COURSE: Medical Microbiology (MBIM 650/720

Lecture 12

TOPIC: Response to Antigen: Processing and Presentation MHC Restriction and Role of the Thymus

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TEACHING OBJECTIVES: Review of different types of antigen recognized by T and B cells
Cell biology and significance of different pathways for antigen processing and presentation by class I and class II MHC
Experimental basis for self MHC restriction
Role of the thymus in determining T cell receptor repertoire
Superantigens as anomalous antigens

SUGGESTED READING: Roitt, Brostoff, Male, 6th Edition, Mosby, 2001 Chapter 6, pp. 106-112, 117; Chapter 12, pp. 192-197

I. REVIEW OF B AND T CELL RECEPTORS FOR ANTIGEN

B cells and T cells recognize different substances as antigens and in a different form. The B cell uses cell surface-bound immunoglobulin as a receptor and the specificity of that receptor is the same as the immunoglobulin that it is able to secrete after activation. B cells recognize the following antigens in **soluble** form: 1) **proteins** (both conformational determinants and determinants exposed by denaturation or proteolysis), 2) **nucleic acids**, 3) **polysaccharides**, 4) some **lipids**, and 5) small chemicals (**haptens**).

In contrast, the overwhelming majority of antigens for T cells are **proteins**, and these must be **fragmented** and recognized in association with MHC products expressed on the surface of nucleated cells, **not** in soluble form. T cells are grouped functionally according to the class of MHC molecules that associate with the peptide fragments of protein: **helper T cells recognize only those peptides associated with class II MHC molecules, and cytolytic T cells recognize only those peptides associated with class I MHC molecules**.

II. ANTIGEN PROCESSING AND PRESENTATION

Antigen processing and presentation are processes that occur within a cell that result in fragmentation (**proteolysis**) of proteins, **association of the fragments with MHC molecules**, and where they can be recognized by the T cell receptor on a T cell. However, the path leading to the association of protein fragments with MHC molecules differs for class I and class II MHC. **MHC class I molecules present degradation products derived from intracellular (endogenous) proteins in the cytosol. MHC class II molecules present fragments derived from extracellular (exogenous) proteins that are located in an intracellular compartment.**

1. **Antigen processing and presentation in cells expressing class I MHC.**

All nucleated cells express class I MHC. As shown in Figure 1, proteins are fragmented in the cytosol by **proteosomes** (a complex of proteins having proteolytic activity) or by other proteases. The fragments are then transported across the membrane of the endoplasmic reticulum by **transporter proteins**. (The transporter proteins and some components of the proteosome have their genes in the MHC complex). Synthesis and assembly of class I heavy chain and beta2 microglobulin occurs in the endoplasmic reticulum. Within the endoplasmic reticulum, the MHC class I heavy chain, beta2microglobulin and peptide form a stable complex that is transported to the cell surface.

2. **Antigen processing and presentation in cells expressing class II MHC.** Whereas all nucleated cells express class I MHC, only a limited group of cells express class II

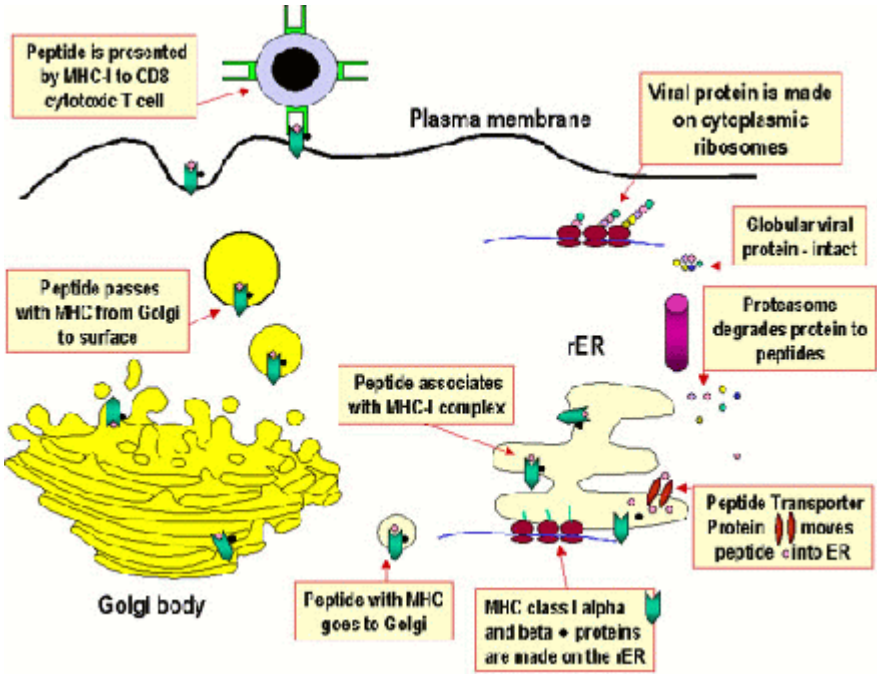

Figure 1: Pathway of class I MHC restricted presentation of an endogenously synthesized antigen. An example of such an antigen would be a viral protein made in the cell as a result of infection.

