Chapter 10 – T Cell Maturation, Activation and Differentiation

T Cell Maturation

As we know, T cell precursors (pro-T cells) originate in the bone marrow and migrate to the thymus via the blood.

The course of maturation of such a cell after it arrives in the thymus can be followed by analysis of its cell surface molecules. These changes in gene expression by the maturing thymocytes are presumably stimulated by interactions with resident cells and their products that they encounter in the thymus. Pro-T cells arriving from the bone marrow already express Thy-1.

Particularly important steps in the process of T cell maturation are as follows (refer to Figure 10-1):

1. During development of the fetal thymus, cells that have down-regulated c-kit and CD44 but still express CD25 – a subunit of the IL-2 receptor – turn on their RAG-1 and RAG2 genes and begin to rearrange their TCR genes. Some cells successfully rearrange their α and β genes, put a αβ-TCR on their surface and leave the thymus to colonize mucosal tissues and skin. These cells, which do not have highly variable sequences, serve an as yet unclear function in these tissues. Commitment to produce αβ-TCRs occurs largely during mouse gestational days 14-17, and thereafter commitment to αβ-TCRs predominates. Only about 5% of the T cells in an adult thymus are γδ-T cells.

2. Most of the cells stop proliferating and rearrange their β chain genes. As seen with the immunoglobulin loci in B cells, if a cell fails to make a productive β chain rearrangement, it dies by apoptosis. If it is successful, the β chain goes to the surface with the CD3 complex, a pre-Tα chain (also called gp33). As with immunoglobulins, the pre-Tα chain complex signals the cell to stop rearranging at the β chain locus, probably as a result of binding to a ligand on thymus stromal cells. The result is allelic exclusion. The cell also proliferates to generate a clone of thymocytes, all of whose members express the same α chain, and these cells also now express both CD4 and CD8. They are now called double-positive (DP) thymocytes because they are CD4+CD8+. RAG1 and RAG2 continue to be transcribed, but the RAG2 protein is degraded and proliferation continues without TCR α chain rearrangement.

3. When proliferation stops, RAG2 protein accumulates and rearrangement at the TCR α locus begins. Once a cell expresses an αβ-TCR, it is now ready for selection processes that determine whether it will survive to populate the secondary lymphoid tissues of the body. Approximately 98% of all thymocytes do not survive the rigors of selection and die by apoptosis.

Demonstration of the role of the thymus in determining self-restriction of T lymphocytes

In the early to mid 1970s, Rolf Zinkernagel and Peter Doherty did an experiment that demonstrated the critical importance of the thymus in determining what is recognized as “self.” It was already known that T cells were focused on something encoded in the MHC. What Zinkernagel and Doherty did (see Figure 10-4) was to cause H-2^{a/b} heterozygous T cell precursors to mature in an H-2^{b} thymus.

The result was that the H-2^{a/b} heterozygous T cells that were generated could only recognize foreign antigens on cells that expresses gene products (class I and III were not known then) encoded by the H-2^{b} haplotype. They called this thymic education, but we now know it was due to positive selection of all αβ-TCRs on class I and class II molecules encoded by the H-2^{b} haplotype (see below).

Thymic Selection

The process is summarized in Figure 10-5. The major steps break down into two phases: positive selection that occurs in the thymic cortex, and negative selection that occurs in the medulla.
**Positive selection** - Thymocytes with αβ-TCRs must first undergo **positive selection** in the thymic cortex. Each cell must have an affinity **above a certain threshold** for a class I MHC/self-peptide or a class II/self-peptide displayed on **cortical epithelial cells**. This allows a protective signal to be sent, without which the cell dies by apoptosis.

While positive selection is going on, RAG1 and RAG2 and TdT continue to be expressed, and TCR α chain rearrangement can still occur. If a cell’s αβ-TCRs does not recognize self MHC/peptide with sufficient affinity, it may escape apoptosis if a second productive TCR α chain rearrangement occurs in time. It can then “retake the positive selection test,” and it may pass on the second try.

The text describes three experiments that provide evidence for positive selection:

1. Mice that lack class I genes due to “knockout” (i.e., targeted recombination) of the gene encoding β2-microglobulin have DN, DP and CD4+ class II specific thymocytes, but no CD8+ class I-specific thymocytes. Similarly, mice in which class II genes have been “knocked out” have DN, DP and CD8+ class II specific thymocytes, but do not develop CD4+ class II specific thymocytes.

