T Cell Development, Activation and Effector Functions

Chapter 9

WHAT'S IN THIS CHAPTER?

Don't fall before you're pushed.

English Proverb

A. T Cell Development

I. Comparison of B and T Cell Development

B and T cells are both lymphocytes and are derived from the same very early hematopoietic progenitors, but their development differs in several important ways:

(1) The thymus is required for the generation of the vast majority of mature peripheral T cells but not for that of mature B cells. T cells developing in thymus are called **thymocytes**.

(2) Naïve B cells are freshly produced at a virtually constant rate for the life of the individual. In contrast, once the involution of the thymus commences around puberty, new naïve T cell production is sharply reduced, and the maturing adult becomes increasingly dependent on the existing repertoire of T cells.

(3) MHC molecules are involved in the establishment of central tolerance of T cells but not B cells. The TCR on a thymocyte must not only be functional (be derived from productive rearrangements of the TCR genes) but must also recognize the host's MHC molecules (so that it can "see" pMHC). This requirement imposes an additional layer of selection on T cells that developing B cells do not experience.

(4) The TCR expressed on a T cell's surface is fixed for the life of the clone and cannot undergo the somatic hypermutation in response to activation that occurs following B cell activation.

(5) Whereas the vast majority of functional B cells result from a single developmental program, functional T cells can result from several different paths. A developing thymocyte may give rise to γδ T cells or αβ T cells, and among these, Th or Tc cells. Once activated by antigen, an αβ T cell clone can further differentiate into subsets that differ slightly in their effector functions. In addition, certain thymocytes have the capacity to differentiate into **regulatory T cells** that can control the responses of activated Th and Tc cells.

Regulatory T cells contribute to peripheral tolerance and are discussed in Chapter 10.

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NOTE: The complex pathways mediating lymphocyte development, activation and differentiation involve many genes that can become targets for mutations leading to primary immunodeficiencies (PIDs). PIDs are discussed in detail in Chapter 15.

II. Colonization of the Thymus

During the early embryonic development of a mammalian fetus, the thymus is empty of hematopoietic progenitors. The fetal thymus must be "colonized" or seeded with hematopoietic progenitors that subsequently proliferate and mature in the thymus into functional naïve T cells. As introduced in Chapter 2, T cells (like all hematopoietic cells) are derived from HSCs. Although HSCs are located in the bone marrow in an adolescent or adult individual, they are generated in the liver in a fetus. In either location, a proliferating HSC can differentiate into early progenitors, including the postulated MPPs and CLPs of the simplified model of hematopoiesis shown in Figure 2-4. These MPPs and CLPs leave the bone marrow or liver and enter the blood circulation. Many immunologists believe that circulating CLPs eventually give rise to a slightly more differentiated progenitor called the **NK/T precursor** that can generate NK cells and T cells but not B cells. When circulating NK/T precursors that express high levels of the chemokine receptors CCR9 and CCR7 exit the blood, they are drawn to the thymic endothelium by the presence of the ligands for these receptors, the chemokines CCL25 and CCL19 (respectively). Once a given NK/T precursor enters the thymus, it may eventually differentiate into αβ or γδ T cells, lymphoid DCs, or NK or NKT cells, depending on the cytokines and stromal cell ligands it encounters.

Development of γδ T, NK and NKT cells from NK/T precursors is described in Chapter 11.

The fetal thymus is colonized by NK/T precursors in a limited number of distinct waves that occur both before and after birth. The earliest prenatal waves migrate from the fetal liver, enter the fetal thymus and give rise only to γδ thymocytes. Subsequent waves of NK/T precursors entering the thymus just before birth and shortly thereafter give rise to both αβ and γδ thymocytes. However, after birth, those NK/T precursors destined to become T cells are more and more biased toward the αβ T cell lineage such that γδ T cells become a minor population. In addition, the bone marrow becomes the dominant site of generation of the NK/T precursors needed to replenish the thymus.

Age-dependent changes in the dominant site of hematopoiesis were illustrated in Figure 2-3.

Study of the developmental path of HSCs to mature T cells in mice has shown that the pattern is generally the same in fetal, neonatal, adolescent and adult animals but displays slower kinetics in adolescents and adults (**Table 9-1**). Importantly, the TdT enzyme responsible for much of the junctional diversity generated during TCR gene rearrangement is not fully active until shortly after birth. Thus, the repertoire of T cell specificities available in the neonate is significantly less diverse than in older individuals.

NOTE: Identifying the factors driving thymic colonization by NK/T precursors is of more than just academic interest. A patient that has undergone a **bone marrow transplant** (BMT) or a **hematopoietic stem cell transplant** (HSCT) in an attempt to treat disease depends on the ability of donor-derived CLPs (or another early progenitor) to generate NK/T precursors able to repopulate his/her thymus and generate mature T cells. On its own, this process can be quite slow, putting the patient at high risk for infection. Clinicians therefore use laboratory methods to manipulate the cells to be transplanted in an effort to accelerate thymic repopulation and T cell generation (see Ch. 17).

III. Thymocyte Maturation in the Thymus

Thymocytes at different developmental stages are morphologically very similar and so are usually distinguished by either their patterns of surface marker expression or by their TCR gene rearrangement status. These parameters have been used to divide thymocyte maturation into three broad phases: the **double negative** phase (DN, stages

TABLE 9-1 Comparison of Murine Thymocyte Development at Different Life Stages

Property	Fetus	Neonate	Post-Neonate*
Origin of NK/T precursors	Fetal liver	Fetal liver	Bone marrow
TCRs in the periphery	Only TCRγδ No TCRαβ	Majority TCRγδ Minority TCRαβ	Majority TCRαβ Minority TCRγδ
Kinetics of progression from NK/T precursors to mature T cells	Fast	Fast	Slow
TdT expression	None	Initiated	Fully active
T cell repertoire diversity	Limited	Limited	Fully diversified
Generation of thymocytes	Continuous	Continuous	Minimal after thymic involution

*Post-neonate includes adolescent and adult mice

DN1–DN4), in which thymocytes express neither CD4 nor CD8; the **double positive** (DP) phase, in which thymocytes express both CD4 and CD8; and the **single positive** (SP) phase, in which thymocytes express either CD4 or CD8 but not both (**Fig. 9-1**). Several selection processes occur during these transitions that remove non-functional and potentially autoreactive thymocytes (see later). Once SP thymocytes emerge from the thymus and enter the circulation and secondary lymphoid tissues, they are considered to be mature naïve $CD4^+$ or $CD8^+$ peripheral T cells.

DiGeorge syndrome is a PID in which thymus development is impaired (see Ch. 15).

i) The Thymic Environment

The development of thymocytes through the DN, DP and SP phases is totally dependent on the stromal cells that make up the thymic architecture (**Fig. 9-2**). As normal thymocytes mature, they pass through a succession of thymic microenvironments characterized by different mixes of stromal cell types. Among the most important of these stromal cells are *cortical thymic epithelial cells (cTECs), medullary thymic epithelial cells (mTECs), thymic DCs* and *thymic fibroblasts*. As discussed later, thymic DCs, cTECs and mTECs are vital for the establishment of T cell central tolerance during the DP phase. cTECs and mTECs also express cell surface ligands for *Notch1*, a cell fate protein expressed on the surface of thymocytes. Once Notch1 has bound to its ligand, the cytoplasmic domain of Notch1 interacts with transcription factors to promote T cell development while suppressing B cell development. Continued Notch1 signaling

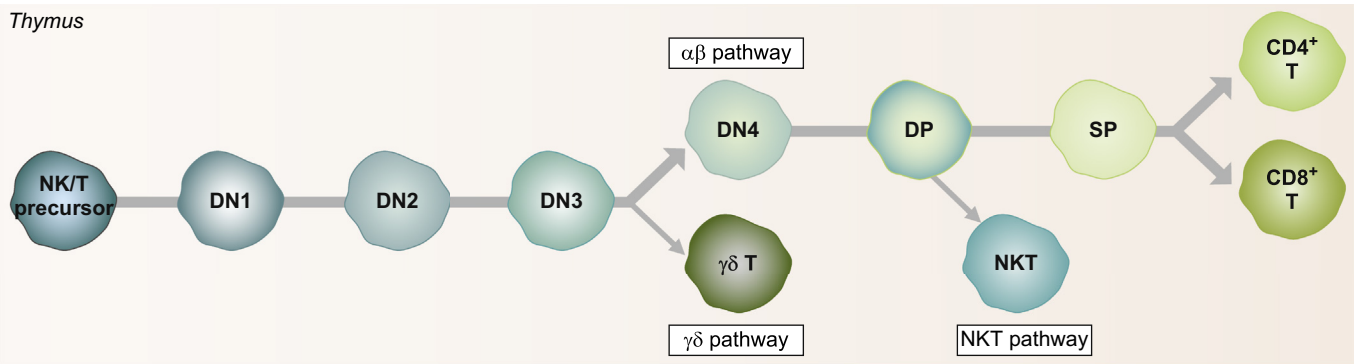

Fig. 9-1
Model of Thymocyte Maturation in the Thymus

This simplified pathway shows the generation of mature naïve αβ and γδ T cells and NKT cells from NK/T precursors through the double negative (DN), double positive (DP) and single positive (SP) stages of thymocyte development. Most DN3 thymocytes become αβ T cells, but some generate γδ T cells. Most DP thymocytes that survive various thymic selection processes become $CD4^+$ or $CD8^+$ SP T cells, but some generate NKT cells. The development of γδ T cells and NKT cells is discussed further in Chapter 11.

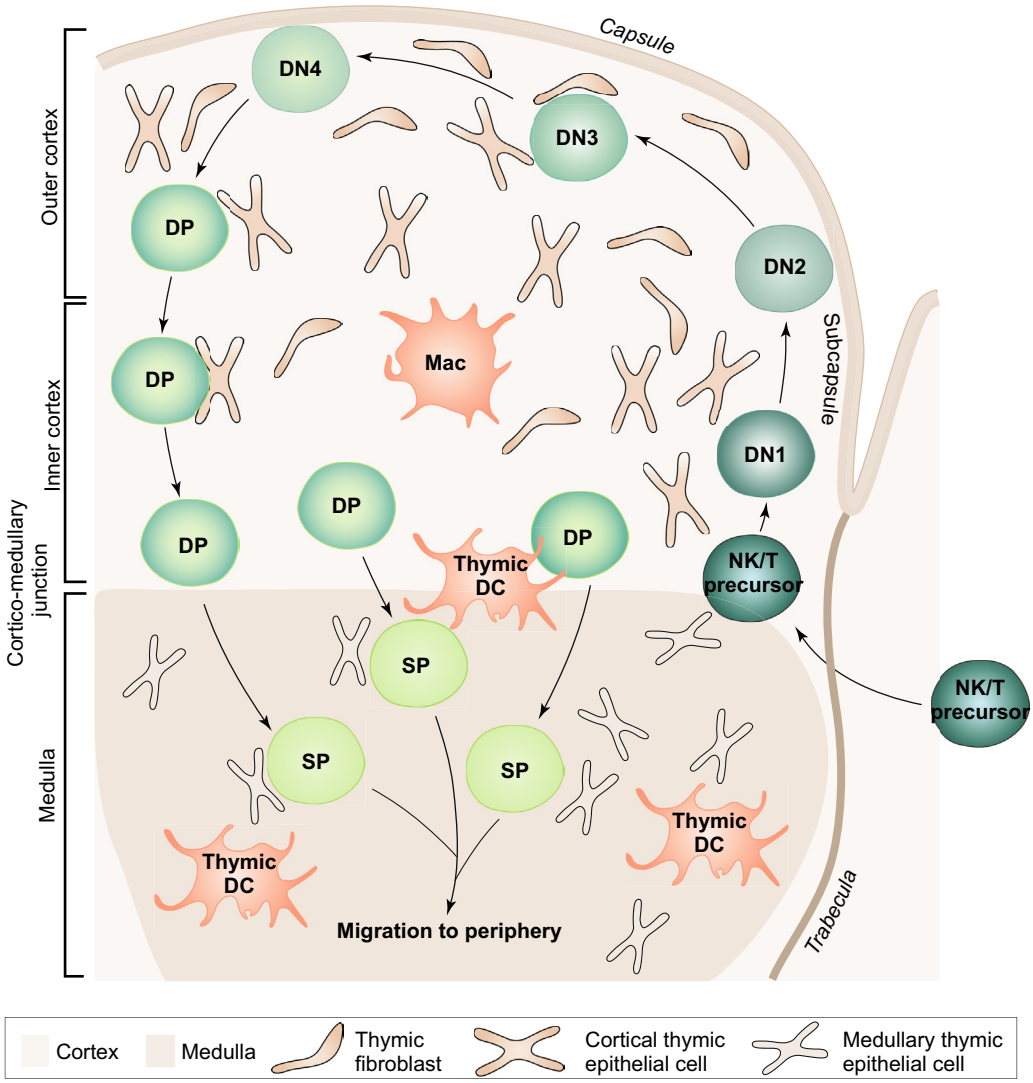

Fig. 9-2
Thymic Microenvironment and Location of Developing Thymocytes

NK/T precursors enter the thymus at its cortico-medullary junction. DN1 thymocytes are found in the inner cortex but soon migrate toward the outer cortex and become DN2 cells. DN3 thymocytes predominate in the outer cortex and make the transition to the DN4 stage in the subcapsular region. These cells then become DP thymocytes and migrate back down through the cortex to the cortico-medullary junction. Only SP thymocytes are found in the medulla. *[Adapted from Blackburn, C.C., & Manley, N.R. (2004).* Nature Reviews Immunology 4, *278–289.]*

is then required to sustain the survival of thymocytes until they pass through the DN stage. Thymic fibroblasts secrete components of the extracellular matrix (such as collagen) that create a scaffolding used to concentrate the cytokines crucial for thymocyte development. Other components secreted by thymic fibroblasts are involved in controlling the adhesion of thymocytes to stromal cells and thus may direct thymocyte migration through the thymus.

