, the naive T cell enlarges into a blast cell and begins undergoing repeated rounds of cell division. As described earlier, activation depends on a signal induced by engagement of the TCR complex and a co-stimulatory signal induced by the CD28-B7 interaction (see Figure 10-13). These signals trigger entry of the T cell into the G_1 phase of the cell

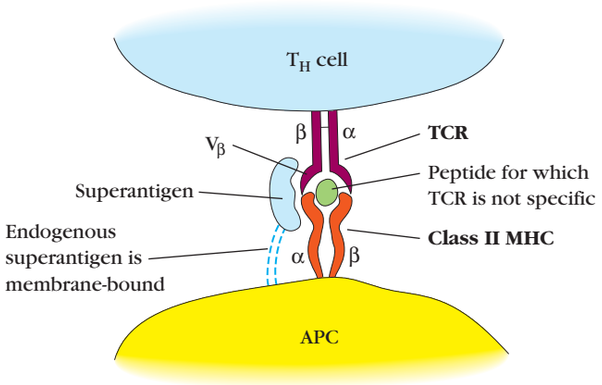


FIGURE 10-16 Superantigen-mediated crosslinkage of T-cell receptor and class II MHC molecules. A superantigen binds to all TCRs bearing a particular $V_β$ sequence regardless of their antigenic specificity. Exogenous superantigens are soluble secreted bacterial proteins, including various exotoxins. Endogenous superantigens are membrane-embedded proteins produced by certain viruses; they include Mls antigens encoded by mouse mammary tumor virus.

cycle and, at the same time, induce transcription of the gene for IL-2 and the α chain of the high-affinity IL-2 receptor. In addition, the co-stimulatory signal increases the half-life of the IL-2 mRNA. The increase in IL-2 transcription, together with stabilization of the IL-2 mRNA, increases IL-2 production by 100-fold in the activated T cell. Secretion of IL-2 and its subsequent binding to the high-affinity IL-2 receptor induces the activated naive T cell to proliferate and differentiate (Figure 10-17). T cells activated in this way divide 2–3 times per day for 4–5 days, generating a large clone of progeny cells, which differentiate into memory or effector T-cell populations.

The various *effector T cells* carry out specialized functions such as cytokine secretion and B-cell help (activated $CD4^+$ T_H cells) and cytotoxic killing activity ($CD8^+$ CTLs). The generation and activity of CTL cells are described in detail in Chapter 14. Effector cells are derived from both naive and memory cells after antigen activation. Effector cells are short-lived cells, whose life spans range from a few days to a few weeks. The effector and naive populations express different cell-membrane molecules, which contribute to different recirculation patterns.

As described in more detail in Chapter 12, $CD4^+$ effector T cells form two subpopulations distinguished by the different panels of cytokines they secrete. One population, called the **T_H1 subset,** secretes IL-2, IFN-γ, and TNF-β. The T_H1 subset is responsible for classic cell-mediated functions, such as delayed-type hypersensitivity and the activation of cytotoxic T lymphocytes. The other subset, called the **T_H2 subset,** secretes IL-4, IL-5, IL-6, and IL-10. This subset functions more effectively as a helper for B-cell activation.

The *memory T-cell* population is derived from both naive T cells and from effector cells after they have encountered antigen. Memory T cells are antigen-generated, generally

TABLE 10-3 Exogenous superantigens and their $V_β$ specificity

		$V_β$ SPECIFICITY	
Superantigen	**Disease***	**Mouse**	**Human**
Staphylococcal enterotoxins			
SEA	Food poisoning	1, 3, 10, 11, 12, 17	nd
SEB	Food poisoning	3, 8.1, 8.2, 8.3	3, 12, 14, 15, 17, 20
SEC1	Food poisoning	7, 8.2, 8.3, 11	12
SEC2	Food poisoning	8.2, 10	12, 13, 14, 15, 17, 20
SEC3	Food poisoning	7, 8.2	5, 12
SED	Food poisoning	3, 7, 8.3, 11, 17	5, 12
SEE	Food poisoning	11, 15, 17	5.1, 6.1–6.3, 8, 18
Toxic-shock-syndrome toxin (TSST1)	Toxic-shock syndrome	15, 16	2
Exfoliative-dermatitis toxin (ExFT)	Scalded-skin syndrome	10, 11, 15	2
Mycoplasma-arthritidis supernatant (MAS)	Arthritis, shock	6, 8.1–8.3	nd
Streptococcal pyrogenic exotoxins (SPE-A, B, C, D)	Rheumatic fever, shock	nd	nd

*Disease results from infection by bacteria that produce the indicated superantigens.

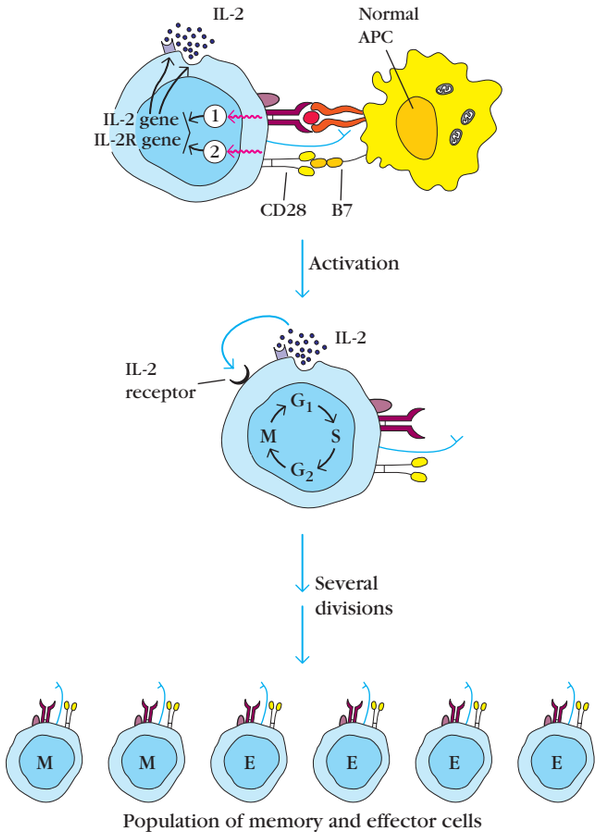


FIGURE 10-17 Activation of a T_H cell by both signal 1 and co-stimulatory signal 2 up-regulates expression of IL-2 and the high-affinity IL-2 receptor, leading to the entry of the T cell into the cell cycle and several rounds of proliferation. Some of the cells differentiate into effector cells, others into memory cells.