2. Fetal thymus organ cultures (FTOC) are produced by dissecting the thymus from a fetal mouse at day 14 of gestation and placing it in tissue culture. The T cell precursors present in the fetal organ go on to develop into T cells. If an anti-class I monoclonal antibody is added to the culture, CD8+ class I-specific thymocytes fail to develop. If an anti-class II monoclonal antibody is added, no CD4+ class II specific thymocytes develop (**Figure 10-6**).

3. Transgenic mouse strains are made that express in developing thymic T lymphocytes the cDNAs encoding an αβ-TCR that recognizes an influenza virus antigen together with an H-2k class I molecule. Expression of transgenes encoding a fully-rearranged αβ-TCR suppresses rearrangement at the mouse’s own TCR α and β chain loci just as we have discussed for allelic exclusion during normal immunoglobulin and T cell receptor locus gene rearrangement. The transgenes were placed on genetic backgrounds containing either the H-2k or the H-2d haplotype. Whereas a high proportion of immature DP thymocytes expressed the transgene in both strains, thymocytes expressing the transgene were found to mature into CD8+ T cells only in the H-2k strain – the only strain in which they could be positively selected.

**Negative selection**

If the αβ-TCR expressed on a cell that survives positive selection binds self-MHV/peptide on bone marrow-derived dendritic cells or macrophages in the thymic medulla too strongly, the cell is stimulated to undergo apoptosis. This is thought to protect the individual from autoimmunity that could result if this cell were permitted to enter the mature T cell population.

Evidence for negative selection comes from another experiment involving a transgenic mouse strain expressing transgenes encoding an already-rearranged αβ-TCR. The TCR recognizes H-Y, an antigen expressed only in males, together with H-2Dd. The haplotype of the transgenic strain is H-2k – the same as the specificity of the transgenic TCR. When thymocytes of male and female mice were tested for expression of the transgenic TCR, all four classes of thymocytes (DN, DP, CD4+ and CD8+) in females expressed it. The males, however, expressed the transgene in DN, DP and CD4+ thymocytes, but not in CD8+ thymocytes. The negative selection occurred in males but not females because only the males expressed H-Y antigen together with H-2Dd in the thymus.

**What determines whether positive or negative selection occurs?**

Alternative models are the **avidity hypothesis** and the **differential signaling hypothesis**.

**Avidity hypothesis** – the strength of the signal received by the thymocyte depends on both the affinity of the TCR for class I/peptide or class II/peptide and the density of the relevant MHC/peptide complex on the cells upon which
selection is occurring. Avidity not strong enough leads to no positive selection and “death by neglect” – no protective signal. Avidity too strong leads to negative selection. Avidity just right leads to survival.

**Differential signaling hypothesis** – the signal given to the thymocyte differs qualitatively, depending on whether the interaction with the MHC/peptide complex is strong or weak.

Experiments done with **fetal thymic organ cultures (FTOC)** provide evidence for both hypotheses. These experiments were performed by K. Hoquist, J. Jameson and M. Bevan and colleagues (see references, Chapter 10).

**Evidence in favor of the avidity hypothesis** - FTOCs were established using a fetal thymus from a strain of mouse mutant in the TAP transporters. Expression of class I MHC molecules on thymic stromal cells is very low, but expression is obtained upon addition of peptides. At low concentrations of peptides, both class I expression and CD8+ thymocytes were seen. At high concentrations of peptides, where greater amounts of MHC/peptide complexes were expressed, the number of CD8+ thymocytes declined – likely due to high avidity and resulting negative selection.

**Evidence in favor of differential signaling** – this experiment makes use of FTOCs using thymus from TAP-deficient strain that is also transgenic for a TCR that recognizes a class I MHC/peptide complex. Earlier studies in which variants of the peptide were tested for ability to activate T cells expressing that particular TCR showed that some peptides (called **agonists**) activated the T cell completely while others (called **antagonists**) gave only a partial activating signal. Results indicated that when no peptide was added, no CD8+ thymocytes expressing the transgenic TCR were obtained. When antagonist peptide, incapable of full activation, was added, transgenic TCR-positive CD8+ thymocytes were obtained. However, when agonist peptides capable of full activation were added, transgenic TCR-positive CD8+ thymocytes were absent, suggesting negative selection.