NOTE: In humans, a mutation of the transcription factor FoxN1 blocks TEC development. Without cTECs and mTECs, mature T cells cannot develop properly from thymocytes, rendering these individuals immunodeficient (see Ch. 15). Similarly, the *nude* mutant mouse strain has a defect in the development of thymic stromal cells and so lacks all mature T cells. This immunodeficient animal is used in many laboratory experiments in which immune system reconstitution is required.

TABLE 9-2 Comparison of Murine and Human Thymocyte Markers

Stage	Murine	Human
DN1	ckit$^+$ CD44$^+$CD25$^-$	CD34$^+$CD38$^-$CD1a$^-$
DN2	ckit$^+$ CD44$^+$CD25$^+$	CD34$^+$CD38$^+$CD1a$^-$
DN3	ckit$^-$ CD44$^-$CD25$^+$	CD34$^+$CD38$^+$CD1a$^+$
DN4	ckit$^-$ CD44$^-$CD25^{lo}	CD34$^-$CD38$^-$CD1a$^+$CD4$^+$
DP-TCRβ	CD4$^+$CD8$^+$	CD4$^+$CD8$^+$
DP-TCRαβ	CD4$^+$CD8$^+$	CD4$^+$CD8$^+$
SP-CD4	CD4$^+$CD8$^-$	CD4$^+$CD8$^-$
SP-CD8	CD4$^-$CD8$^+$	CD4$^-$CD8$^+$

ii) DN Phase

As mentioned above, the earliest thymocytes are said to be in the double negative or *DN phase* because they express neither CD4 nor CD8. These cells are also negative for TCR expression, cannot bind pMHC and do not carry out effector functions. Within the DN phase are four subsets of thymocytes labeled DN1–4. In mice, these subsets are distinguished from each other by their expression of the surface markers c-kit (a cytokine receptor), CD44 (an adhesion protein) and CD25 (the α chain of the IL-2 receptor). Note that IL-2 is not required for thymocyte development, and the function of CD25 in thymocytes is unknown. In humans, expression patterns of the markers CD34 (a putative adhesion protein), CD38 (an adhesion protein) and CD1a (an MHC-like protein) distinguish the DN1–4 thymocyte subsets. Major markers expressed by developing human and murine T cells are listed in **Table 9-2**, and additional molecules expressed during murine thymocyte development appear in **Figure 9-3**. The following sections discuss the better-studied developmental path of murine thymocytes.

a) DN1 Subset

Murine DN1 thymocytes express both c-kit and CD44 (but not CD25) and reside in the thymic cortex near its junction with the medulla (refer to **Fig. 9-2**). The TCR

	DN Phase				DP Phase		SP Phase	
Subset / Marker	DN1	DN2	DN3	DN4	TCRβ	TCRαβ	CD4$^+$	CD8$^+$
c-kit	+	+	–	–	–	–	–	–
CD44	+	+	–	–	–	–	–	–
CD25	–	+	+	Low	–	–	+	+
CD3	–	+	+	+	+	+	+	+
Rearranging TCR genes	–	–	*TCRB* *TCRG* *TCRD*	*TCRB*	*TCRA*	–	–	–
pTα	–	–	+	+	Low	–	–	–
RAG	–	–	+	Low	+	+	–	–
TdT	–	–	+	Low	+	+	–	–
TCR	–	–	–	–	–	+	+	+
CD4	–	–	–	Low	Med	+	+	–
CD8	–	–	–	Low	Med	+	–	+

Figure 9-3
Markers Characterizing the Phases of αβ T Cell Development in the Mouse

The expression status of various molecules can be used to monitor the phases of T cell development as a given thymocyte moves through each phase sequentially. pTα = pre-T alpha chain (see main text).

genes remain in germline configuration. DN1 thymocytes are small and closely packed together among the cTECs. cTECs supply *stem cell factor (SCF)* that binds to c-kit on DN1 cells and delivers a survival signal. Without c-kit signaling, the maturation process ceases and the DN1 cells die. The transcription factor GATA-3 is also vital for the generation of DN1 thymocytes from thymic NK/T precursors.

b) DN2 Subset

Murine DN2 thymocytes express CD25 as well as c-kit and CD44 and are sometimes known as *pro-T cells* (progenitor T cells). These thymocytes start to migrate toward the subcapsule of the thymus and thus are present primarily in the outer cortex. The TCR genes remain in germline configuration. DN2 thymocytes commence expression of the CD3 chains, but it is unclear whether these proteins have a signaling function at this stage. Under the influence of IL-7 and SCF, DN2 thymocytes start to proliferate rapidly.

c) DN3 Subset

Murine DN3 thymocytes lose their expression of c-kit and CD44 but continue to express CD25. These cells stop proliferating and remain in the outer cortex. The DN3 stage is critical in T cell development because five key events occur: (1) DN3 thymocytes become restricted to the T lineage and eventually generate mature αβ and γδ T cells. (2) The *TCRG*, *TCRD* and *TCRB* loci commence V(D)J recombination with concomitant upregulation of RAG and TdT. (3) DN3 thymocytes that eventually generate mature αβ T cells express a functional **pre-TCR** complex that allows them to determine if a functional TCRβ chain has been produced. (4) Successful rearrangement at the *TCRB* locus induces the cessation of further rearrangements at the *TCRG* and *TCRD* loci in these cells. (5) These DN3 thymocytes become *early pre-T cells* that are fully committed to the αβ T cell lineage and express a diverse repertoire of TCRβ chains.

Signals that commit DN3 thymocytes to the γδ T cell lineage are discussed in Chapter 11.

In DN3 thymocytes that eventually generate αβ T cells, the *TCRB* locus is the first to undergo V(D)J recombination. Some rearrangement of the *TCRG* loci may also occur, but functional chains are not produced. As introduced in Chapter 8, the productivity of a given rearranged TCRβ gene in a DN3 thymocyte is tested by the synthesis of the candidate TCRβ chain and the formation of a pre-TCR analogous to the pre-BCR structure in pre-B cells. The pre-TCR counterpart of the surrogate light chain in the pre-BCR is the **pre-T alpha chain** (pTα). pTα is an invariant protein first expressed in DN3 thymocytes that develop into αβ T cells; there is no equivalent in DN3 thymocytes that generate γδ T cells. pTα functions as a "surrogate TCRα chain" and brings the newly translated TCRβ chain (and the CD3 signaling chains) to the thymocyte membrane to form a pre-TCR complex. The pre-TCR complex acts as a sensor so that if the TCRβ chain is functional, the cell receives a survival/differentiation signal that involves Notch1 signaling. The cell also starts to proliferate vigorously, generating a clone of DN4 thymocytes that will eventually become αβ T cells. The process of testing newly produced TCRβ chains is called **β-selection**, and cells that survive β-selection are said to have passed the **pre-TCR checkpoint** (**Fig. 9-4**). The successful rearrangement of the *TCRB* gene on one chromosome signals to the cell to suppress V(D)J rearrangement of the *TCRB* locus on the other chromosome and rearrangement of both *TCRG* loci. If *TCRB* rearrangement on both chromosomes has been unsuccessful, the cell neither attempts to rearrange its *TCRA* genes nor becomes a γδ T cell; instead, it dies by apoptosis. Indeed, only 10% of DN3 thymocytes successfully rearrange their TCRβ genes, are β-selected and enter the cell cycle. β-selection is thus directly linked to the proliferation of thymocytes that can proceed further in maturation.

d) DN4 Subset

Murine DN4 thymocytes, also called *late pre-T cells*, are slightly larger in size than DN3 cells and are concentrated in the subcapsular region of the thymic cortex (refer to **Fig. 9-2**). DN4 cells contain a functionally rearranged TCRβ gene; downregulate their expression of CD25, RAG and TdT; and start to express very low levels of CD4 and CD8.

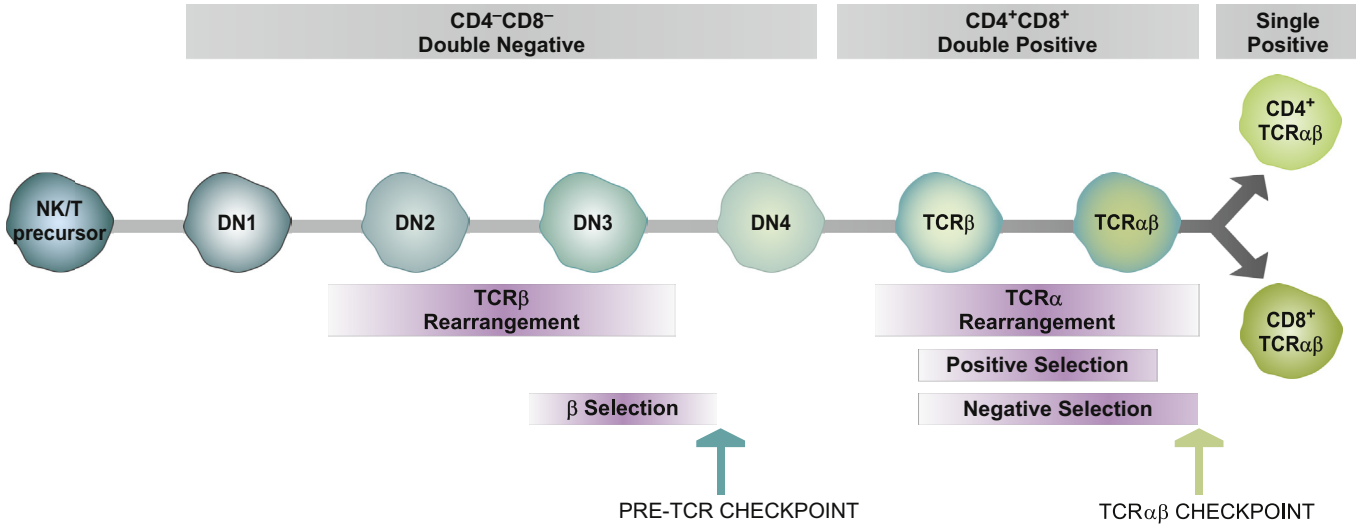

Fig. 9-4
Checkpoints of T Cell Development

Under the influence of particular cytokines and intercellular contacts, NK/T precursors pass through the DN1–DN4 stages of the double negative phase ($CD4^-CD8^-$). The pre-TCR checkpoint marks the end of this phase and entry into the double positive ($CD4^+CD8^+$) phase. After passing through the TCRαβ checkpoint, the developing thymocytes enter the single positive phase and become mature $CD4^+CD8^-$ or $CD4^-CD8^+$ T cells. *[Adapted from Yeung, R.S., Ohashi, P., & Mak, T.W. (2007). T cell development. In Ochs, H.D., Smith, C.I., & Puck, J.M., Eds.,* Primary Immunodeficiency Diseases. A Molecular and Genetic Approach*, 2nd ed. Oxford University Press Inc., New York.]*

NOTE: A group of PIDs characterized by **severe combined immunodeficiency** (SCID) result from a failure in the V(D)J recombination required in both B cells and T cells. *Alymphocytosis* results from null mutations of either the RAG-1 or RAG-2 genes, while *Omenn syndrome* is caused by amino acid substitution mutations in the RAG genes that reduce their activity to 1–25% of normal. *Artemis SCID* is caused by mutations of the Artemis gene, which is important for both V(D)J recombination and DNA repair (see Ch. 15).

iii) The DP Phase

In both humans and mice, the DP phase of αβ T cell development is dominated by the thymic selection processes that shape the mature αβ T cell repertoire. CD4 and CD8 expression levels are steadily upregulated, and these coreceptors play increasingly important roles in directing thymocyte development. As they mature, DP thymocytes move from the subcapsular region through the outer cortex and back through the inner cortex toward the medulla (refer to **Fig. 9-2**).

a) TCRαβ Pool Expansion and TCRA Locus Rearrangement

Early DP thymocytes receive signals through their pre-TCRs that drive their rapid proliferation. These signals appear to depend upon the assembly of pre-TCR itself and not upon interaction with a specific ligand. RAG and TdT expression resume, and V(D)J recombination in both *TCRA* loci commences, resulting in the deletion of the *TCRD* loci (refer to Ch. 8). With the production of the first TCRα chains, pTα expression is gradually downregulated. Newly synthesized (but untested) TCRα chains combine with the proven TCRβ chains to form complete TCRαβ heterodimers (which may or may not be functional). *TCRA* rearrangement continues on both chromosomes until *positive selection* (see below) delivers a survival signal to those thymocytes with functional TCRs that recognize the host's MHC molecules with moderate affinity.

b) Thymic Selection and the Establishment of Central T Cell Tolerance

The establishment of central T cell tolerance requires that thymocytes with TCRs that recognize self antigen be eliminated before they leave the thymus. Central T cell