long-lived, quiescent cells that respond with heightened reactivity to a subsequent challenge with the same antigen, generating a *secondary response.* An expanded population of memory T cells appears to remain long after the population of effector T cells has declined. In general, memory T cells express many of the same cell-surface markers as effector T cells; no cell-surface markers definitively identify them as memory cells.

Like naive T cells, most memory T cells are resting cells in the G_0 stage of the cell cycle, but they appear to have less stringent requirements for activation than naive T cells do. For example, naive T_H cells are activated only by dendritic cells, whereas memory T_H cells can be activated by macrophages, dendritic cells, and B cells. It is thought that the expression of high levels of numerous adhesion molecules by memory T_H cells enables these cells to adhere to a broad spectrum of antigen-presenting cells. Memory cells also display recirculation patterns that differ from those of naive or effector T cells.

A $CD4^+CD25^+$ Subpopulation of T cells Negatively Regulates Immune Responses

Investigators first described T cell populations that could suppress immune responses during the early 1970s. These cells were called suppressor T cells (T_s) and were believed to be $CD8^+$ T cells. However, the cellular and molecular basis of the observed suppression remained obscure, and eventually great doubt was cast on the existence of $CD8^+$ suppressor T cells. Recent research has shown that there are indeed T cells that suppress immune responses. Unexpectedly, these cells have turned out to be $CD4^+$ rather than $CD8^+$ T cells. Within the population of $CD4^+CD25^+$ T cells, there are regulatory T cells that can inhibit the proliferation of other T cell populations in vitro. Animal studies show that members of the $CD4^+CD25^+$ population inhibit the development of autoimmune diseases such as experimentally induced inflammatory bowel disease, experimental allergic encephalitis, and autoimmune diabetes. The suppression by these regulatory cells is antigen specific because it depends upon activation through the T cell receptor. Cell contact between the suppressing cells and their targets is required. If the regulatory cells are activated by antigen but separated from their targets by a permeable barrier, no suppression occurs. The existence of regulatory T cells that specifically suppress immune responses has clinical implications. The depletion or inhibition of regulatory T cells followed by immunization may enhance the immune responses to conventional vaccines. In this regard, some have suggested that elimination of T cells that suppress responses to tumor antigens may facilitate the development of anti-tumor immunity. Conversely, increasing the suppressive activity of regulatory T cell populations could be useful in the treatment of allergic or autoimmune diseases. The ability to increase the activity of regulatory T cell populations might also be useful in suppressing organ and tissue rejection. Future work on this regulatory cell population will seek deeper insights into the mechanisms by which members of $CD4^+CD25^+$ T cell populations regulate immune responses. There will also be determined efforts to discover ways in which the activities of these populations can be increased to diminish unwanted immune responses and decreased to promote desirable ones.

Antigen-Presenting Cells Have Characteristic Co-Stimulatory Properties

Only professional antigen-presenting cells (dendritic cells, macrophages, and B cells) are able to present antigen together with class II MHC molecules and deliver the co-stimulatory signal necessary for complete T-cell activation that leads to proliferation and differentiation. The principal co-stimulatory molecules expressed on antigen-presenting cells are the glycoproteins B7-1 and B7-2 (see Figure 10-13). The professional antigen-presenting cells differ in their ability to display antigen and also differ in their ability to deliver the co-stimulatory signal (Figure 10-18).

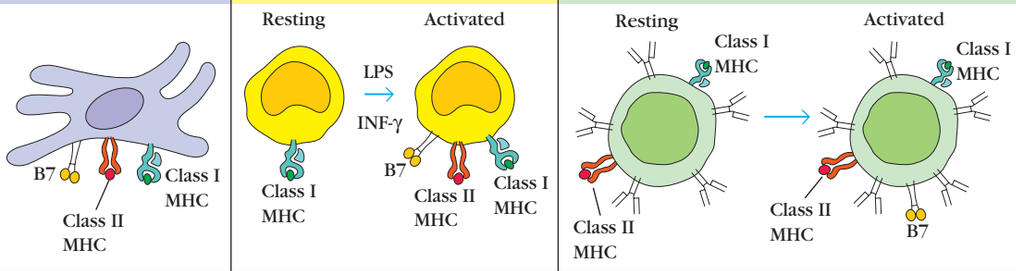
	Dendritic cell	Macrophage		B Lymphocyte	
					
Antigen uptake	Endocytosis phagocytosis (by Langerhans cells)	Phagocytosis	Phagocytosis	Receptor-mediated endocytosis	Receptor-mediated endocytosis
Class II MHC expression	Constitutive (+++)	Inducible (–)	Inducible (++)	Constitutive (++)	Constitutive (+++)
Co-stimulatory activity	Constitutive B7 (+++)	Inducible B7 (–)	Inducible B7 (++)	Inducible B7 (–)	Inducible B7 (++)
T-cell activation	Naive T cells Effector T cells Memory T cells	(–)	Effector T cells Memory T cells	Effector T cells Memory T cells	Naive T cells Effector T cells Memory T cells

FIGURE 10-18 Differences in the properties of professional antigen-presenting cells affect their ability to present antigen and induce T-cell activation. Note that activation of effector and memory T cells does not require the co-stimulatory B7 molecule.