The issue of how positive and negative selection are accomplished is still not resolved.

Also not resolved is the question the mechanism by which DP thymocytes whose TCR is positively selected on class I shut off CD4 and become mature CD8+ T cells. Similarly, the mechanism by which DP thymocytes that recognize class II shut off CD8 and become mature CD8+ T cells.

**T**<sub>HELPER</sub> (T<sub>H</sub>) **Cell Activation**

The question we will address now is how a mature T cell circulating through the body becomes activated to participate in an immune response upon contact with the MHC/peptide complex recognized by its αβ-TCR. The result is that it leaves the G<sub>0</sub> state and enters the cell cycle, proliferates and generates **effector** cells that mediate the immune activity and **memory** cells that survive to give a stronger response if the invading organism or antigen ever returns. We will focus here on T<sub>H</sub> cells, though the process for T<sub>C</sub> cells is very similar.

**A general discussion of signal transduction**

**Signal transduction** is the process by which signals that impinge upon a cell lead to its activation. The text talks about “common themes of signal transduction,” and these are important to review.

1. **Involvement of a receptor.** For extracellular ligands that are either bound to another cell (e.g., a MHC/peptide complex) or are soluble but cannot enter the cell, the receptor will generally be a transmembrane protein or protein complex (e.g., the αβ-TCR/CD3 complex). In the case of lipid-soluble ligands like **steroids**, these penetrate the cell and bind to intracellular receptors to transduce a signal.

2. **The generation of second messengers.** Interaction of the receptor with an extracellular ligand activates in processes that generate, within the cell, other molecules that carry the signal further. Second messengers very common in immune cell activation are **diacylglycerol (DAG)** and **inositol-1,4,5-triphosphate (IP<sub>3</sub>)** that are generated by cleavage of **phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>)**, a phospholipid that is a constituent of the cell’s plasma membrane.
3. Action of protein kinases – enzymes that phosphorylate (or attach a phosphate group to) tyrosine, serine or threonine side chains of intracellular proteins – and protein phosphatases – enzymes that remove phosphate groups (or dephosphorylate) the same amino acids. Some of the kinases we will be concerned with are members of a large family called the src (pronounced “sark”) family (e.g., Lck, Fyn), while others, like ZAP-70, belong to a different family.

4. The induced assembly of intracellular complexes of proteins that move the signal inward to the nucleus and perhaps other cell organelles. Important in assembling such complexes are conserved domains shared by many proteins called SH2 and SH3 domains. SH2 domains of proteins mediate binding of the protein to a region containing phosphotyrosine on another protein, often in a way that is specific for the target protein. SH3 domains of proteins mediate binding to proline-rich regions of a target protein, often specifically. Some proteins referred to as adaptor proteins appear to be made up only of Sh2 and SH3 domains, and these have as their only function the ability to link two or more proteins of a multi-protein complex together.

5. Signal cascades, in which a signal that impinges upon one enzyme is amplified by that enzyme’s action upon multiple molecules of the next protein in the cascade, and onward to a third protein, etc.

6. Involvement of different types of G proteins – polypeptides that are active when they bind GTP (or guanosine triphosphate) and inactive when that GTP is either hydrolyzed to GDP or the GTP is exchanged for a GDP. Additional proteins are involved in this process that are referred to as GAPs (for guanosine nucleoside activating proteins) or GEFs (guanosine nucleoside exchange factors). We will not talk about the large or small G proteins in any detail, but will refer to two small G protein, called Ras and Rac that pass signals on to two important cascades within the cell.

**Summary of T_H cell signal transduction**

Refer to Figures 10-11 and 10-12 as you consider these steps.

1. The process generally begins in a secondary lymphoid organ when a T_H cell’s TCRs encounter MHC class II/peptide complexes that are bound with sufficient avidity to initiate the process of T cell activation. The αβ-TCRs and their associated CD3 complexes cluster together upon encountering and binding MHC class II/peptide. CD4 binds to a constant portion of the class II molecules and brings to the complex the Lck kinase associated with its cytoplasmic domain. Also, CD45 (also known as leukocyte common antigen) joins the complex, and its intracellular domain consists of two tyrosine phosphatase domains. Finally, among the molecules we will be discussing, the src family tyrosine kinase, Fyn, joins the complex. Interestingly, both Lck and Fyn are able to by modified at their amino-termini to contain a lipid group – a palmitoyl group for Lck, and a myristoyl group for Fyn. These appear to be necessary for successful T cell activation, and likely function to help Lck and Fyn associate with the plasma membrane.