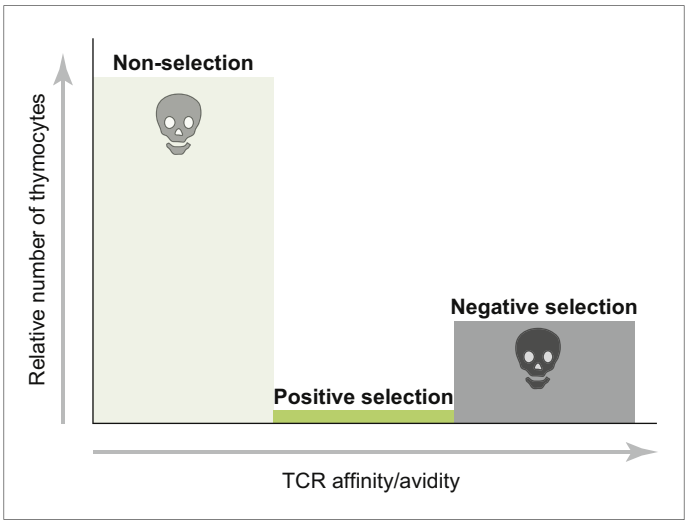

Fig. 9-5
Affinity/Avidity Model of Thymic Selection

The overall affinity/avidity of the interaction between a developing thymocyte's TCR and self pMHC results in intracellular signaling whose nature and level determine the thymocyte's fate. Most thymocytes bear TCRs that fail to bind to self pMHC or do so very weakly; these cells are non-selected and die by apoptosis. Thymocytes with TCRs that bind strongly to self pMHC are negatively selected and also die. Thymocytes with TCRs interacting with self pMHC with moderate affinity/avidity are positively selected and survive. *[Adapted from Yeung, R.S., Ohashi, P., & Mak, T.W. (2007). T cell development. In Ochs, H.D., Smith, C.I., & Puck, J.M., Eds.,* Primary Immunodeficiency Diseases. A Molecular and Genetic Approach, *2nd ed. Oxford University Press Inc., New York.]*

tolerance is established by the processes of **non-selection** (or "neglect"), **positive selection** and **negative selection**. Mature T cells must recognize both MHC and peptide simultaneously to mount an immune response, meaning that the TCRs of a DP thymocyte must bind to the host's MHC molecules (self MHC) with at least moderate affinity. T cells that fail to produce functional TCRs, or produce TCRs that have no affinity for self MHC, cannot be activated in the periphery and thus are useless with respect to defending the host. These cells, which can number as high as 80% of developing thymocytes, are therefore "non-selected" and undergo apoptosis (**Fig. 9-5**). Negative selection removes from the T cell repertoire DP cells whose TCRs bind strongly to pMHCs involving self peptides bound to self MHC. The activation of such T cells in the periphery could spark a damaging autoimmune response in the host. Strong binding of a TCR to self pMHC triggers signaling in the thymocyte that induces its apoptosis. It is estimated that almost 20% of developing thymocytes are "deleted" from the thymus in this way. Positive selection preserves the 1–2% of developing thymocytes whose TCRs recognize self pMHCs neither too strongly nor too weakly. It is a very fine line that separates ligands that are bound to a given TCR too avidly from those that are bound just tightly enough, but only in the second case is a survival signal delivered that allows positively selected thymocytes to proliferate and mature further. Once released to the periphery, it is this small population of new T cells that is most likely to bear TCRs recognizing non-self peptide on self MHC, which is exactly what an APC or target cell will present when an immune response is required. The few remaining potentially autoreactive clones that escape thymic deletion, and clones that recognize self antigens that emerge only later in life (after thymic involution), are neutralized by the mechanisms of peripheral tolerance (see Ch. 10).

Immunologists continue to debate exactly when and where in the thymus each type of selection occurs. Some maintain that positive selection takes place primarily in the cortex and is mediated mainly by cTECs, whereas negative selection occurs later when the DP cells approach the medulla and is mediated by mTECs. Others believe that both positive and negative selection can occur in either the cortex or the medulla, and that these processes are temporally independent. What is now clear is that, regardless

of timing, mTECs have a specialized role to play during negative selection that establishes central tolerance to *tissue-specific antigens*. Tissue-specific antigens are self antigens that are normally expressed only in particular host tissues, such as insulin in the pancreas and saliva proteins in the salivary glands. mTECs express a transcription factor called AIRE (*autoimmune regulator*) that allows the expression of many of these tissue-specific antigens in the thymus, so that thymocytes bearing TCRs that recognize these antigens can be deleted. Once transcribed and translated within an mTEC, a given tissue-specific antigen may be processed through the endogenous pathway and presented on MHC class I, or processed via autophagy and presented on MHC class II. In either case, a DP thymocyte bearing a TCR specific for this antigen is negatively selected.

NOTE: Both humans and mice with a genetic deficiency of AIRE show symptoms of autoimmune disease because, without this transcription factor, tissue-specific antigens are not expressed in the thymus. Thymocytes capable of responding to these tissue-specific antigens are therefore not deleted and escape to the periphery where they can mount autoreactive responses against host tissues expressing these antigens (see Ch.19). Inhibition of autophagy in the thymus has also been associated with autoimmunity in mouse models.

c) Nature of Signaling during Thymic Selection

The intracellular signals received by thymocytes during non-selection, positive selection and negative selection depend on the overall affinity/avidity of the interaction between the TCRs of a DP thymocyte and the pMHCs presented by the thymic APC it encounters. The level of intracellular signaling triggered by this interaction is also influenced by the level of aggregation of the TCRs and coreceptor molecules, by the type of thymic APC presenting the pMHC (thymic DC, mTEC or cTEC), and by the costimulatory molecules expressed by the thymic APC. As noted earlier, the majority of DP thymocytes are non-selected because their TCRs cannot interact at all with the pMHCs presented by thymic APCs. Some of these thymocytes have out-of-frame rearrangements of the Vα and Jα TCR gene segments such that no TCRα protein can be produced. Other thymocytes have undergone successful V(D)J recombination, but the TCR produced has a conformation that simply cannot bind to self MHC with any level of affinity. In both cases, TCR signaling is not triggered, and these DP cells proceed down a default path of apoptosis and die in the cortex.

When pMHC co-ligates a TCR and its coreceptor, molecules of Lck kinase associated with the cytoplasmic tail of the coreceptor begin to phosphorylate the ITAMs in the CD3 chains. A negatively selecting pMHC presented by a thymic APC (often an mTEC) binds to the TCR and coreceptor for a longer period of time than a positively selecting pMHC (often presented by a cTEC), enabling complete CD3 ITAM phosphorylation. These phosphorylated ITAMs in turn recruit molecules of ZAP70 kinase, which participates in signaling driving new gene expression. The amount of ZAP70 that accumulates around the base of the TCR complex appears to dictate the direction of selection: the binding of negatively selecting pMHCs results in the recruitment of three times more ZAP70 molecules to the TCR than does the binding of a positively selecting pMHC. The sum total of all the signaling downstream of a TCR bound to a negatively selecting pMHC results in the expression of pro-apoptotic genes that induce cell death. In contrast, the signaling triggered by TCR engagement by a positively selecting pMHC is sufficient to induce the transcription of anti-apoptotic genes that rescue the thymocyte from death. While several molecular events have been identified as contributing to positive selection signaling, how they all fit together remains unclear. Ca^{2+} flux and the activity of Erk kinase are extremely important for positive selection, but it is not understood how they are regulated by the weak binding of a DP thymocyte's TCR to the self pMHC of a thymic APC. A relatively recently discovered molecule important for positive selection is Themis ("thymocyte expressed molecule involved in selection"). Themis is expressed in DN4 thymocytes and early DP thymocytes and is required for passage of a thymocyte through the *TCRαβ checkpoint*

(see next section). Themis is rapidly phosphorylated after TCR engagement and is downregulated after positive selection, but its precise molecular function is unknown.

d) The TCRαβ Checkpoint

DP thymocytes that have survived the gauntlet of thymic selection have passed the second developmental checkpoint, the **TCRαβ checkpoint** (refer to **Fig. 9-4**). These positively selected DP cells express a fully functional TCRαβ and both CD4 and CD8, and can thus interact with both MHC class I and class II. It is these thymocytes that proceed to the final phase of αβ T cell development. As described in Chapter 11, it is also these cells that will eventually give rise to NKT cells.

iv) The SP Phase

The SP phase is entered when the class of MHC recognized by a positively selected DP thymocyte becomes fixed by the loss of expression of either CD4 or CD8. This cell and its immediate progeny will thus be either $CD4^+$ or $CD8^+$ SP thymocytes. Which coreceptor is lost and which is retained on the thymocyte surface is determined by complex intracellular signaling pathways and the class of MHC molecule that has participated in the positive selection of the DP thymocyte. As described in Chapter 8, CD4 and CD8 molecules bind to specific regions (outside the TCR binding site) on MHC class II and MHC class I molecules, respectively. If the TCR of the DP thymocyte has bound to a peptide-MHC class I complex presented by the selecting thymic APC, there is an interaction between the MHC molecule and the CD8 coreceptor that causes CD8 expression to be retained and CD4 expression to be lost. Conversely, if the DP thymocyte's TCR has interacted with a peptide-MHC class II complex, an interaction occurs between the MHC molecule and CD4 coreceptor such that CD4 expression is retained while CD8 expression is lost. Thus, the descendants of SP $CD4^+$ thymocytes will bind to pMHCs containing MHC class II, and descendants of SP $CD8^+$ thymocytes will bind pMHCs containing MHC class I. We stress that this discrimination is not due to differences in the type of TCR expressed by $CD4^+$ and $CD8^+$ T cells; all TCRs expressed by all αβ T cells are derived from the same pool of TCRα genes and TCRβ genes.

In addition to TCR-pMHC interaction, there are likely many more downstream intracellular signaling events involved in determining CD4/CD8 lineage commitment. For example, there is some evidence that positively selected DP thymocytes expressing higher levels of ZAP70 become $CD8^+$ SP thymocytes, whereas those with lower ZAP70 levels become $CD4^+$ SP thymocytes. In addition, activation of a transcription factor called *cKrox* may promote $CD4^+$ SP development at the expense of $CD8^+$ SP cells. Other studies have suggested that cytokines within the thymus, particularly IL-7, may nudge DP thymocytes to commit to the $CD8^+$ SP lineage.

Once committed, both $CD4^+$ and $CD8^+$ SP thymocytes loiter in the medulla of the thymus for a short time (2–3 days in the mouse) before they receive a final proliferative signal and expand their numbers. These progeny exit the thymus into the blood and travel to the secondary lymphoid organs, taking up residence as fully functional mature $CD4^+$ or $CD8^+$ T cells. They survive in these locations (in the apparent absence of significant antigenic stimulation) for at least 5–7 weeks, and are poised to react upon meeting their specific antigens. For reasons that are not yet understood, the eventual effector function acquired by a T cell clone is largely dependent on which coreceptor it expresses, such that the vast majority of $CD4^+$ SP thymocytes differentiate into mature naïve Th cells and $CD8^+$ SP thymocytes usually differentiate into mature naïve Tc cells.

B. T Cell Activation

Like B cells, the complete activation of naïve T cells generally requires three signals: (1) the engagement of the antigen receptor by antigen, (2) costimulation, and (3) the receipt of cytokines. However, these signals differ slightly between B and T cell activation, and between naïve Th and Tc cell activation. Additional differences in the activation of effector and memory T cells also exist and are addressed in Section C of this chapter.

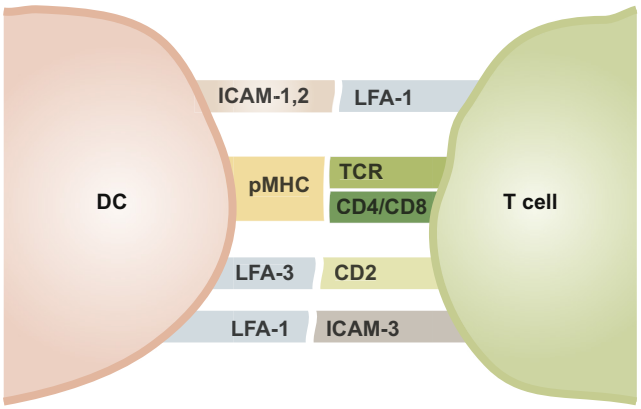

Fig. 9-6
Important Adhesion Contacts between Human T Cells and DCs

In addition to the interaction between the pMHC displayed by a mature DC and the TCR complex plus coreceptor molecule present on a naïve T cell surface, binding between the indicated pairs of adhesion receptors and counter receptors allows the T cell enough time to determine whether its TCR recognizes the pMHC.

I. Meeting of Naïve T Cells and DCs

The activation of most naïve T cells takes place in the paracortex of the lymph nodes, the secondary lymphoid organs where antigen-loaded mature DCs congregate and through which naïve T cells recirculate. As described in Chapter 7, immature migratory and lymphoid-resident DCs are experts at capturing entities from the surrounding microenvironment. If the host is experiencing infection or inflammation, pro-inflammatory cytokines and DAMPs/PAMPs that engage the PRRs of these DCs will be present such that these cells are induced to mature. Different pathogens will influence the panel of cytokines the maturing DC will eventually produce, and thus the direction of T cell differentiation (see later). A maturing migratory DC enters a lymph node via an afferent lymphatic vessel and settles in the paracortical region surrounding the HEVs within the node. Maturing lymphoid-resident DCs are already in this location. In both cases, the mature DCs process their captured antigens and display antigenic peptides derived from them on MHC class II via exogenous processing and on MHC class I via cross-presentation. If a DC has become infected by the pathogen, intracellular antigens may also be processed via the endogenous pathway and presented on MHC class I or displayed on MHC class II via autophagy.

Meanwhile, as described in Chapter 2, naïve Th and Tc cells are recirculating in the blood and throughout the secondary lymphoid tissues. In most cases, a naïve T cell enters the node via its HEVs and inspects the pMHCs displayed by the mature DCs in the immediate vicinity of these vessels. The T cell "crawls" slowly over the surface of a DC in a process facilitated by several adhesion molecule pairs (**Fig. 9-6**). These molecules loosely hold the T cell and DC together so that the fit of a particular pMHC in the TCR's binding site can be evaluated. pMHCs that are bound with sufficient affinity/avidity by the TCR have the potential to activate the T cell.

Look back at Figures 7-3 and 2-17 for illustrations of DC and lymphocyte migration, respectively.

NOTE: The crawling of a T cell over a DC's surface has been visualized in a lymph node using live tissue imaging based on two-photon laser microscopy. To see an example, you can visit the following website: https://www.youtube.com/watch?v=PsOZkfj-DTA&feature.