Dendritic cells constitutively express high levels of class I and class II MHC molecules as well as high levels of B7-1 and B7-2. For this reason, dendritic cells are very potent activators of naive, memory, and effector T cells. In contrast, all other professional APCs require activation for expression of co-stimulatory B7 molecules on their membranes; consequently, resting macrophages are not able to activate naive T cells and are poor activators of memory and effector T cells. Macrophages can be activated by phagocytosis of bacteria or by bacterial products such as LPS or by IFN-γ, a T_H1-derived cytokine. Activated macrophages up-regulate their expression of class II MHC molecules and co-stimulatory B7 molecules. Thus, activated macrophages are common activators of memory and effector T cells, but their effectiveness in activating naive T cells is considered minimal.

B cells also serve as antigen-presenting cells in T-cell activation. Resting B cells express class II MHC molecules but fail to express co-stimulatory B7 molecules. Consequently, resting B cells cannot activate naive T cells, although they can activate the effector and memory T-cell populations. Upon activation, B cells up-regulate their expression of class II MHC molecules and begin expressing B7. These activated B cells can now activate naive T cells as well as the memory and effector populations.

Cell Death and T-Cell Populations

Cell death is an important feature of development in all multicellular organisms. During fetal life it is used to mold and sculpt, removing unnecessary cells to provide shape and form. It also is an important feature of lymphocyte homeostasis, returning T- and B-cell populations to their appropriate levels after bursts of antigen-induced proliferation. Apoptosis also plays a crucial role in the deletion of potentially autoreactive thymocytes during negative selection and in the removal of developing T cells unable to recognize self (failure to undergo positive selection).

Although the induction of apoptosis involves different signals depending on the cell types involved, the actual death of the cell is a highly conserved process amongst vertebrates and invertebrates. For example, T cells may be induced to die by many different signals, including the withdrawal of growth factor, treatment with glucocorticoids, or TCR signaling. Each of these signals engages unique signaling pathways, but in all cases, the actual execution of the cell involves the activation of a specialized set of proteases known as *caspases*. The role of these proteases was first revealed by studies of developmentally programmed cell deaths in the nematode

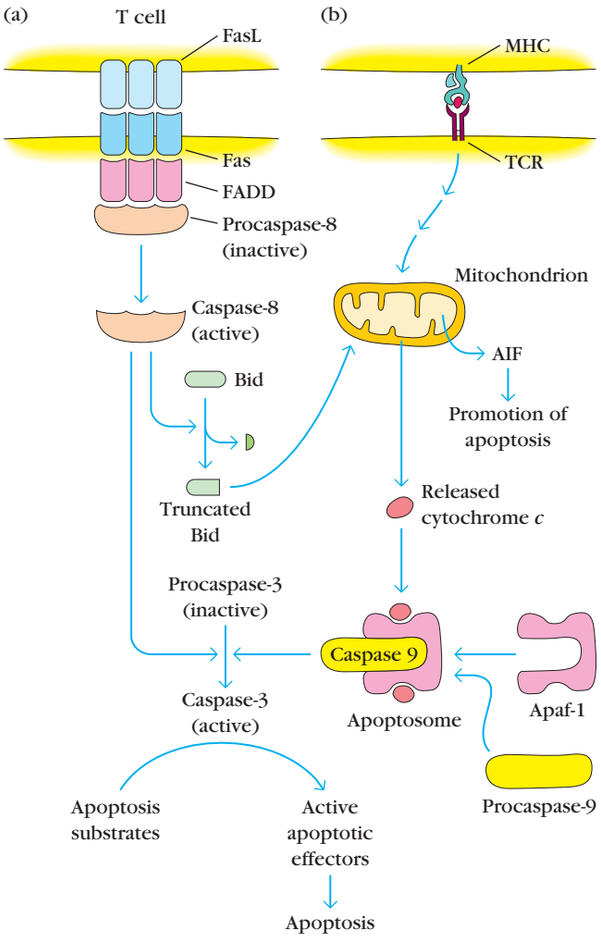


FIGURE 10-19 Two pathways to apoptosis in T cells. (a) Activated peripheral T cells are induced to express high levels of Fas and FasL. FasL induces the trimerization of Fas on a neighboring cell. FasL can also engage Fas on the same cell, resulting in a self-induced death signal. Trimerization of Fas leads to the recruitment of FADD, which leads in turn to the cleavage of associated molecules of procaspase 8 to form active caspase 8. Caspase 8 cleaves procaspase 3, producing active caspase 3, which results in the death of the cell. Caspase 8 can also cleave Bid to a truncated form that can activate the mitochondrial death pathway. (b) Other signals, such as the engagement of the TCR by peptide-MHC complexes on an APC, result in the activation of the mitochondrial death pathway. A key feature of this pathway is the release of AIF (apoptosis inducing factor) and cytochrome *c* from the inner mitochondrial membrane into the cytosol. Cytochrome *c* interacts with Apaf-1 and subsequently with procaspase 9 to form the active apoptosome. The apoptosome initiates the cleavage of procaspase 3, producing active caspase 3, which initiates the execution phase of apoptosis by proteolysis of substances whose cleavage commits the cell to apoptosis. *[Adapted in part from S. H. Kaufmann and M. O. Hengartner, 2001.* Trends Cell Biol. ***11:****526.]*

C. elegans, where the death of cells was shown to be totally dependent upon the activity of a gene that encoded a cysteine protease with specificity for aspartic acid residues. We now know that in mammals there are at least 14 cysteine proteases or caspases, and all cell deaths require the activity of at least a subset of these molecules. We also know that essentially every cell in the body produces caspase proteins, suggesting that every cell has the potential to initiate its own death.