2. All of the above components as well as others appear to cluster in what appear to be detergent-resistant domains of the plasma membrane referred to as rafts. This compartment of the plasma membrane is recognized by its resistance to dissociation by a 0.5% solution of the detergent, Triton X-100, and 1 mM sodium vanadate (Na3VO4). Other proteins in the rafts are receptors whose signal transduction capabilities are mediated through G proteins, as well as proteins whose association with the membrane by means of GPI (or glycosylphosphatidylinositol) linkages. The αβ-TCRs and their associated CD3 and αβ complexes must be recruited to these rafts for T cell activation to occur. This localized accumulation of molecules is referred to as an immunological synapse, and its structure is still being deciphered. One interesting aspect relates to a point we made in Figure 9-3 where we said the αβ-TCRs are rather small and do not extend far from the cell surface. It appears that the molecules involved in association of a T cell with an APC arrange themselves hierarchically in the immunological synapse, with “short” molecules like the αβ-TCRs and the class II MHC/peptide complexes on the opposing cells towards the middle of the synapse. ‘Taller” molecules like CD45 and adhesion molecules (see Figure 9-13b) towards the outer regions of the synapse.

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3. As shown in Figures 10-11 and 10-12, CD45 uses its phosphatase activity to remove inhibitory phosphates from Lck and Fyn. As a result, these kinases phosphorylate ITAMs on the CD3 and αβ cytoplasmic domains, and the tyrosine kinase, ZAP-70, associates with the ITAMs of αβ using its SH2 domains. ZAP-70 then is phosphorylated by Lck or Fyn, and in this activated form, it transfers the activation signal to several different pathways within the cell.

4. The best known of these pathways involves the phosphorylation of phospholipase Cγ (PLCγ). The resulting phosphorylated PLCγ is associated with the rafts. The phosphorylated PLCγ then cleaves membrane phosphatidylinositol-4,5-bisphosphate (PIP2) to DAG and IP3, which act as second messengers.

1. The DAG activates protein kinase C, a serine kinase that, among other things, phosphorylates a protein in the cytoplasm called IκB. IκB is complexed with and thereby inactivates a potent transcription factor called NF-κB. The phosphorylated IκB dissociates from NF-κB, becomes ubiquitinated, and is destroyed by proteasomes. The released free NF-κB travels to the nucleus where it participates in gene activation.

2. The IP3 triggers a rise in the intracellular concentration of calcium ion (Ca^{2+}) both by causing its release from intracellular stores in the endoplasmic reticulum and by stimulating influx from outside the cell. This happens very soon after αβ-TCR crosslinking (Figure 10-10). Among a number of consequences of the increased intracellular Ca^{2+} concentration is the activation of the regulatory protein calmodulin. One important function of calmodulin is to activate an intracellular serine phosphatase called calcineurin, which dephosphorylates NF-ATc-phosphate, the inactive form of the transcription factor, NF-AT, that resides in the cytoplasm. Once dephosphorylated, the NF-AT travels to the nucleus where it participated in activation of the gene encoding IL-2, an important cytokine, as well as other genes.

Interference with the activation of NF-AT is an important means by which drugs are used to inhibit the immune system in patients receiving tissue transplants. The drug, cyclosporin A (CsA), binds to an intracellular protein called cyclophilin. The CsA/cyclophilin complex inhibits calcineurin, resulting in its inability to dephosphorylate NF-ATc-phosphate. Without NF-AT, gene activation is incomplete, and T cell activation is inhibited. The immunosuppressed patient will now be less likely to reject his or her tissue graft, though may be more susceptible to infection.

3. Besides cleavage of PIP2 and generation of the second messengers DAG and IP3, other events occur that lead to cascades of intracellular activity. “Docking proteins” such as grb-2 also bind to phosphorylated ITAMS using the SH2 domains and lead to activation of a protein kinase cascade called the Ras/MAP kinase pathway. The net effect of this cascade is the phosphorylation and activation of members of the Fos and Jun families of transcription factors that form AP1 heterodimers, important in gene activation.