II. Signal 1

Signal 1 is delivered when specific pMHCs displayed on a DC surface bind to multiple copies of a TCR expressed on a naïve Th or Tc cell surface (**Fig. 9-7**). The engagement of a TCR by pMHC likely leads to a conformational change of its associated CD3 chains that allows the phosphorylation of the CD3 ITAMs by molecules of Lck kinase associated with the CD4 and CD8 cytoplasmic domains. This aggregation of pMHC-bound TCRs coupled with the conformational shift in CD3 chains may deflect large molecules

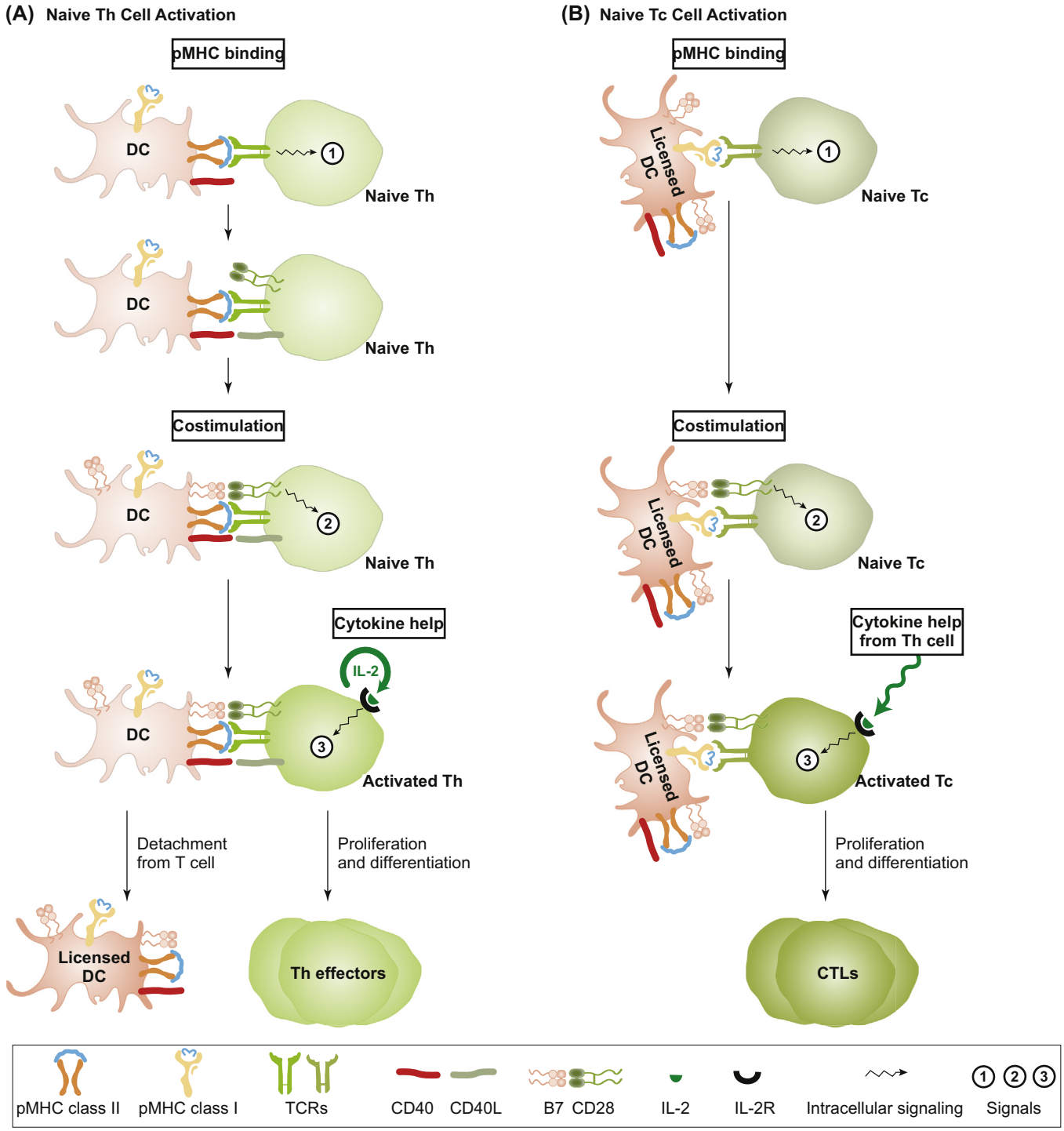

Fig. 9-7
Three Signal Model of Naïve Th and Tc Cell Activation

(A) For naïve Th cells, the binding of the TCR to antigenic peptide displayed on MHC class II by a mature DC delivers signal 1 to the Th nucleus and also upregulates CD40L on the Th cell surface. The binding of this CD40L to CD40 on the DC upregulates the latter's expression of B7 molecules. Signal 2 to the Th nucleus is delivered when these B7 molecules interact with costimulatory CD28 molecules on the Th cell. Expression of cytokine receptors is upregulated, and the Th cell commences production of IL-2. Signal 3 is delivered when the Th cell's IL-2 receptors are engaged by IL-2. The DC, which is now considered to be "licensed" due to its upregulated B7 expression, detaches from the Th cell. The activated Th cell proliferates and generates daughter cells that differentiate into Th effector cells. **(B)** A naïve Tc cell receives signal 1 when its TCR binds to peptide presented on MHC class I expressed by a licensed DC. Costimulation is mediated even in the absence of CD40/CD40L binding because the B7 already expressed on the licensed DC binds to the upregulated CD28 on the Tc cell. Signal 2 is thus delivered to the Tc cell nucleus and results in upregulated IL-2R expression. Signal 3 is delivered when IL-2 produced by a Th cell engages the Tc cell's IL-2Rs. The Tc cell detaches from the licensed DC, proliferates and generates daughter cells that differentiate into effector CTLs. For simplicity, the CD4/CD8 coreceptors and the aggregation of TCRs necessary for T cell activation are not shown.

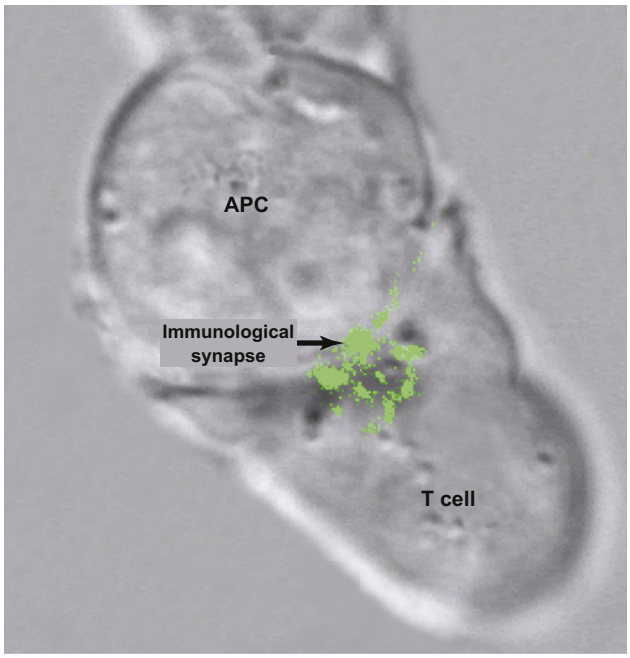

Plate 9-1
The Immunological Synapse

Green dots indicate points of contact at the interface between an APC and a T cell. In the case of naïve T cell activation, the APC is a DC. *[Reproduced by permission of Vincent Das and Andres Alcover, Institut Pasteur.]*

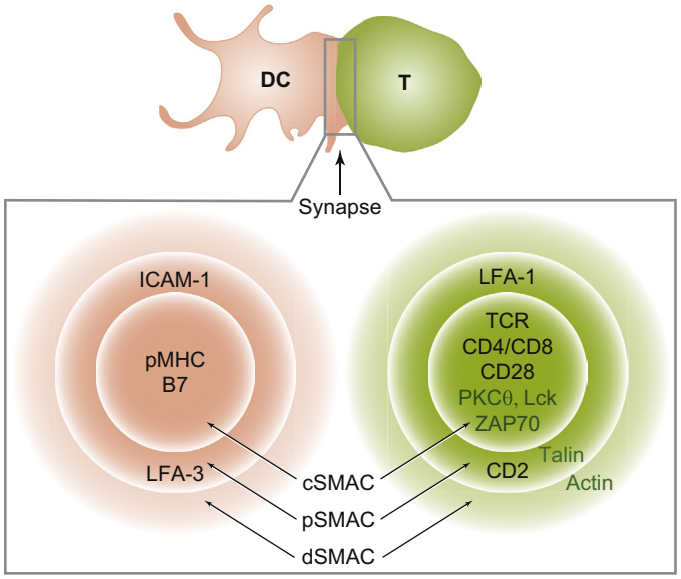

Fig. 9-8
The Supramolecular Activation Complex (SMAC) in the Immunological Synapse

The expansion box shows a cross-sectional view of the three layers of the SMAC (central, peripheral and distal) at the interface between a DC and a naïve T cell. Cell surface molecules are shown in black, whereas intracellular molecules are shown in gray.

(such as CD45 phosphatase) that normally inhibit TCR signaling in the absence of specific pMHC. Additional intracellular signaling enzymes are then recruited to the cytoplasmic tails of the CD4 or CD8 coreceptor and the CD3 chains. Together, these enzymes mediate a cascade of chemical reactions that leads to the activation of several other enzymes. When this activation cascade occurs for multiple TCRs, the T cell receives signal 1.

Because the affinity of binding between a given pMHC and a TCR is relatively low (has a high "off" rate), a single pMHC does not engage a single TCR long enough to achieve complete activation of a naïve T cell. Neither is a transient interaction between a few pMHC–TCR pairs sufficient. Sustained interaction between the naïve T cell and DC for several hours is needed to properly trigger the intracellular signaling pathways within the T cell that lead to the activation of the nuclear transcription factors necessary for new gene transcription. The TCRs and pMHCs required for sustained signaling are gathered together by the formation of an **immunological synapse** at the interface between the T cell and DC (**Plate 9-1**).

Both cells undergo rearrangements of their actin cytoskeletons that are induced by the initial TCR–pMHC binding. The T cell also undergoes polarization, in that its *microtubule organizing center (MTOC)* and Golgi apparatus move toward the site of contact with the DC. A parallel reorganization of these cytoplasmic organelles may also be occurring in the DC. In the T cell at least, these alterations result in the formation of three concentric rings, each containing various signaling, adhesion and cytoskeletal molecules that cluster around the TCR–pMHC pairs (**Fig. 9-8**). The inner ring is called the **central supramolecular activation cluster (cSMAC)** and is composed mainly of the aggregated TCRs and costimulatory molecules. The middle ring is called the **peripheral supramolecular activation cluster (pSMAC)** and contains the signaling adaptor talin as well as large numbers of integrins and other adhesion molecules. The outer ring, called the **distal supramolecular activation cluster**, mainly contains actin-based cytoskeletal structures as well as large proteins excluded from the inner area of TCR aggregation. On the side of the T cell opposite from the immunological synapse is the *distal pole complex*, which is thought to actively sequester negative signaling regulators away from the site of TCR aggregation.

III. Signal 2

In most cases, the engagement of TCRs by pMHCs is not sufficient to fully activate a naïve Th or Tc cell, and signal 2 in the form of costimulatory signaling is required. Occasionally, a Tc cell will encounter a pMHC (usually derived from a virus) that delivers such a strong signal 1 that costimulation is not required; this response is then independent of both costimulation and Th cell help.

In the case of Th cells, the receipt of signal 1 leads to the upregulation of the important costimulatory molecule *CD28* on the Th cell's surface (refer to **Fig. 9-7A**). However, in order for CD28 to convey signal 2 to the Th cell nucleus, it must bind to its ligand **B7** on the surface of the DC presenting the activating pMHC. When the immunological synapse first starts to form, the mature DC involved does not express optimal levels of B7. A critical consequence of the delivery of signal 1 to a Th cell and the initial binding of CD28 to B7 is the upregulation of the transmembrane protein *CD40 ligand* (CD40L) on the Th cell surface. Once CD40L on the Th cell engages CD40 expressed by the DC, the DC greatly increases its expression of B7 and thus its binding to CD28 on the Th cell. As a result, a vigorous signal 2 is delivered that enhances the activatory intracellular signaling that is occurring within the T cell as a result of signal 1.

Although they upregulate CD28, most Tc cells do not express CD40L even after receiving signal 1. Thus, Tc cells cannot induce a DC to initiate CD40 signaling and upregulate B7 expression. Instead, Tc cells rely on CD28 engagement resulting from interaction with a DC that *already* expresses B7 due to a previous interaction with an antigen-activated Th cell. Some immunologists say that these DCs have been "licensed" for Tc activation (refer to **Fig. 9-7B**). This licensing of DCs by Th cells is one component of the T cell help provided by Th cells for Tc responses.

B7 actually refers to two closely related costimulatory proteins: B7-1 (CD80) and B7-2 (CD86).

For both Tc and Th cells, CD28 signaling lowers the T cell activation threshold necessary to activate new gene transcription in the T cell and push it to proliferate and differentiate. In the absence of CD28 costimulation, naïve T cells are anergized instead of activated and fail to respond to pMHC (see Ch. 10). Costimulation via CD28 has several important molecular effects: (1) IL-2R expression is induced on the T cell surface, allowing the cell to receive signal 3. (2) Th cells start to secrete large quantities of IL-2, as well as other important cytokines and chemokines. (3) The expression or upregulation of additional costimulatory and regulatory molecules is induced in both Th and Tc cells. (4) Intracellular signaling supporting T cell survival, proliferation, and metabolism is promoted.