Cells protect themselves from apoptotic death under normal circumstances by keeping caspases in an inactive form within a cell. Upon reception of the appropriate death signal, certain caspases are activated by proteolytic cleavage and then activate other caspases in turn, leading to the activation of *effector caspases.*This catalytic cascade culminates in cell death. Although it is not well understood how caspase activation directly results in apoptotic death of the cell, presumably it is through the cleavage of critical targets necessary for cell survival.

T cells use two different pathways to activate caspases (Figure 10-19). In peripheral T cells, antigen stimulation results in proliferation of the stimulated T cell and production of several cytokines including IL-2. Upon activation, T cells increase the expression of two key cell-surface proteins involved in T-cell death, Fas and Fas ligand (FasL). When Fas binds its ligand, FasL, FADD (Fas-associated protein with death domain) is recruited and binds to Fas, followed by the recruitment of procaspase 8, an inactive form of caspase 8. The association of FADD with procaspase 8 results in the proteolytic cleavage of procaspase 8 to its active form; caspase 8 then initiates a proteolytic cascade that leads to the death of the cell.

Outside of the thymus, most of the TCR-mediated apoptosis of mature T cells is mediated by the Fas pathway. Repeated or persistent stimulation of peripheral T cells results in the coexpression of both Fas and Fas ligand, followed by the apoptotic death of the cell. The Fas/FasL mediated death of T cells as a consequence of activation is called *activation-induced cell death* (AICD) and is a major homeostatic mechanism, regulating the size of the pool of T cells and removing T cells that repeatedly encounter self antigens.

The importance of Fas and FasL in the removal of activated T cells is underscored by *lpr/lpr* mice, a naturally occurring mutation that results in non-functional Fas. When T cells become activated in these mice, the Fas/FasL pathway is not operative; the T cells continue to proliferate, producing IL-2 and maintaining an activated state. These mice spontaneously develop autoimmune disease, have excessive numbers of T cells, and clearly demonstrate the consequences of a failure to delete activated T cells. An additional mutation, *gld/gld*, is also informative. These mice lack functional FasL and display much the same abnormalities found in the *lpr/lpr* mice. Recently, humans with defects in Fas have been reported. As expected, these individuals display characteristics of autoimmune disease. (See the Clinical Focus box.)

Fas and FasL are members of a family of related receptor/ligands including tumor necrosis factor (TNF) and its

ligand, TNFR (tumor necrosis factor receptor). Like Fas and FasL, membrane-bound TNFR interacts with TNF to induce apoptosis. Also similar to Fas/FasL-induced apoptosis, TNF/TNFR-induced death is the result of the activation of caspase 8 followed by the activation of effector caspases such as caspase 3.

In addition to the activation of apoptosis through death receptor proteins like Fas and TNFR, T cells can die through other pathways that do not activate procaspase 8. For example, negative selection in the thymus induces the apoptotic death of developing T cells via a signaling pathway that originates at the TCR. We still do not completely understand why some signals through the TCR induce positive selection and others induce negative selection, but we know that the strength of the signal plays a critical role. A strong, negatively selecting signal induces a route to apoptosis in which mitochondria play a central role. In mitochondrially dependent apoptotic pathways, cytochrome *c,* which normally resides in the inner mitochondrial membrane, leaks into the cytosol. Cytochrome *c* binds to a protein known as Apaf-1 (apoptotic protease-activating factor-1) and undergoes an ATP-dependent conformational change and oligomerization. Binding of the oligomeric form of Apaf-1 to procaspase 9 results in its transformation to active caspase 9. The complex of cytochrome *c*/Apaf-1/caspase 9, called the *apoptosome,* proteolytically cleaves procaspase 3 generating active caspase 3, which initiates a cascade of reactions that kills the cell (Figure 10-19). Finally, mitochondria also release another molecule, AIF (apoptosis inducing factor), which plays a role in the induction of cell death.

Cell death induced by Fas/FasL is swift, with rapid activation of the caspase cascade leading to cell death in 2–4 hours. On the other hand, TCR-induced negative selection appears to be a more circuitous process, requiring the activation of several processes including mitochondrial membrane failure, the release of cytochrome *c,* and the formation of the apoptosome before caspases become involved. Consequently, TCR-mediated negative selection can take as long as 8–10 hours.

An important feature in the mitochondrially induced cell death pathway is the regulatory role played by Bcl-2 family members. Bcl-2 and Bcl-XL both reside in the mitochondrial membrane. These proteins are strong inhibitors of apoptosis, and while it is not clear how they inhibit cell death, one hypothesis is that they somehow regulate the release of cytochrome c from the mitochondria. There are at least three groups of Bcl-2 family members. Group I members are anti-apoptotic and include Bcl-2 and Bcl-xL. Group II and Group III members are pro-apoptotic and include Bax and Bak in Group II and Bid and Bim in Group III. There is clear evidence that levels of anti-apoptotic Bcl-2 family members play an important role in regulating apoptosis in lymphocytes. Bcl-2 family members dimerize, and the anti-apoptotic group members may control apoptosis by dimerizing with pro-apoptotic members, blocking their activity. As indicated in Figure 10-19, cleavage of Bid, catalyzed by caspase 8 generated by the Fas pathway, can turn on the mitochondrial pathway. Thus signals initiated through Fas can also involve the mitochondrial death pathway.

While it is apparent there are several ways a lymphocyte can be signaled to die, all of these pathways to cell death converge upon the activation of caspases. This part of the cell-death pathway, the execution phase, is common to almost all death pathways known in both vertebrates and invertebrates, demonstrating that apoptosis is an ancient process that has been conserved throughout evolution.