The importance for T cell activation of stimulation of protein kinase C by DAG and increase in intracellular Ca^{2+} concentration caused by IP3 is underlined by the fact that achievement of these conditions by artificial means actually sets the activation machinery in motion. Addition to resting T cells of ionomycin, a calcium ionophore that allows entry of Ca^{2+} into the cells, and phorbol ester (actually, phorbol myristate acetate), an activator of protein kinase C, causes them to enter the cell cycle and exhibit a number of characteristics of T cell activation.

The steps described above and in Figures 10-11 and 10-12 only provide a hint of the complex and inter-related steps that are part of T cell activation. Other cascades that we will not discuss are known to be involved, and signals delivered through other surface receptors influence the outcome of the activation process. We will now discuss the necessity for a co-stimulatory signal that is required for full T cell activation.
The co-stimulatory signal delivered through CD28

Interaction of a resting class II-specific T cell’s αβ-TCRs with class II MHC/peptide on an APC provides the initial signal for T cell activation. However, if this is the only signal the T cell receives, the cell is rendered anergic – it is still alive, but unresponsive to antigen (see Figure 10-15). To be activated, the T cell must receive a second signal, and that is delivered through CD28, a homodimeric transmembrane molecule on the T cell surface (see Figures 10-14 and 10-15).

To deliver the second signal, CD28 must interact with a member of the B7 family of ligands on the surface of APCs – B7-1 or B7-2, monomeric members of the immunoglobulin supergene family that are expressed only on professional APCs. Dendritic cells always express B7-1 and B7-2, and activated (but not resting) macrophages and B cells do as well.

The pathway of signal transduction through CD28 is not well understood, but one result is phosphorylation of Jun kinase, and phosphorylated Jun participates in formation of the transcription factor, AP-1 (see Figure 10-12).

What is the importance of this “second signal?” Its main importance is that there be a check on T cell activation so that when it occurs, it is intentional. We said earlier that expression of class II MHC molecules can be induced to occur on non-professional APCs such as endothelial cells that form the walls of blood vessels. If only one signal were needed for activation of TH cells, T cell activation might be induced upon contact and the secretion of cytokines might lead to inflammation and damage. The requirement for a second signal through CD28 and the fact that CD28 is not expressed at such sites lessens the chance of this happening.

In summary, the requirement for a second signal restricts TH activation to the surface of an APC that expresses both class II MHC molecules and CD28.

Negative regulation by CTLA-4

TH cells also express a CD28-related molecule called CTLA-4 that also binds B7-1 and B7-2 with an affinity that is much higher than that for CD28. CTLA-4 in resting TH cells is stored intracellularly, but it is transported to the cell surface shortly after the TH cell receives an activating stimulus. CTLA-4 interaction with B7-1 or B7-2 delivers a negative signal to the TH cell. Thus the means to put the brakes on the TH cell response appears very quickly following activation.

The braking action by CTLA-4 does not take effect until the positive response has run a reasonable course. However, since CTLA-4 has a much higher affinity for the B7 ligands than CD28, there has to be a large amount of B7 expressed on dendritic cells and activated APCs to overcome the effect of CTLA-4’s braking action and allow T cell activation to continue.

Negative regulation of T cell activation by CTLA-4 is very important. Mice in which the CTLA-4 gene has been “knocked out” die at 3-4 weeks of age due to massive T cell lymphoproliferation.

Thus TH cell activation in the body must be carefully regulated. Two reagents to interfere with co-stimulation are being tested as agents that might be used to modify immune reactivity when desirable.

1. A monoclonal antibody directed against CTLA-4. Such an antibody might block stimulation through CTLA-4 and delay or abolish the signal for negative regulation. This has been considered as an agent to enhance the T cell response against tumors.