NOTE: The potentially destructive power of T cells must be tightly controlled to ensure it is applied only where and when it is necessary. The TCR and costimulatory signaling pathways are therefore subject to negative regulation at multiple steps. The two most important negative regulators of T cell activation are *PD-1* (programmed death-1) and *CTLA-4* (cytotoxic T lymphocyte associated molecule 4). While PD-1 is expressed by T cells, B cells and some DCs, CTLA-4 expression is exclusive to T cells. PD-1 expression on a T cell is induced within hours of its activation, while expression of the PD-1 ligands PD-L1 and PD-L2 on the DC surface is induced by inflammatory cytokines. When PD-1 and the TCR are both engaged, PD-1 transmits an inhibitory signal that shuts down early steps of the TCR signaling pathway. In contrast to PD-1, CTLA-4 is not expressed on the T cell surface until 1–2 days after activation is initiated by TCR/pMHC interaction, giving the adaptive response time to eliminate the threat before T cell activation is damped down. CTLA-4 competes with CD28 for binding to the B7 costimulatory ligands. Because CTLA-4 has a much higher affinity for B7 proteins than does CD28, CTLA-4 displaces CD28 and recruits inhibitory molecules to the TCR complex.

IV. Signal 3

A naïve Th or Tc cell that has received signals 1 and 2 upregulates the receptors (particularly IL-2R) which permit it to receive signal 3 in the form of cytokines, chemokines and growth factors (refer to **Fig. 9-7**). Activated Th cells produce many of the cytokines that can bind to these newly expressed receptors, the chief among them being IL-2. IL-2 is believed to deliver the most important signal for the proliferation of newly activated naïve T cells. Although a Th cell on its own can make sufficient IL-2 to meet its requirements (i.e., carries out *autocrine* IL-2 production), a Tc cell usually cannot. Thus, another component of T cell help provided to Tc cells by Th cells is the production of IL-2 (and possibly other cytokines) necessary for Tc proliferation.

A naïve T cell that achieves activation proliferates and generates daughter T cells that differentiate into effector T cells. Effector T cells differ from naïve T cells in several important ways besides function, including tissue of residence, preferred APC, costimulatory requirements, duration of TCR signaling needed for activation, dominant metabolic pathways, rate of cell division, sensitivity to cell death mechanisms, and life span. The next two sections address the properties of Th effector cells and CTLs.

C. Th Cell Differentiation and Effector Function

I. Overview

Once a naïve Th cell is fully activated, it starts to produce copious amounts of IL-2 and proliferates vigorously. The progeny generated are called *Th0* cells. About 48–72 hours after the original antigenic stimulation of the naïve Th cell, these Th0 cells terminally differentiate into various subsets of resting effector Th cells. Of these subsets, *Th1* cells, *Th2* cells and *Th17* cells are arguably the most important. Other Th effector subsets include *Th9* cells, *Th22* cells and follicular Th (*fTh*) cells. Th0 cells can also generate *induced regulatory T cells,* which function in peripheral tolerance and so are introduced here and discussed further in Chapter 10.

The type of effector Th subset generated from a proliferating Th0 cell is determined by (1) the cytokines and other factors present in the immediate microenvironment and (2) the nature of the DC by which the original naïve Th cell was activated. Different pathogens supply PAMPs that bind to different PRRs, causing the DCs expressing these receptors to mature into subtly different subsets. These DCs then secrete different panels of cytokines and deliver various intercellular signals that help to direct Th differentiation such that the Th effectors best-suited for eliminating the pathogen are produced. Some Th effector subsets secrete cytokines that facilitate effector functions

specialized for the disposal of intracellular invaders, whereas the cytokines produced by other Th effector subsets promote effector functions designed to counter extracellular threats. In any case, following their generation and differentiation in a secondary lymphoid tissue, most resting Th effectors migrate back to the site of inflammation containing the antigen that sparked the activation of the original naïve Th cell. In this site, presentation of the same antigenic pMHCs by an APC (which can be a DC, macrophage or B cell at this stage) activates these Th effectors and causes them to take action in the form of secreting their subset-specific panels of cytokines.

NOTE: After newly produced naïve T cells migrate from the bone marrow and enter the secondary lymphoid tissues, they adopt a resting state in which the metabolic demands of perusing APCs for foreign pMHCs are met by ATP generated via mitochondrial oxidative phosphorylation of glucose and glucosamine. However, when the naïve T cell is activated and undergoes the proliferation and differentiation needed to generate effector T cells, an entirely new set of metabolic requirements arises that is met by a shift from oxidative phosphorylation to glycolysis. Glycolysis is much more efficient at providing the energy needed for the synthesis of new proteins, sugars, lipids and nucleotides, and for the execution of T cell effector functions. At the conclusion of the primary response, surviving effector T cells and memory T cells revert to quiescence, decrease glycolysis, and resume energy generation via oxidative phosphorylation.

II. Differentiation of Th Effector Cell Subsets

Th cell differentiation has been best studied in mice. Many of the cytokines and transcription factors involved in human Th cell differentiation overlap.

The differentiation paths of the most important Th effector cell subsets identified to date are summarized in **Figure 9-9**, including relevant cytokines and transcription factors. It should be noted, however, that accumulating evidence suggests that these differentiation paths are not fixed for the life of a particular T cell clone, and that one type of effector can become another type if circumstances change and its transcriptional programs shift in response.

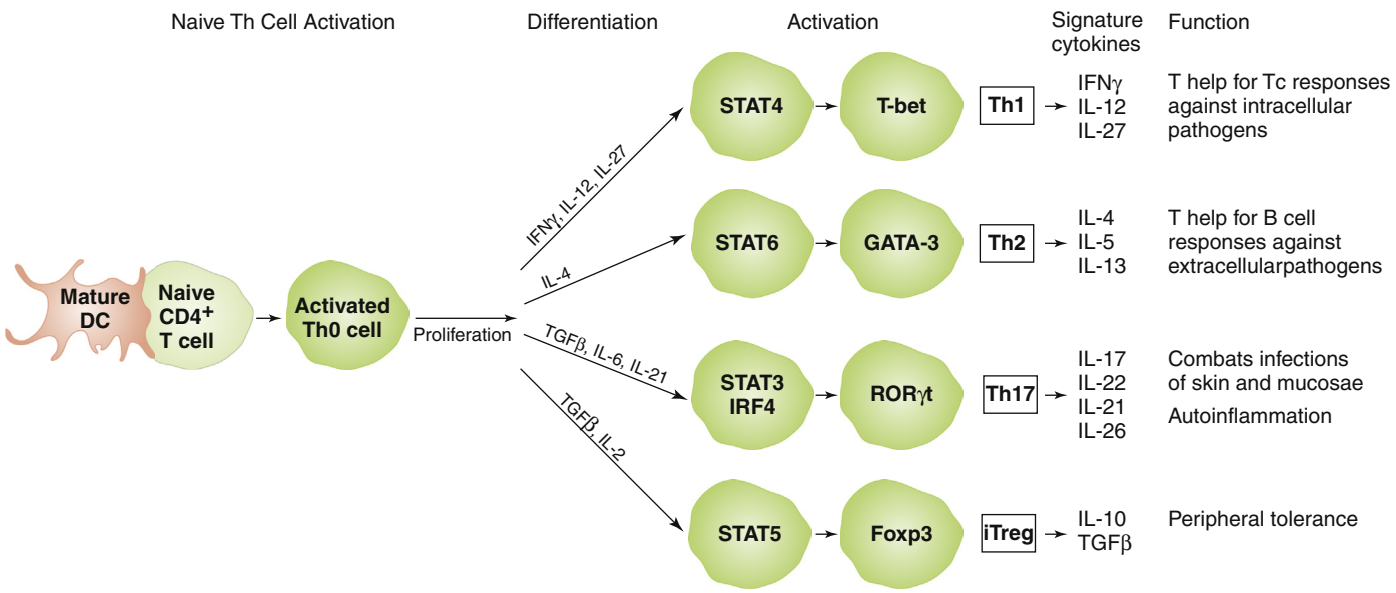

Fig. 9-9
Th Effector Cell Differentiation and Functions

A naïve Th cell activated by a mature DC proliferates and generates daughter cells that are induced to differentiate into the indicated Th effector subsets depending on the cytokines in the immediate microenvironment. For a given Th subset, the indicated cytokines activate the indicated transcription factors within a Th0 cell to direct differentiation down a specific path. Once differentiated, each Th subset produces a signature cytokine profile that mediates its effector function. Not all Th subsets are shown.

i) Th1 Cells

Intracellular pathogens such as viruses and intracellular bacteria trigger macrophages and DCs to produce IFNγ, IL-12 and IL-27. A Th0 cell that has its TCR engaged by specific pMHC and encounters these cytokines experiences activation of the transcription factor STAT4. STAT4 drives a gene expression program that causes the Th0 cell to commit to the Th1 subset 5–7 days after antigen stimulation. IL-18 then supports the survival and proliferation of the newly produced Th1 effector cells. Once in the site of inflammation where their effector function is required, Th1 cells are stimulated by antigen in this site and activate the transcription factor T-bet. T-bet drives IFNγ production and opposes intracellular signaling promoting Th2 differentiation.

ii) Th2 Cells

Most extracellular pathogens do not induce IL-12 production by macrophages and DCs. Instead, these invaders stimulate an unknown cell type (which might be a mast cell or NKT cell) to secrete IL-4. In the absence of IL-12 and IFNγ but in the presence of IL-4, a Th0 cell experiences activation of the transcription factor STAT6. STAT6 drives a gene expression program that causes the Th0 cells to generate Th2 effectors. IL-4 then sustains the survival and proliferation of the newly produced Th2 cells. Once in the inflammatory site where they are needed, Th2 cells stimulated by specific antigen activate the transcription factor GATA-3. GATA-3 both drives the production of the Th2 signature cytokines IL-4, IL-5 and IL-13 and reciprocally opposes intracellular signaling promoting Th1 differentiation.

iii) Th17 Cells

Th17 effector cells counter infections of the skin and mucosae (particularly in the lung and intestine) that are initiated by certain species of extracellular bacteria and fungi. Th0 cells exposed to a combination of the immunosuppressive cytokine TGFβ plus the pro-inflammatory cytokines IL-6 and/or IL-21 produced by local innate leukocytes experience activation of the transcription factors STAT3 and IRF4, which implement a gene expression program resulting in Th17 effector generation. IL-23 appears to be required for the continued survival and terminal differentiation of Th17 cells into fully functioning effectors. In an inflammatory site, antigen-stimulated Th17 effectors activate the transcription factor RORγt that drives a gene expression program resulting in the production of IL-17, IL-21, IL-22 and IL-26. Both IFNγ (produced by Th1 cells) and IL-4 (produced by Th2 cells) suppress Th17 differentiation.

Th17 cells were first identified through studies of autoimmune disease in mice. Although these disorders had been previously blamed on the actions of dysregulated Th1 cells, it has turned out that the autoinflammatory lesions in these animals contain large numbers of Th17 cells. Indeed, knockout mice lacking IL-17R are resistant to the induction of experimental autoimmune diseases. In humans, elevated levels of IL-17 have been detected in the blood and tissues of patients with autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, psoriasis, and inflammatory bowel disease (see Ch. 19). Moreover, T cells isolated from the affected tissues of these patients show the phenotype of Th17 cells.

iv) Other Th Effector Subsets

Th9 cells help to combat infections by helminth worms. Th0 cells exposed to TGFβ and IL-4 differentiate into Th9 effectors that secrete IL-9 upon antigen stimulation. *Th22 cells* help to protect the skin and mucosae, and also promote wound healing after trauma. Th0 cells exposed to IL-6 plus TNF differentiate into Th22 cells that secrete IL-22. IL-22 combines with other cytokines to induce skin cells to produce antimicrobial peptides. *Follicular Th (fTh) cells* is the name some immunologists have given to the subset of Th cells that provide help in the form of cytokine secretion and intercellular contacts to antigen-activated B cells within germinal centers (see Ch. 5). Within the T cell zone of a lymph node, a Th0 cell that is exposed to IL-6 plus IL-27 and/or IL-12 switches expression of its chemokine receptors and makes its way into the GC of a

B cell follicle. In this location, the T cell receives additional differentiation signals from antigen-presenting GC B cells and becomes an fTh effector cell.

In addition to these Th effector subsets, Th0 cells can differentiate into *induced regulatory T (iTreg) cells* if exposed to TGFβ plus IL-2. As mentioned earlier, regulatory T cells are T cells that can shut down the functions of effector T cell subsets, suppressing the adaptive response. Once differentiated, iTreg cells activate the Foxp3 transcription factor, which induces the secretion of IL-10 and TGFβ. IL-10 and TGFβ then act on effector T cells to curtail their responses. Interestingly, IL-6 and IL-21 are potent repressors of TGFβ–driven Foxp3 expression, so that Th0 cells exposed to TGFβ are induced to become Th17 cells rather than iTreg cells if IL-6 and/or IL-21 is also present. Thus, the balance between the generation of Th17 cells and iTreg cells is fine-tuned by the surrounding microenvironment.

NOTE: The plasticity of Th subsets is an issue of growing interest. It has become clear that Th cell fate decisions are influenced by two general mechanisms of gene expression regulation: *microRNA* expression and *epigenetic modification*. MicroRNAs (miRNAs) are short, single-stranded pieces of RNA that do not encode a protein themselves but repress the expression of particular mRNAs by promoting their degradation and inhibiting their translation. Epigenetic modifications involve the methylation of cytosine and guanine residues in genomic DNA in the promoter and enhancer regions of genes. The presence of these methylated CpG motifs blocks gene transcription. At least *in vitro*, different Th effector subsets show different patterns of miRNA expression and CpG methylation, and these patterns often change when the Th cell clone switches effector fates.