Peripheral γδ T Cells

In 1986, a small population of peripheral-blood T cells was discovered that expressed CD3 but failed to stain with monoclonal antibody specific for the αβ T-cell receptor, indicating an absence of the αβ heterodimer. Many of these cells eventually were found to express the γδ receptor.

gd T Cells Are Far Less Pervasive Than ab T Cells

In humans, less than 5% of T cells bear the γδ heterodimer, and the percentage of γδ T cells in the lymphoid organs of mice has been reported to range from 1% to 3%. In addition to their presence in blood and lymphoid tissues, they also appear in the skin, intestinal epithelium, and pulmonary epithelium. Up to 1% of the epidermal cells in the skin of mice are γδ T cells. In general, γδ T cells are not MHC-restricted, and most do not express the coreceptors CD4 and CD8 present on populations of αβ T cells. Although the potential of the γ and δ TCR loci to generate diversity is great, very little diversity is found in this type of T cell. In fact, as pointed out in Chapter 9, most of the γδ T cells in humans have an identical combination of γδ chains (γ9 and δ2).

γδ T Cells Recognize Nonpeptide Ligands

Not all T cells are self-MHC restricted and recognize only peptide antigens displayed in the cleft of the self-MHC molecule. Indeed, Chapters 2 and 8 describe αβ TCR-bearing T cells (NK1-T cells and CD1-restricted T cells) that are not restricted by conventional MHC molecules. In one study, a γδ T-cell clone was found to bind directly to a herpes-virus protein without requiring antigen processing and presentation together with MHC. Human γδ T cells have been reported that display MHC-independent binding of a phospholipid derived from *M. tuberculosis*, the organism responsible for tuberculosis (see Chapter 9). This finding suggests that in many cases the TCR receptors of γδ T cells bind to epitopes in much the same way that the immunoglobulin receptors of B cells do. The fact that most human γδ T cells all have the same specificity suggests that like other components of the innate immune system, they recognize and respond to

CLINICAL FOCUS

Failure of Apoptosis Causes Defective Lymphocyte Homeostasis

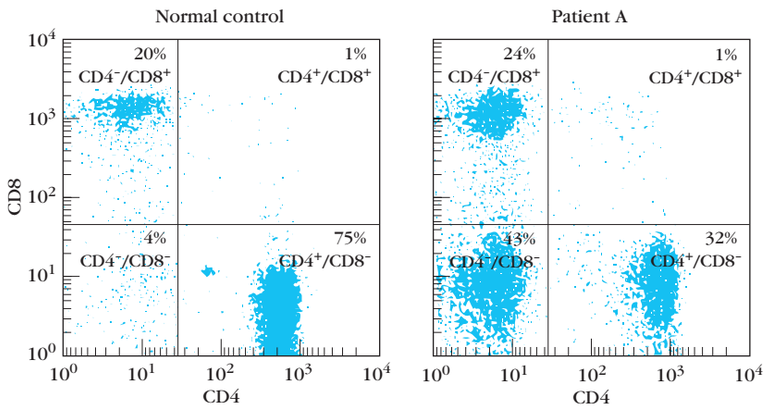
The maintenance of appropriate numbers of various types of lymphocytes is extremely important to an effective immune system. One of the most important elements in this regulation is apoptosis mediated by the Fas/FasL ligand system. The following excerpts from medical histories show what can happen when this key regulatory mechanism fails.

Patient A: A woman, now 43, has had a long history of immunologic imbalances and other medical problems. By age 2, she was diagnosed with the Canale-Smith syndrome (CSS), a severe enlargement of such lymphoid tissues as lymph nodes (lymphadenopathy) and spleen (splenomegaly). Biopsy of lymph nodes showed that, in common with many other CSS patients, she had greatly increased numbers of lymphocytes. She had reduced numbers of platelets (thrombocytopenia) and, because her red blood cells were being lysed, she was anemic (hemolytic anemia). The reduction in numbers of platelets and the lysis of red blood cells could be traced to the action of circulating antibodies that reacted with these host components. At age 21, she was diagnosed with grossly enlarged pelvic lymph nodes that had to be removed. Ten years later, she was again found to have an enlarged abdominal mass, which on surgical removal turned out to be a half-pound lymph-node aggregate. She has continued to have mild lymphadenopathy and, typical of these patients, the lymphocyte populations of enlarged nodes had elevated numbers of T cells (87% as opposed to a normal range of 48%–67% T cells). Examination of these cells by flow cytometry and fluorescent antibody staining revealed an excess of double-negative T cells (see illustration below). Also, like many patients with Canale-Smith syndrome, she has had cancer, breast cancer at age 22 and skin cancer at ages 22 and 41.

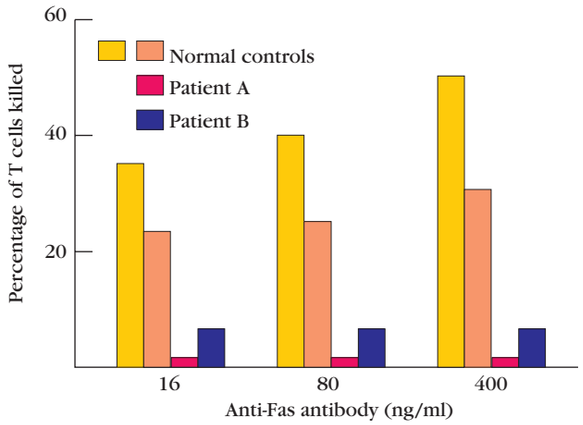
Patient B: A man who was eventually diagnosed with Canale-Smith syndrome had severe lymphadenopathy and splenomegaly as an infant and child. He was treated from age 4 to age 12 with corticosteroids and the immunosuppressive drug mercaptopurine. These appeared to help, and the swelling of lymphoid tissues became milder during adolescence and adulthood. At age 42, he died of liver cancer.