2. An artificial fusion protein consisting of the extracellular domains of CTLA-4 fused to the Fc portion of an immunoglobulin (CTLA-4-Ig). This reagent might block the B7-1 and B7-2 on dendritic cells and activated APCs and thereby inhibit immune stimulation of T cells. This might be used in instances of autoimmunity.
Activation of TH cells by superantigens

Superantigens are protein molecules that activate TH cells directly without requiring that the T cell’s αβ-TCR specifically recognize a class II MHC/peptide combination on the APC. Superantigens bypass the need for specific recognition by binding non-covalently to both a site on the class II molecules on APCs and to a portion of the Vβ region of certain αβ-TCRs (Figure 10-16). Such binding brings the class II and αβ-TCRs together and TH cell activation occurs.

Different superantigens recognize differed Vβ regions, and all TH cells bearing those Vβ regions become activated. As a result, responses to superantigens can be quite strong, since as many as 10% of an individual may become activated upon encounter with a particular superantigen.

Superantigens can be subdivided into two general classes:

1. **Exogenous superantigens**, such as the exotoxins manufactured by gram-positive bacteria such as staphylococcal enterotoxins, toxic shock syndrome toxin (TSST-1). These cause release of potent cytokines that lead to system-wide toxicity and major symptoms;

2. **Endogenous superantigens**, such as the minor lymphocyte stimulating (Mls) antigens encoded in the genomes of certain strains of mice. These endogenous superantigens are expressed on APCs, and they have the effect of causing deletion of T cells whose αβ-TCRs contain certain classes of Vβ regions. This deletion is accomplished by negative selection in the thymus, where crosslinking of class II molecules and Vβ regions by the endogenous superantigen (also expressed on the APCs) gives too strong a signal to the developing TH cell. The cell dies by apoptosis, just as a self-reactive, potentially autoimmune T cell is eliminated during normal thymic negative selection. The result is absence from the peripheral T cell population of T cells with αβ-TCRs containing the affected Vβ regions.

Time course of TH cell activation and differentiation to effector cells

When the naïve TH cell encounters class II/peptide complexes recognized by its αβ-TCR together with CD28 on an appropriate APC, it initiates a primary response. Within 48 hours it enlarges to become a “blast,” and it secretes the cytokine, IL-2, and expresses the high affinity IL-2 receptor on its surface. The autocrine signaling loop of IL-2 and the IL-2R causes the cell to undergo multiple rounds of proliferation.

This generates relatively short-lived effector cells that participate in immune reactions such as helper activity (the TH2 subset) and delayed type hypersensitivity (the TH1 subset) of CD4-positive cells, or cytotoxic killing (for CD8-positive Tc cells) (Figure 10-17). It also generates memory cells that persist in the body and give a quicker, stronger response if the body is challenged by the same organism again. There is evidence to suggest that antigen actually persists in the body as well and is necessary to keep the memory cells alive. Memory cells do not display the same patterns of circulation in the body as naïve cells. We will come back to this later.

Final notes

I will not discuss certain topics in lecture, but you should make note of them in your studying. They are as follows:

1. A good comparison of the activities of different APCs with respect to T cell activation is presented in Figure 10-18.

2. When T cells are no longer needed, they die by apoptosis. This will be discussed further in Chapters 14 and 15. Briefly, however, inside the thymus, apoptosis is stimulated by glucocorticoid hormones and, experimentally, by ionizing radiation. TCR-mediated apoptosis can also be induced, and this appears to require a family of receptors called Nur77/Nor-1 to occur. Outside the thymus, however, the Fas pathway is utilized to induce apoptosis, or activation induced cell death (AICD). Components of this pathway include a receptor (Fas) and a ligand (FasL). Genetically-engineered mice that either lack a functional Fas gene (Fas—) or a FasL
gene exhibit **massive lymphoproliferative disorders** due to failure of activated T lymphocytes to undergo apoptosis when they are no longer needed. This is summarized in the **Clinical Focus** section on pp. 262-263 of the Kuby text.

3. Finally, we do not really understand the function of T cells bearing γδ-TCRs (the so-called **γδ-T cells**). Large numbers of them are found in the skin (**intraepidermal lymphocytes**) and in the intestinal epithelium (**intraepithelial lymphocytes** or **IELs**). The intraepidermal lymphocytes do not express either CD4 or CD8, while IELs express CD8. This is surprising since CD8 is a coreceptor for class I MHC molecules, and γδ-T cells have no known involvement in MHC-associated recognition. Though there are some hints as to what they do, the function of these cells remains to be clearly elucidated.