III. Activation of Th Effector Cells

i) Localization

Once differentiated, Th effector cells may remain in the lymph node to supply T cell help to naïve Tc cells in the paracortex and naïve B cells in the primary follicles. Alternatively, Th effectors may leave the node and bolster defense of the diffuse lymphoid tissues under the skin and mucosae (SALT and MALT), or follow chemokine gradients to sites of inflammation. All Th effector subsets initially express CCR7, which acts within a lymph node to permit migration of the effector T cells from the paracortex to the edges of the primary lymphoid follicles (where naïve B cells are located). However, as the response progresses, fully differentiated Th1, Th2 and Th17 cells express different panels of chemokine receptors and thus exhibit differential trafficking patterns. Later members of Th1 effector clones express CCR1, CCR5 and CXCR3 that draw the Th1 cells to sites of inflammation in the peripheral tissues where defense against intracellular pathogens is usually required. In contrast, Th2 effectors begin to preferentially express CCR3, CCR4 and CCR8. These receptors direct Th2 cells to sites such as the mucosae where responses against extracellular pathogens and toxins are needed. CCR6 expressed by Th17 cells also promotes migration to the SALT and MALT as well as to inflamed tissues.

ii) Interaction with APCs

Effector Th cells encountering pMHCs presented by APCs either in the lymph node or in the site of attack are activated essentially in the same way as naïve Th cells but with some important differences. Compared to naïve Th cells, effector Th cells express higher levels of adhesion molecules that stabilize the immunological synapse more rapidly, facilitating TCR triggering. While an estimated 20–30 hours of sustained TCR signaling is required for naïve T cell activation, only 1 hour is required for effector Th cell activation. Effector Th cells are thus activated by significantly lower quantities of pMHC. In addition, far less costimulation by the APC is required. As a result, effector Th cells respond efficiently to pMHC presented by DCs, macrophages or B cells or sometimes even by non-hematopoietic cells such as gut or skin epithelial cells. In general, B cells are the principal APCs presenting antigen to Th2 cells, whereas macrophages predominate as APCs in interactions with Th1 and Th17 cells.

iii) Differential Costimulatory Requirements

While CD28-B7 interaction is the major costimulatory mechanism for naïve T cell activation, effector T cells appear to require only low levels of CD28-B7 costimulation for activation. Nevertheless, CD28 engagement on effectors is critical because it reduces the time required to achieve activation and avoids prolonged stimulation. In addition, CD28 signaling downregulates the expression of chemokine receptors, preventing the effector cell from migrating away from the site where antigen has been encountered. Two supplementary costimulatory pairs that are important for effector T cell activation are OX40-OX40L and ICOS-ICOSL. In Th1 responses, *OX40* expressed on a Th1 cell surface binds to *OX40 ligand* (OX40L) expressed on APCs. Similarly, the *inducible costimulatory* (ICOS) molecule, which is upregulated on Th2 and Th17 cells only after activation, binds to *ICOS ligand* (ICOSL) expressed on APCs. ICOS is rarely expressed by Th1 effectors.

IV. Functions of Th Effector Cells

A brief comparison of the properties of Th1, Th2 and Th17 effectors is given **Table 9-3**.

i) Th1 Effector Functions

Th1 effectors supply T cell help to Tc and B cells providing cell-mediated and humoral defense against intracellular pathogens. Th1 cells secrete a panel of cytokines dominated by IL-2, IFNγ and lymphotoxin (LT) (sometimes called *Th1 cytokines*). IL-2 drives T and B cell proliferation and enhances ROI production by macrophages. IFNγ and LT hyperactivate macrophages and spur them to secrete additional cytokines, undertake vigorous phagocytosis and upregulate NO production. IFNγ also increases NK cell and macrophage expression of high affinity FcγR molecules that promote ADCC and influences B cells to switch to the production of the Ig isotypes most effective against intracellular pathogens (IgG1 and IgG3 in humans). IgG1 and IgG3 are the antibodies best suited for opsonization, phagocytosis and complement activation, and also bind with high affinity to FcR on NK cells, macrophages and other phagocytes, further increasing ADCC. In addition, Th1 cytokines increase the antigen-presenting potential of macrophages by upregulating MHC class II and TAP. Th1 cells support the activation of Tc cells by producing IL-2 and by providing CD40/CD40L contacts for **DC licensing**.

TABLE 9-3 Comparison of the Properties of Th Effector Cells

Property	Th1 Effectors	Th2 Effectors	Th17 Effectors
Cytokines important for differentiation	IL-2, IL-12, IFNγ, IL-27	IL-4	TGFβ, IL-6, IL-21
Transcription factors important for differentiation	STAT4	STAT6	STAT3, IRF4
Transcription factors important for effector function	T-bet	GATA-3	RORγt
Distinguishing surface markers	IL-12R	IFNγR	IL-23R
Chemokine receptors	CXCR3, CCR5, CCR1	CCR3, CCR4, CCR8	CCR4, CCR5, CCR6, CXCR6
Preferred APCs	Macrophages	B cells	Macrophages
Costimulation	CD28/B7 (low) OX40/OX40L	CD28/B7 (very low) ICOS/ICOSL	CD28/B7 (low) ICOS/ICOSL
Cytokines secreted	IFNγ, IL-2, LT	IL-4, IL-5, IL-13, IL-10, IL-6, IL-3, IL-1	IL-17, IL-21, IL-22, IL-26
Type of immune response promoted	Humoral, cell-mediated	Humoral	Inflammatory
Pathogens combatted	Intracellular	Extracellular	Bacteria and fungi not eliminated by Th1/Th2
Associated with	Transplant rejection	Allergy	Autoimmune disease

ii) Th2 Effector Functions

Th2 differentiation is usually induced upon invasion by extracellular pathogens. Th2 cells tend to promote humoral responses because these cells secrete IL-3, IL-4, IL-5, IL-6, IL-10 and IL-13 (sometimes called *Th2 cytokines*). A major function of Th2 cells is to establish CD40–CD40L contacts with B cells and to secrete IL-4 and IL-5, cytokines that induce switching to the Ig isotypes most effective against extracellular pathogens. Such isotypes, which include IgG4 in humans, are those best suited for neutralization. IgG4 is not very proficient at complement activation or ADCC, which is an advantage in combatting pathogens in mucosal sites where the inflammation induced by these effector functions could be damaging (see Ch. 12). IL-4 and IL-13 inhibit pro-inflammatory cytokine production, downregulate NO production, and decrease FcγR expression on macrophages, blocking ADCC. However, IL-4 upregulates MHC class II expression on APCs such as macrophages, DCs and B cells, and thereby contributes to Th cell stimulation. IL-4 and IL-13 also enhance the humoral response by stimulating B cell proliferation. IL-5 promotes the growth, differentiation and activation of eosinophils crucial for the elimination of large parasites such as helminth worms. IL-3, IL-4 and IL-10 combine to promote the activation and proliferation of mast cells, also effective against large parasites. In general, however, IL-10 acts as a brake on immune responses and balances the stimulation exerted by other cytokines. For example, IL-10 inhibits the pro-inflammatory functions of macrophages and abrogates their production of IL-12 and MHC class II. IL-10 also downregulates B7 expression on macrophages and DCs.

iii) Th17 Effector Functions

Until recently, Th17 cells were most often thought of in the context of promoting autoimmune disease. Obviously, this is unlikely to be their physiological function! Rather, the massive inflammatory responses mounted by these cells, which are dominated by IL-17, IL-21, IL-22 and IL-26, are designed to protect mucosal surfaces against pathogens that can resist assault by Th1 and Th2 cells. Pathogens triggering strong Th17 cell-mediated responses include several bacterial species, such as *Borrelia burgdorferi* and *Klebsiella pneumoniae*, as well as the AIDS-associated organism *Pneumocystis carinii* and the fungus *Candida albicans* (among others). IL-17 produced by Th17 cells induces nearby non-hematopoietic cells to produce destructive pro-inflammatory cytokines such as TNF, IL-1 and IL-6.

T cell responses to specific pathogens are discussed in Chapter 13.

iv) Th Effector Cell Cross-Regulation and Amplification

Because of the cytokines they produce, various Th cell subsets can cross-regulate each other's differentiation and activities, either positively or negatively. For example, Th1 cells produce large amounts of IL-2 that can promote the proliferation of both Th1 and Th2 cells. However, the IFNγ produced by Th1 cells has a direct antiproliferative effect on Th2 cells and inhibits further Th2 differentiation. On the other hand, IFNγ stimulates macrophages to produce IL-12, which promotes Th1 differentiation. Th2 cells do not make substantial amounts of IL-2 or IFNγ and instead secrete IL-4, IL-13 and IL-10. These cytokines suppress IFNγ and IL-2 secretion by Th1 cells, inhibit further Th1 differentiation and downregulate macrophage production of IL-12. In an example of positive feedback, the IL-4 produced by Th2 cells promotes the continued differentiation of this subset. A similar effect is seen for Th17 cells, in that the IL-21 (but not IL-17) produced by activated Th17 cells supports continued Th17 cell differentiation. IL-21 also helps to repress the expression of Foxp3 that drives Treg differentiation.

V. Nature of Th Responses

Among immunologists, it is said that an immune response has either a Th1, Th2 or Th17 character or phenotype, depending on the predominant Th subset and cytokines observed in the host during that response. An attack on a host by intracellular

pathogens stimulates the production by DCs and macrophages of cytokines favoring Th1 development, leading to the mounting of a *Th1 response*. Conversely, invasion by extracellular pathogens most often promotes the development of a *Th2 response*, or, depending on the specific invader, a *Th17 response*. Immunological disease states also tend to have a Th1, Th2 or Th17 phenotype. For example, allergies are associated with a prevalence of Th2 cells, whereas Th1 cells dominate in transplant rejection, and Th17 cells are associated with many autoimmune disorders. Despite these generalizations, however, the overall phenotype of an immune response to a given pathogen can change with time. For example, mice infected with the parasite causing malaria first develop a Th1 response designed to deal with the intracellular stage of the infection. Th1 effectors are generated that secrete IFNγ, which activates macrophages to secrete cytotoxic cytokines and produce large quantities of NO. IFNγ also induces B cells to switch to the production of parasite-specific IgG2a antibodies. However, 10 days after the initial attack, the parasite adopts an extracellular phase that triggers a Th2 response characterized by high serum levels of IL-4, IL-10 and parasite-specific IgG1 antibodies. Both types of Th responses are needed to keep the pathogen in check.

D. Tc Cell Differentiation and Effector Function

I. Overview

Cytotoxic T cell responses can be thought of as occurring in five stages:

(1) Activation of the naïve Tc cell by a licensed DC in a secondary lymphoid tissue;
(2) Proliferation and differentiation of the activated Tc cell into daughter cells called *pre-CTLs*;
(3) Differentiation of a pre-CTL in an inflammatory site into an *"armed" CTL*;
(4) Activation of the armed CTL by encounter with specific non-self peptide presented by MHC class I on a target cell; and
(5) CTL-mediated destruction of the target cell as well as other cells displaying the identical pMHC.

Target cells of CTLs include cells infected with intracellularly replicating pathogens, tumor cells and foreign cells entering the body as part of a tissue transplant. We emphasize that an activated Tc cell has no lytic powers at all: only its mature CTL progeny develop cytotoxicity.

NOTE: Until relatively recently, it was thought that activated naïve $CD8^+$ Tc cells developed only into CTLs with ability to lyse target cells. There is now *in vitro* and *in vivo* evidence that naïve Tc cells exposed to specific antigen in the presence of cytokines (TGFβ, IL-6, IL-21) that influence Th17 effector cell differentiation can result in the development of so-called *Tc17 cells*. Tc17 cells show greatly repressed cytotoxicity functions and instead secrete copious amounts of IL-17. Tc17 cells have been found along with Th17 cells in the lesions of mice with autoimmune diseases and in the lungs of mice challenged with a lethal dose of influenza virus.

II. Generation and Activation of CTLs

i) Differentiation of CTLs

In the presence of IL-2 secreted by an activated Th cell, an activated naïve Tc cell proliferates and generates pre-CTL precursor cells (**Fig. 9-10**). These pre-CTLs leave the lymph node and travel to the site of pathogen attack. In the presence of IL-12, IFNγ and IL-6 produced by activated macrophages and DCs, the pre-CTLs differentiate into mature CTLs whose cytoplasm contains cytotoxic granules. These mature CTLs (that

have yet to encounter antigen) are said to be "armed" and do not need to carry out any additional protein synthesis to be effective killers. This effector generation process is completed within 24–48 hours of TCR stimulation of the original Tc cell. Importantly, because pre-CTL differentiation into armed CTLs requires inflammatory cytokines, the development of cytotoxicity and the power of the CTL response are reserved for situations in which a threat is actually present.

ii) Activation of Armed CTLs and Conjugate Formation

Within an inflammatory site, an armed CTL binds weakly to one host cell after another in search of its specific pMHC. The armed CTL detaches without incident if the affinity of TCR–pMHC binding is too low. However, should the armed CTL encounter a pMHC for which it is specific (the TCR binds with sufficient affinity), the host cell becomes a