Patient C: An 8-year-old boy, the son of patient B, was also afflicted with Canale-Smith syndrome and showed elevated T-cell counts and severe lymphadenopathy at the age of seven months. At age 2 his spleen became so enlarged that it had to be removed. He also developed hemolytic anemia and thrombocytopenia. However, although he continued to have elevated T-cell counts, the severity of his hemolytic anemia and thrombocytopenia have so far been controlled by treatment with methotrexate, a DNA- synthesis-inhibiting drug used for immunosuppression and cancer chemotherapy.

Recognition of the serious consequences of a failure to regulate the number of lymphocytes, as exemplified by these case histories, emerged from detailed study of several children whose enlarged lymphoid tissues attracted medical attention. In each of these cases of Canale-Smith syndrome, examination revealed grossly enlarged lymph nodes that were 1–2 cm in girth and sometimes large enough to distort the local anatomy. In four of a group of five children who were studied intensively, the



Flow-cytometric analysis of T cells in the blood of Patient A and a control subject. The relative staining by an anti-CD8 antibody is shown on the *y* axis and the relative staining by an anti-CD4 antibody appears on the *x* axis. Mature T cells are either $CD4^+$ or $CD8^+$. While almost all of the T cells in the control subject are $CD4^+$ or $CD8^+$, the CSS patient shows high numbers of double-negative T cells (43%), which express neither CD4 nor CD8. The percentage of each category of T cells is indicated in the quadrants. *[Adapted from Drappa et al., 1996,* New England Journal of Medicine ***335:****1643.]*



Fas-mediated killing takes place when Fas is crosslinked by FasL, its normal ligand, or by treatment with anti-Fas antibody, which artificially crosslinks Fas molecules. This experiment shows the reduction in numbers of T cells after induction of apoptosis in T cells from patients and controls by crosslinking Fas with increasing amounts of an anti-Fas monoclonal antibody. T cells from the Canale-Smith patients (A and B) are resistant to Fas-mediated death. *[Adapted from Drappa et al., 1996,* New England Journal of Medicine ***335:****1643.]*

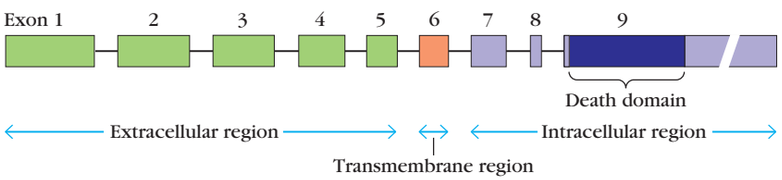
spleens were so massive that they had to be removed.

Even though the clinical picture in Canale-Smith syndrome can vary from person to person, with some individuals suffering severe chronic affliction and others only sporadic episodes of illness, there is a common feature, a failure of activated lymphocytes to undergo Fas-mediated apoptosis. Isolation and sequencing of Fas genes from a number of patients and more than 100 controls reveals that CSS patients are heterozygous ($fas^{+/-}$) at the *fas* locus and thus carry one copy of a defective *fas* gene. A comparison of Fas-mediated cell death in T cells from normal controls who do not carry mutant Fas genes with death induced in T cells from CSS patients, shows a marked defect in Fas-induced death (see illustration above). Characterization of the Fas genes so far seen in CSS patients reveals that they have mutations in or around the region encoding the death-inducing domain (the "death domain") of this protein (see illustration below). Such mutations result in the production of Fas protein that lacks biological activity but still competes with normal Fas molecules for interactions with essential components of the Fas-mediated death pathway. Other mutations have been found in the extracellular domain of Fas, often associated with milder forms of CSS or no disease at all.

A number of research groups have conducted detailed clinical studies of CSS patients, and the following general observations have been made:

- The cell populations of the blood and lymphoid tissues of CSS patients show dramatic elevations (5-fold to as much as 20-fold) in the numbers of lymphocytes of all sorts, including T cells, B cells, and NK cells.
- Most of the patients have elevated levels of one or more classes of immunoglobulin (hyper-gammaglobulinemia).
- Immune hyperactivity is responsible for such autoimmune phenomena as the production of autoantibodies against red blood cells, resulting in hemolytic anemia, and a depression in platelet counts due to the activity of anti-platelet auto-antibodies.

These observations establish the importance of the death-mediated regulation of lymphocyte populations in lymphocyte homeostasis. Such death is necessary because the immune response to antigen results in a sudden and dramatic increase in the populations of responding clones of lymphocytes and temporarily distorts the representation of these clones in the repertoire. In the absence of cell death, the periodic stimulation of lymphocytes that occurs in the normal course of life would result in progressively increasing, and ultimately unsustainable, lymphocyte levels. As the Canale-Smith syndrome demonstrates, without the essential culling of lymphocytes by apoptosis, severe and life-threatening disease can result.



Map of *fas* locus. The *fas* gene is composed of 9 exons separated by 8 introns. Exons 1–5 encode the extracellular part of the protein, exon 6 encodes the transmembrane region, and exons 7–9 encode the intracellular region of the molecule. Much of exon 9 is responsible for encoding the critical death domain. *[Adapted from G. H. Fisher et al., 1995,* Cell ***81:****935.]*

molecular patterns that are found in certain pathogens but not in humans. Thus they may play a role as first lines of defense against certain pathogens, expressing effector functions that help control infection and secreting cytokines that promote an adaptive immune response by αβ T cells and B cells.