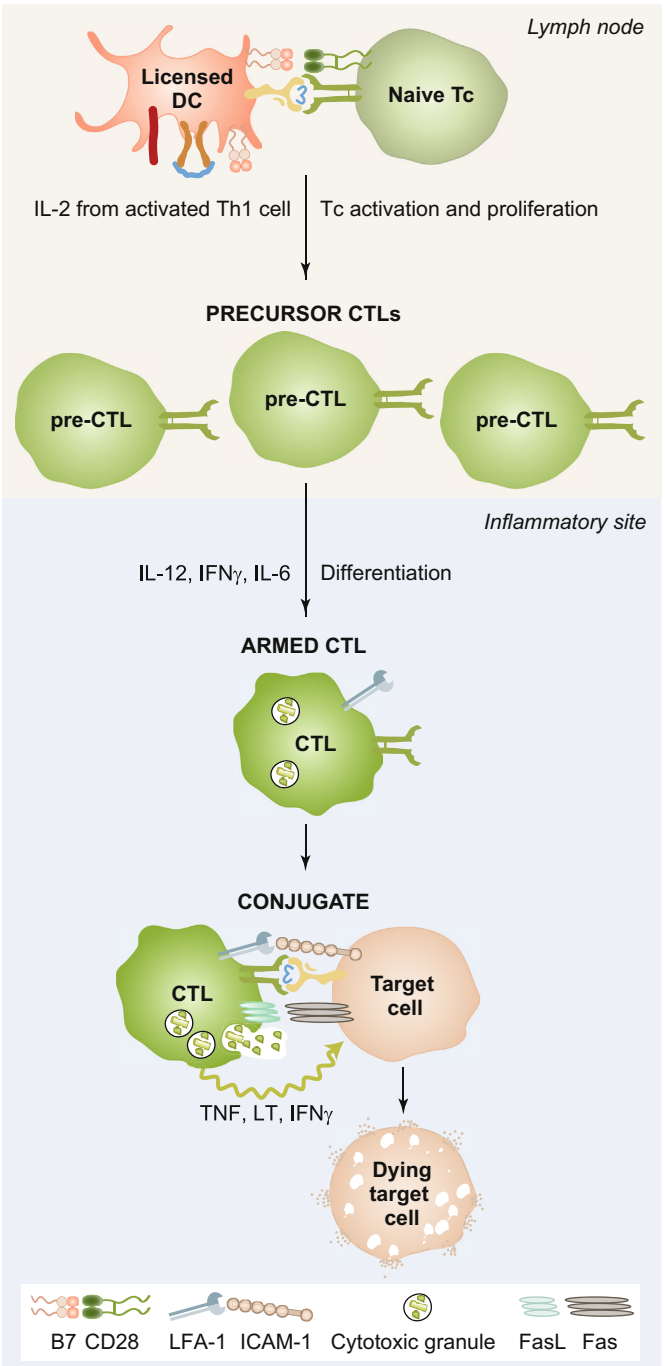

Fig. 9-10
CTL Generation and Cytotoxicity

A naïve Tc cell encountering a licensed DC presenting cognate pMHC in the presence of IL-2 is activated, proliferates and generates pre-CTLs. In the presence of the indicated cytokines, the pre-CTLs differentiate into armed CTLs containing cytoplasmic granules and expressing FasL. When the TCR of an armed CTL is engaged by specific pMHC displayed by a target cell, the apoptotic death of this cell is induced via cytotoxic granule release, Fas ligation or the secretion of cytotoxic cytokines. The ultimate death of the target cell occurs after the CTL has detached.

target. Stimulation of the TCR of an armed CTL rapidly increases the binding affinity of adhesion molecule pairs between the CTL and the target cell, forming a bicellular conjugate (refer to **Fig. 9-10**). The CTL then delivers a "lethal hit" of chemical mediators that rapidly causes target cell death. This speed is important to ensure the killing of an infected cell before too many of the progeny of the replicating pathogen can escape to new cells. Much lower concentrations of specific pMHC and the engagement of far fewer TCRs are required to activate armed CTLs compared to naïve Tc cells: only the engagement of a single TCR by a single specific pMHC is needed, and no costimulation is required.

III. Mechanisms of Target Cell Destruction

Target cell destruction by CTLs can occur via the *granule exocytosis pathway,* the *Fas pathway*, and/or the release of *cytotoxic cytokines* such as TNF and LT (**Fig. 9-11**). The pathway used depends on the nature of the attacking intracellular pathogen, but granule exocytosis accounts for the majority of target cell killing by CTLs.

i) Granule Exocytosis

The granule exocytosis pathway refers to the release of the contents of the CTL's cytotoxic granules. Soon after conjugate formation, the cytoskeleton of the CTL reorganizes

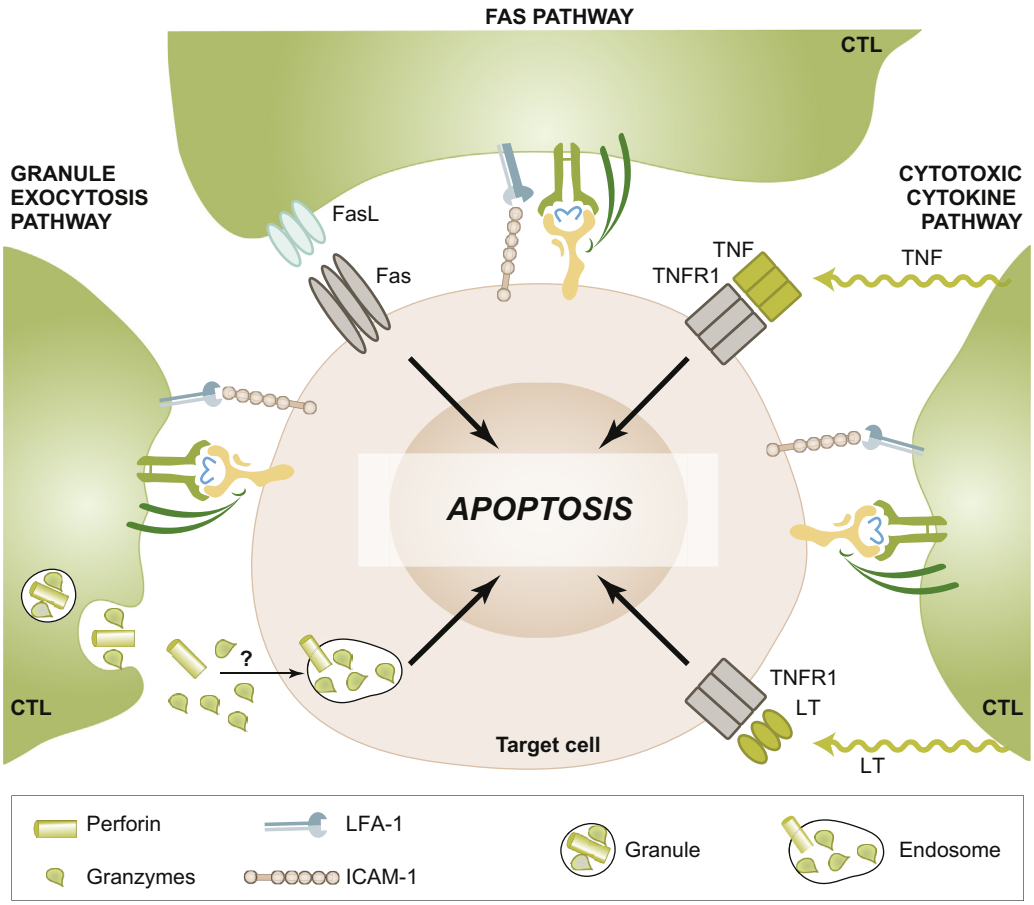

Fig. 9-11
Mechanisms of CTL Cytotoxicity
The three major ways a CTL can kill a target cell are illustrated. In the granule exocytosis pathway, granules are exocytosed by a CTL in close proximity to the target cell membrane. The granzymes and perforin released from the granule are endocytosed by the target cell and captured in endosomes. The perforin forms pores in the endosomal membrane, causing the granzymes to be released into the cytoplasm. In the Fas pathway, FasL expressed by the armed CTL binds to Fas expressed by the target cell. In the cytotoxic cytokine pathway, TNF or LT secreted by the CTL binds to TNFR1 on the target cell. Each of these three pathways triggers apoptosis that may or may not involve caspases.

so that its cytotoxic granules are brought to the site of CTL–target cell contact. The granules fuse with the CTL membrane, and the cytotoxic contents of the granules are directionally exocytosed toward the target cell membrane. Among these granule contents are *perforin* and *granzymes.* Perforin is a pore-forming protein, and the granzymes are a family of serine proteases. It is not clear how these proteins actually enter the target cell, but after they do, they are immediately confined to its endocytic system. Perforin then facilitates the release of the granzymes from the endolysosomal vesicles into the cytoplasm of the target cell. Granzyme A initiates a caspase-independent pathway of DNA damage, while granzyme B triggers classical caspase-mediated apoptosis. Upon the degradation of its DNA and other important intracellular substrates, the target cell dies. This form of death is called **perforin/granzyme-mediated cytotoxicity**.

ii) Fas Pathway

Fas is a transmembrane "death receptor" that is widely expressed on mammalian cells. Engagement of Fas on a target cell by *Fas ligand* (FasL) expressed by an armed CTL results in the death of the target cell. Naïve Tc cells do not express FasL, but after activation by encounter with antigen, FasL is synthesized and stored in specialized transport vesicles in differentiating CTLs. Upon conjugate formation, the FasL-containing transport vesicles fuse with the CTL plasma membrane and anchor FasL on the CTL surface. The FasL engages Fas on the target cell and induces its apoptosis.

iii) Cytotoxic Cytokines

CTLs can kill target cells by producing **cytotoxic cytokines**, particularly TNF and LT. Apoptosis of a target cell can be induced by the binding of TNF produced by a CTL to TNF receptor 1 (TNFR1) on the target cell surface. LT, which also binds to TNFR1, has a similar effect. CTLs also secrete IFNγ, whose action in this context is more indirect. IFNγ stimulates B cells to produce antibodies that facilitate killing via ADCC or complement activation. As well, IFNγ upregulates MHC class I on nearby host cells, enhancing antigen display and making target cells more visible to scanning CTLs.

IV. Dissociation

About 5–10 minutes after delivery of a lethal hit, the adhesion molecules on the CTL resume a low affinity conformation that allows the CTL to dissociate from the damaged target cell. The target cell succumbs to apoptosis within 3 hours of dissociation, while the CTL commences synthesis of new cytotoxic granules and moves off to inspect other host cells. A single armed (and re-armed) CTL can attach to many host cells in succession, delivering lethal hits without sustaining any damage itself. How the CTL avoids self-destruction by its granule contents is a mystery. There is some evidence suggesting that the plasma membrane of CTLs differs slightly from that of most target cells in its lipid and/or protein composition and that this difference may confer resistance to the effects of granule exocytosis.

E. Termination of Effector T Cell Responses

The Th effector cells and CTLs that are actively proliferating and employed in eliminating a pathogen that sparked a primary immune response are sustained by signals delivered by inflammatory cytokines (such as IL-12) and transcription factors (such as Id2 and Bcl-3). However, after the effectors have removed the threat, there is no further need for their presence. Continued exposure to the inflammatory environment in the absence of antigen causes the effectors to downregulate IL-7R and IL-15R, reducing their ability to receive survival signals. Three mechanisms then act in concert to further bias the balance of pro-apoptotic/anti-apoptotic gene expression and induce effector cell death: **activation-induced cell death (AICD), cytokine "withdrawal,"** and **T cell clonal exhaustion.**

AICD is a form of apoptosis induced when the intracellular signaling triggered by TCR engagement by antigen becomes prolonged. This extended signaling is thought to induce the transcription of pro-apoptotic genes such as FasL and TNFR1 within the effector T cell, and to decrease its expression of anti-apoptotic molecules. These changes set the effector T cell up to be killed by contact with Fas or TNF expressed by a neighboring cell. In the early stages of a primary response, effector T cells are protected from AICD by a constant low level of CD28–B7 signaling. This minimal costimulation initially ensures that pro-apoptotic genes are not transcribed to a level that overwhelms the effects of anti-apoptotic genes.

Cytokine withdrawal is a mechanism that was first observed for IL-2 *in vitro*. Clones of T cells die if all IL-2 is removed from the culture medium after activation. It seems that this phenomenon also happens *in vivo*, when antigen is being mopped up and less of it is around to stimulate DCs and other APCs to secrete cytokines. In the absence of antigen and these cytokines, T cell activation cannot be sustained, IL-2 production falls, and effector T cells die. In this case, the induction of T cell apoptosis does not involve Fas or TNF. Rather, the lack of cytokines prevents cytokine receptor engagement and blocks the delivery of a vital survival signal. In the absence of this survival signal, pro-apoptotic gene expression dominates and kills the cell.

Although most effector T cells eventually succumb to AICD or cytokine withdrawal, entire activated T cell clones are sometimes eliminated by "clonal exhaustion." In this case, continuous exposure to antigen causes the T cells to divide so relentlessly into effectors that they burn out metabolically without generating memory cells. Effector cells, as well as the memory cells that normally would have dealt with a subsequent assault, are completely absent from the host.

As noted previously, effector T cell responses can also be suppressed by regulatory T cells in a manner that does not involve killing the effector T cell (see Ch. 20).

F. Memory T Cells

I. Types of Memory T Cells

For both $CD4^+$ and $CD8^+$ T cells, about 5–10% of the antigen-specific progeny T cells generated in a primary response survive AICD or IL-2 withdrawal. These cells are, or give rise to, long-lived memory T cells. Memory T cells recognize the same pMHC as naïve and effector T cells but have properties intermediate between them (**Table 9-4**). Memory T cells are usually found in a resting state but occasionally undergo self-renewal to ensure their long-term survival. Upon a second assault by the same pathogen, memory T cells mount a secondary response that is faster and stronger than the primary response. These differences are attributable to the localization, increased numbers and enhanced capacities of memory T cells, and the significantly faster rate at which they differentiate into effector cells when activated by antigen.

Research over the past decade has revealed that there are at least two major classes of memory T cells: **effector memory T (Tem)** cells and **central memory T (Tcm)**

TABLE 9-4 Comparison of Properties of Naïve, Effector and Memory T Cells

Property	Naïve T Cell	Effector T Cell	Memory T Cell
Preferred tissue	Secondary lymphoid tissues	Peripheral tissues, inflammatory sites, secondary lymphoid tissues	Peripheral tissues, secondary lymphoid tissues, diffuse lymphoid tissues, bone marrow, inflammatory sites
Preferred APCs	DCs	Macrophages, B cells, DCs	DCs, B cells, macrophages
Required costimulation	CD28/B7 (high)	CD28/B7 (low to none)	Minimal to none
Duration of TCR signaling for activation	20–30 hours	<1 hour	<1 hour
Cell division	Slow	Rapid	Moderate
Sensitivity to AICD	Low	High	High
Life span	5–7 weeks	2–3 days	Up to 50 years

cells. These subsets differ in some important properties. Firstly, in the absence of specific antigen, Tem cells appear to have a shorter life span than Tcm cells. Secondly, after a primary response, differences in homing receptor expression between these populations lead to differences in their distribution throughout the body. In particular, Tcm cells express high levels of the lymph node homing molecules CD62L and CCR7. Accordingly, these cells tend to migrate through the lymph nodes and other secondary lymphoid tissues, thereby maintaining a long-term, central reservoir of memory cells. In contrast, Tem cells express only low levels of CD62L and CCR7, so that they circulate mainly through non-lymphoid tissues where pathogens are likely to attack a second time. Such tissues include the lung, intestines, reproductive tract, liver and fat. Tem cells also express homing and chemokine receptors that allow their active recruitment into sites of inflammation. As a result, Tem cells constantly patrol the peripheral tissues and are able to migrate quickly into sites of infection. Together, Tem and Tcm cells ensure the ability of the host to mount a strong and immediate response upon subsequent exposure to a previously encountered pathogen. The cytokine milieu in which the original naïve T cell was activated appears to influence whether its progeny will have a greater Tem or Tcm character. For example, exposure to high levels of inflammatory cytokines during the primary response can suppress CD62L and CCR7 expression, thus favoring the generation of Tem cells. Researchers continue to debate the precise origin of Tem and Tcm cells because the transcription factors, cytokines and possible intercellular interactions directing the naïve to memory cell transition are as yet unclear.

II. Memory T Cell Activation and Differentiation

Naïve Th and Tc cells are activated exclusively by pMHCs presented by DCs, and this most often takes place in lymph nodes or other secondary lymphoid tissues, In contrast, memory T cells have less stringent requirements. Memory Th cells not only are dispersed in a broader range of anatomical sites than are naïve Th cells, but can also respond to pMHC presented by DCs, B cells or macrophages. Similarly, memory Tc cells can respond to infected host cells located almost anywhere in the body provided that these cells display the appropriate pMHC on their surfaces. In terms of signaling, the activation of both memory Th and memory Tc cells more closely resembles that of an effector T cell than a naïve T cell. Activation can occur at very low concentrations of antigen with only minimal costimulation (if any), and the duration of TCR signaling required is much shorter. Although naïve Tc cells usually require T cell help from antigen-specific Th cells to become activated, memory Tc cells often do not. Once activated, most memory Th and memory Tc cells proliferate more readily and for longer periods than their naïve counterparts.

Note: While memory T cells in general proliferate more readily than naïve T cells, it appears that Tcm cells proliferate to a much greater extent than Tem cells.

With respect to differentiation, most activated memory Th and Tc cells follow much the same pathways as naïve Th and Tc cells but complete them more quickly (within 24 hours as opposed to 4–5 days). Some immunologists maintain that many memory T cells are not really resting but instead are maintained in a type of "pre-activation" state (which may correlate with their intermediate marker phenotype). This theory holds that pre-activation may make it easier for the cells to immediately differentiate into new effectors capable of quickly combatting an aggressive pathogen. Thus, for example, Tem cells of the $CD4^+$ Th phenotype that are recruited to a site of attack give rise to effector cells that rapidly produce cytokines such as IFNγ , IL-4 and IL-5. Their Tcm counterparts in the secondary lymphoid organs undergo rapid differentiation and produce copious amounts of IL-2. Similarly, $CD8^+$ Tem cells in peripheral tissues exhibit high levels of granzyme B expression and, once activated, quickly generate CTLs that can immediately engage in cytolytic activity.

III. Memory T Cell Life Span

Most memory T cells persist in the host for at least several months and often years, greatly exceeding the longevity of both naïve and effector T cells. The maintenance of the cellular pool depends on IL-7 because this cytokine drives the expression of anti-apoptotic molecules that protect against AICD. IL-2 and IL-15 also support the survival of memory T cells over the long-term, allowing them to proliferate as needed to maintain homeostasis. Studies of $CD4^+$ memory T cells have shown that Tem cells express lower levels of IL-2R, IL-7R and IL-15R on their surfaces than Tcm cells, likely accounting for the shorter life spans and less prolific proliferation of Tem cells.

The length of the life span of a memory T cell clone varies with the nature of the antigen that provoked the primary response. We see evidence of this variability in the immunization schedules of different vaccines (see Ch. 14). Just one dose of some vaccines (e.g., against the polio virus) provides immunity for life, whereas "booster" doses of other vaccines (e.g., against the bacterium causing tetanus) must be given every few years to maintain protection. While the cytokines needed to maintain memory T cell pools are now quite well defined, immunologists are still divided over whether the persistence of memory lymphocytes also requires a periodic low level of stimulation by tiny amounts of residual antigen. Such stimulation might help to induce the expression of base levels of anti-apoptotic molecules that would permit the memory T cell to survive. It may be that the requirement for stimulation varies with the antigen: significant numbers of memory T cells can be detected 15 years after infection with at least some viruses, with no evidence of re-infection or persistence of viral antigen.

Can memory T cells protect a host forever? Studies of the aging of the immune system indicate that memory T cells arising from a given clone can be stimulated only so many times before they fail to proliferate in response to antigen. As well, the production of new naïve T cells by the thymus declines precipitously after involution, also curtailing the generation of new memory T cells. Thus, as an individual ages, the numbers of both naïve and memory T cells that can be activated to generate effector T cells is ultimately limited, and the host becomes increasingly susceptible to pathogens toward the end of his/her life. In general, the longevity of T cell memory in humans pales in comparison to that of B cell memory, where antibodies raised during a natural infection by certain pathogens have been documented in some individuals as persisting for over 50 years.

We have now described all the cellular components of an adaptive immune response and have discussed how that response removes non-self entities. In the next chapter, we examine peripheral tolerance, a collection of mechanisms that control those mature naïve lymphocytes in the periphery which escaped the establishment of central tolerance and whose antigen receptors are directed against self antigens.

Chapter 9 Take-Home Message

- HSCs give rise to NK/T precursors that colonize the thymus and generate thymocytes. Thymocytes mature through the DN, DP and SP phases defined by CD4/CD8 expression.
- Development of αβ T cells is controlled by two checkpoints: the pre-TCR checkpoint and the TCRαβ checkpoint.
- Thymic selection establishing central T cell tolerance involves the non-selection, positive selection or negative selection of DP thymocytes. Selection is determined by the affinity/avidity of TCR binding to pMHCs presented by TECs.
- Thymocytes that survive selection lose expression of one coreceptor to become SP thymocytes, eventually exiting to the periphery as mature $CD4^+$ Th and $CD8^+$ Tc cells.
- Naïve Th and Tc cells are activated by engagement of multiple copies of their TCRs by pMHCs presented on mature DCs in the lymph node.
- Th cell activation involves the formation of the immunological synapse between the naïve Th cell and the DC. The synapse allows the sustained triggering of multiple TCRs that deliver "signal 1."
- "Signal 2" is delivered by costimulatory contacts such as CD28–B7, whereas "signal 3" is delivered by the binding of cytokines such as IL-2.
- Signal 1 induces the phosphorylation of the ITAMs in the CD3 chains, and signals 2 and 3 trigger subsequent intracellular signaling that leads to the new gene transcription necessary to support proliferation and effector cell differentiation.
- Naïve Tc cells are activated by interaction with DCs that have been "licensed" by Th cells to express B7.
- An activated naïve T cell proliferates and differentiates into effector T cells that eliminate antigens, and into memory T cells that mediate secondary immune responses.
- Effector and memory T cells require lower levels of TCR engagement and costimulation than do naïve cells, and express different adhesion molecules and chemokine receptors.
- Depending on the cytokines in the microenvironment, activated Th cells generate Th effector subsets that secrete different panels of cytokines. These cytokines either act against pathogens or support B cell and Tc cell activation. Regulatory T cells may also be produced.
- Activated Tc cells generate armed CTL effectors that kill tumor cells and infected target cells by perforin- and granzyme-mediated cytotoxicity, Fas ligation, and/or secretion of cytotoxic cytokines.
- The duration of Th effector and CTL responses is controlled by AICD, cytokine withdrawal, and T cell exhaustion. Regulatory T cells can suppress effector T cell responses.
- Memory T cells (both $CD4^+$ and $CD8^+$) remain in the host after the majority of effector T cells responding to an antigen have died off. Tem cells patrol peripheral tissues and sites of inflammation, whereas Tcm cells recirculate among the secondary lymphoid organs. Upon a second exposure to an antigen, Tem and Tcm cells rapidly generate new effector T cells that can act in the tissue where the original naïve T cell was activated.

Did You Get It? A Self-Test Quiz

Section A.I–II

1) Give four ways in which B cell and T cell development differ.
2) What cell types arise from NK/T precursors?
3) Which waves of NK/T precursors entering the thymus give rise to αβ T cells?
4) Why is T cell diversity in the neonatal repertoire less than in the adult repertoire?

Section A.III

1) What are cTECs and mTECs, and why are they important?
2) Why is Notch1 described as a "cell fate protein"?
3) How do thymic fibroblasts contribute to T cell development?
4) What are the four stages of the DN phase of T cell development? How are these phases similar in surface marker expression? How do they differ?
5) What is SCF, and what is its function in the thymus?
6) What five key events occur during the DN3 stage?
7) Describe the composition and function of the pre-TCR complex.
8) What is β-selection, and why is it important?
9) Name the three components of thymic selection and describe how affinity/avidity of TCR engagement defines each of them.
10) Why is negative selection essential for the establishment of central T cell tolerance?
11) What is AIRE, and why is it important for thymic selection?
12) What factors influence the intracellular signaling triggered by TCR engagement during thymic selection?
13) Give two reasons why a thymocyte might be non-selected.
14) What are the effects on thymic selection of the expression of anti- and pro-apoptotic genes?

Did You Get It? A Self-Test Quiz—Continued

15) What is the TCRαβ checkpoint?

16) Describe how coreceptor expression in SP thymocytes determines which class of MHC the T lineage clone will later respond to?

17) Name two molecules (other than the coreceptors) that influence SP thymocyte lineage determination.

18) Do newly produced $CD4^+$ and $CD8^+$ mature T cells need antigenic stimulation to survive?

19) How does SP thymocyte coreceptor expression determine the mature T cell's effector function?

Section B

1) What are the three signals of naïve T cell activation?

2) Briefly outline how naïve T cells and antigen-bearing DCs meet in the lymph node.

3) Name two adhesion molecule pairs that help hold T cells and DCs together.

4) Why is sustained TCR triggering necessary to activate naïve T cells?

5) What is the immunological synapse?

6) Distinguish between the cSMAC, pSMAC and dSMAC.

7) What is the most important costimulatory interaction for naïve Th cells, and why is it necessary?

8) What is DC licensing, and why is it necessary?

9) Give three effects of CD28-mediated costimulation.

10) What is the function of CTLA-4? PD-1? How does the timing of these functions differ?

11) What are the two most important components of Th cell help for naïve Tc cells?

Section C

1) Name four types of effector T cells derived from Th0 cells.

2) Which cytokines drive Th1 differentiation, and what is their source? Th2 differentiation? Th17 differentiation?

3) Name three transcription factors important for Th cell differentiation.

4) What pathogens are countered by Th1 cells? Th2 cells? Th9 cells?

5) What class of diseases is associated with the normal function of Th17 cells?

6) What are fTh cells, and what do they do?

7) Are iTreg cells effector cells? If not, why not?

8) How does the localization of Th1, Th2 and Th17 cells differ, and what is the basis for these differences?

9) Give two ways in which the activation of Th effectors differs from that of naïve cells.

10) What molecules provide supplementary costimulation for Th1 cells? Th2 cells?

11) Give two ways in which Th1 cells support cell-mediated immunity and two ways in which Th2 cells support humoral immunity.

12) Why is IL-10 considered to act as a "brake" on immune responses?

13) Give two examples of Th effector cells that can cross-regulate each other.

14) Name two factors influencing the nature of a Th response.

Section D

1) Can you define these terms? pre-CTL, armed CTL, Tc17 cell, granule exocytosis, dissociation

2) Describe the five stages of cytotoxic T cell responses.

3) How does the activation of the armed CTL differ from that of a naïve Tc cell?

4) What are perforin and granzymes, and what do they do?

5) Describe how CTLs use the Fas pathway to kill target cells.

6) Name three cytotoxic cytokines and describe how they contribute to target cell elimination.

Section E

1) Name two molecules contributing to the survival of proliferating effector T cells.

2) Distinguish between AICD and T cell exhaustion.

3) What happens if IL-2 is withdrawn from a culture of activated T cells?

4) What T cell subset can control the responses of effector T cells?

Section F

1) Give three reasons why memory T cells react faster and stronger than naïve T cells.

2) How is the phenotype of memory T cells intermediate between naïve and effector T cell phenotypes?

3) Compare the properties of Tem and Tcm cells.

4) Name three receptors important for memory T cell survival.

5) Give two reasons why older individuals have less effective immune systems.

Can You Extrapolate? Some Conceptual Questions

1) Where in the thymus (outer cortex vs. inner cortex vs. medulla) would you expect to find thymocytes with the following characteristics? (Hint: Figures 9-2 and 9-3 may be helpful.)
   a) Active rearrangement of the *TCRB*, *TCRG* and *TCRD* genes.
   b) Expression of CD3 and TCRαβ, but no expression of Tdt or RAG.
   c) Expression of TDt and RAG but no expression of CD25, plus interaction with thymic DCs.
2) Before newly developed medicines are approved for public use, they are routinely tested for both "efficacy" (will they work adequately?) and for "safety" (will they cause unwarranted harm?). If you think of the process of thymic selection in terms of "approving" the release of mature T cells to the body, how would you relate non-selection and negative selection to efficacy and safety?
3) At a site of infection, an immature migratory DC captures antigen and binds DAMPs and PAMPs to its PRRs, triggering maturation of the DC. Once in the local lymph node, the mature DC activates a naïve $CD4^+$ Th cell, triggering it to proliferate and give rise to activated Th0 cells. What type of Th effectors would you expect to develop from these Th0 cells if
   a) The local environment has high levels of TGFβ and IL-6.
   b) The transcription factors STAT4 and T-bet are expressed during T cell activation.
   c) The local environment has high levels of IL-4.

Would You Like To Read More?

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