SUMMARY

- Progenitor T cells from the bone marrow enter the thymus and rearrange their TCR genes. In most cases these thymocytes rearrange αβ TCR genes and become αβ T cells. A small minority rearrange γδ TCR genes and become γδ T cells.
- The earliest thymocytes lack detectable CD4 and CD8 and are referred to as double-negative cells. During development, the majority of double-negative thymocytes develop into $CD4^+CD8^-$ αβ T cells or $CD4^-CD8^+$ αβ T cells.
- Positive selection in the thymus eliminates T cells unable to recognize self-MHC and is the basis of MHC restriction. Negative selection eliminates thymocytes bearing high-affinity receptors for self-MHC molecules alone or self-antigen plus self-MHC and produces self-tolerance.
- T_H-cell activation is initiated by interaction of the TCR-CD3 complex with a peptide-MHC complex on an antigen-presenting cell. Activation also requires the activity of accessory molecules, including the coreceptors CD4 and CD8. Many different intracellular signal-transduction pathways are activated by the engagement of the TCR.
- T-cells that express CD4 recognize antigen combined with a class II MHC molecule and generally function as T_H cells; T cells that express CD8 recognize antigen combined with a class I MHC molecule and generally function as T_C cells.
- In addition to the signals mediated by the T-cell receptor and its associated accessory molecules (signal 1), activation of the T_H cell requires a co-stimulatory signal (signal 2) provided by the antigen-presenting cell. The co-stimulatory signal is commonly induced by interaction between molecules of the B7 family on the membrane of the APC with CD28 on the T_H cell. Engagement of CTLA-4, a close relative of CD28, by B7 inhibits T-cell activation.
- TCR engagement with antigenic peptide-MHC may induce activation or clonal anergy. The presence or absence of the co-stimulatory signal (signal 2) determines whether activation results in clonal expansion or clonal anergy.
- Naive T cells are resting cells (G_0) that have not encountered antigen. Activation of naive cells leads to the generation of effector and memory T cells. Memory T cells, which are more easily activated than naive cells, are responsible for secondary responses. Effector cells are short lived and perform helper, cytotoxic, or delayed-type hypersensitivity functions.
- The T-cell repertoire is shaped by apoptosis in the thymus and periphery.
- γδ T cells are not MHC restricted. Most in humans bind free antigen, and most have the same specificity. They may function as part of the innate immune system.

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USEFUL WEB SITES

http://www.ncbi.nlm.nih.gov/Omim/
http://www.ncbi.nlm.nih.gov/htbinpost/Omim/getmim

The Online Mendelian Inheritance in Man Web site contains a subsite that features ten different inherited diseases associated with defects in the TCR complex or associated proteins.

http://www.ultranet.com/~jkimball/BiologyPages/A/Apoptosis.html
http://www.ultranet.com/~jkimball/BiologyPages/B/B_and_Tcells.html

These subsites of John Kimball's Biology Pages Web site provide a clear introduction to T-cell biology and a good basic discussion of apoptosis.

http://www.bioscience.org/knockout/knochome.htm

Within the Frontiers in Bioscience Database of Gene Knockouts, one can find information on the effects of knockouts of many genes involved in the development and function of cells of the T cells.

Study Questions

CLINICAL FOCUS QUESTION Over a period of several years, a group of children and adolescents are regularly dosed with compound X, a life-saving drug. However, in addition to its beneficial effects, this drug interferes with Fas-mediated signaling.

a. What clinical manifestations of this side effect of compound X might be seen in these patients?
b. If white blood cells from an affected patient are stained with a fluorescein-labeled anti-CD4 and a phycoerythrin-labeled anti-CD8 antibody, what might be seen in the flow-cytometric analysis of these cells? What pattern would be expected if the same procedure were performed on white blood cells from a healthy control?

1. You have a $CD8^+$ CTL clone (from an H-2^k mouse) that has a T-cell receptor specific for the H-Y antigen. You clone the αβ TCR genes from this cloned cell line and use them to prepare transgenic mice with the H-2^k or H-2^d haplotype.

a. How can you distinguish the immature thymocytes from the mature $CD8^+$ thymocytes in the transgenic mice?
b. For each transgenic mouse listed in the table below, indicate with (+) or (−) whether the mouse would have immature double-positive and mature $CD8^+$ thymocytes bearing the transgenic T-cell receptor.

Transgenic mouse	Immature thymocytes	Mature $CD8^+$ thymocytes
H-2^k female		
H-2^k male		
H-2^d female		
H-2^d male		

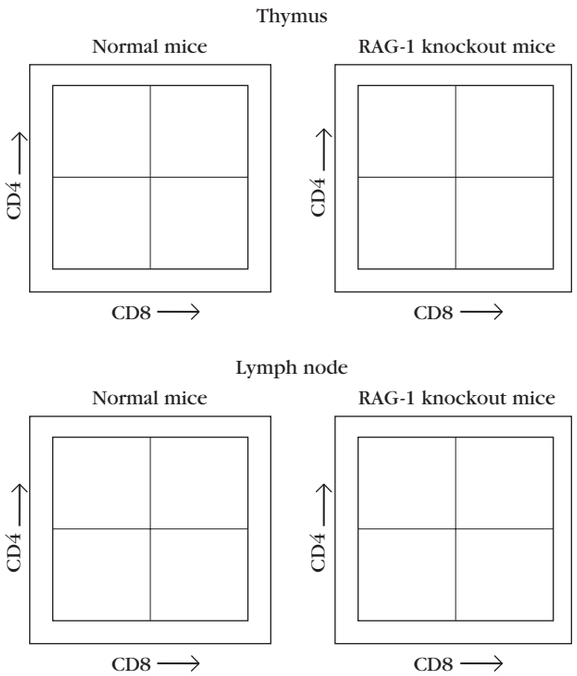
c. Explain your answers for the H-2^k transgenics.
d. Explain your answers for the H-2^d transgenics.

2. Cyclosporin and FK506 are powerful immunosuppressive drugs given to transplant recipients. Both drugs prevent the formation of a complex between calcineurin and Ca^{2+}/calmodulin. Explain why these compounds suppress T-cell–mediated aspects of transplant rejection. Hint: see Figure 10-11.

3. Antigenic activation of T_H cells leads to the release or induction of various nuclear factors that activate gene transcription.

a. What transcription factors that support proliferation of activated T_H cells are present in the cytoplasm of resting T_H cells in inactive forms?
b. Once in the nucleus, what might these transcription factors do?

4. You have fluorescein-labeled anti-CD4 and rhodamine-labeled anti-CD8. You use these antibodies to stain thymocytes and lymph-node cells from normal mice and from RAG-1 knockout mice. In the diagrams below, draw the FACS plots that you would expect.



5. In order to demonstrate positive thymic selection experimentally, researchers analyzed the thymocytes from normal H-2^b mice, which have a deletion of the class II *IE* gene, and from H-2^b mice in which the class II *IA* gene had been knocked out.

a. What MHC molecules would you find on antigen-presenting cells from the normal H-2^b mice?
b. What MHC molecules would you find on antigen-presenting cells from the IA knockout H-2^b mice?
c. Would you expect to find $CD4^+$ T cells, $CD8^+$ T cells, or both in each type of mouse? Why?

6. In his classic chimeric-mouse experiments, Zinkernagel took bone marrow from mouse 1 and a thymus from mouse 2 and transplanted them into mouse 3, which was thymectomized and lethally irradiated. He then challenged the reconstituted mouse with LCM virus and removed its spleen cells. These spleen cells were then incubated with LCM-infected target cells with different MHC haplotypes, and the lysis of the target cells was monitored. The results of two

Experiment	Bone-marrow donor	Thymectomized, x-irradiated recipient	Lysis of LCM-infected target cells		
			H-2^d	H-2^k	H-2^b
A	C57BL/6 × BALB/c	C57BL/6 × BALB/c	+	−	−
B	C57BL/6 × BALB/c	C57BL/6 × BALB/c	−	−	+

such experiments using H-2^b strain C57BL/6 mice and H-2^d strain BALB/c mice are shown in the table on the above.

a. What was the haplotype of the thymus-donor strain in experiment A and experiment B?
b. Why were the H-2^b target cells not lysed in experiment A but were lysed in experiment B?
c. Why were the H-2^k target cells not lysed in either experiment?

7. Fill in the blank(s) in each statement below (a–k) with the most appropriate term(s) from the following list. Terms may be used once, more than once, or not at all.

protein phosphatase(s)	CD8	Class I MHC	CD45
protein kinase(s)	CD4	Class II MHC	B7
CD28	IL-2	IL-6	CTLA-4

a. Lck and ZAP-70 are _____ .
b. _____ is a T-cell membrane protein that has cytosolic domains with phosphatase activity.
c. Dendritic cells express _____ constitutively, whereas B cells must be activated before they express this membrane molecule.
d. Calcineurin, a _____ , is involved in generating the active form of the transcription factor NFAT.
e. Activation of T_H cells results in secretion of _____ and expression of its receptor, leading to proliferation and differentiation.
f. The co-stimulatory signal needed for complete T_H-cell activation is triggered by interaction of _____ on the T cell and _____ on the APC.
g. Knockout mice lacking class I MHC molecules fail to produce thymocytes bearing _____ .
h. Macrophages must be activated before they express _____ molecules and _____ molecules.
i. T cells bearing _____ are absent from the lymph nodes of knockout mice lacking class II MHC molecules.
j. PIP_2 is split by a _____ to yield DAG and IP_3.
k. In activated T_H cells, DAG activates a _____ , which acts to generate the transcription factor NF-κB.
l. _____ stimulates and _____ inhibits T-cell activation when engaged by _____ or on antigen-presenting cells.

8. You wish to determine the percentage of various types of thymocytes in a sample of cells from mouse thymus using the indirect immunofluorescence method.

a. You first stain the sample with goat anti-CD3 (primary antibody) and then with rabbit FITC-labeled anti-goat Ig (secondary antibody), which emits a green color. Analysis of the stained sample by flow cytometry indicates that 70% of the cells are stained. Based on this result, how many of the thymus cells in your sample are expressing antigen-binding receptors on their surface? Would all be expressing the same type of receptor? Explain your answer. What are the remaining unstained cells likely to be?
b. You then separate the $CD3^+$ cells with the fluorescence-activated cell sorter (FACS) and restain them. In this case, the primary antibody is hamster anti-CD4 and the secondary antibody is rabbit PE-labeled anti-hamster-Ig, which emits a red color. Analysis of the stained $CD3^+$ cells shows that 80% of them are stained. From this result, can you determine how many T_C cells are present in this sample? If yes, then how many T_C cells are there? If no, what additional experiment would you perform in order to determine the number of T_C cells that are present?

9. Many of the effects of engaging the TCR with MHC-peptide can be duplicated by the administration of ionomycin plus a phorbol ester. Ionomycin is a Ca^{2+} ionophore, a compound that allows calcium ions in the medium to cross the plasma membrane and enter the cell. Phorbol esters are analogues of diacylglycerol (DAG). Why does the administration of phorbol and calcium ionophores mimic many effects of TCR engagement?

10. What effects on cell death would you expect to observe in mice carrying the following genetic modifications? Justify your answers.

a. Mice that are transgenic for BCL-2 and over-express this protein.
b. Mice in which caspase 8 has been knocked out.
c. Mice in which caspase 3 has been knocked out.

11. Several basic themes of signal transduction were identified and discussed in this chapter. What are these themes? Consider the signal-transduction processes of T-cell activation and provide an example for each of six of the seven themes discussed.