MHC, which includes the antigen presenting cells (APC). The principal APC are macrophages, dendritic cells (Langerhans cells), and B cells, and the expression of class II MHC molecules is either constitutive or inducible, especially by interferon-gamma in the case of macrophages.

As shown in Figure 2, exogenous proteins taken in by endocytosis are fragmented by proteases in an endosome. The alpha and beta chains of MHC class II, along with an invariant chain, are synthesized, assembled in the endoplasmic reticulum, and transported through the Golgi and trans-Golgi apparatus to reach the endosome, where the invariant chain is digested, and the peptide fragments from the exogenous protein are able to associate with the class II MHC molecules, which are finally transported to the cell surface.

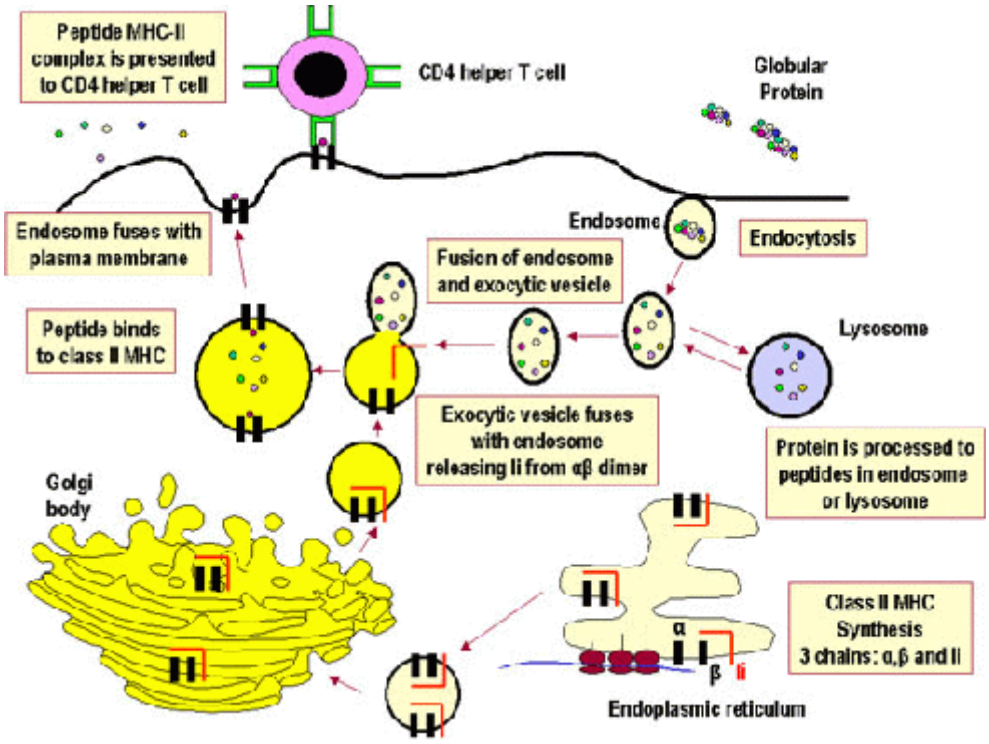

Figure 2: Pathway of class II MHC-restricted presentation of an exogenous antigen

3. Other points concerning antigen processing and presentation

   a. One way of rationalizing the development of two different pathways is that each ultimately stimulates the population of T cell that is most effective in eliminating that type of antigen.

      Viruses replicate within nucleated cells in the cytosol and produce **endogenous** antigens that can associate with class I MHC. By killing these infected cells, **cytolytic T cells** help to control the spread of the virus.

      Bacteria mainly reside and replicate extracellularly. By being taken up and fragmented inside cells as **exogenous** antigens that can associate with class II MHC molecules, **helper Th2 T cells** can be activated to assist B cells to make antibody against bacteria, which limits the growth of these organisms.

      Some bacteria grow intracellularly inside the vesicles of cells like macrophages. **Inflammatory Th1 T cells** help to activate macrophages to kill the intracellular bacteria.

   b. Fragments of **self**, as well as **non-self**, proteins associate with MHC molecules of both classes and are expressed at the cell surface.

   c. Which protein fragments bind is a function of the chemical nature of the groove for that specific MHC molecule.

III. SELF MHC RESTRICTION

In order for a T cell to recognize and respond to a foreign protein antigen, it must recognize the MHC on the presenting cell as **self** MHC. This is termed **self MHC restriction**. Helper T cells recognize antigen in context of class II **self** MHC. Cytolytic T cells recognize antigen in context of class I **self** MHC. The process whereby T cells become restricted to recognizing self MHC molecules occurs in the **thymus**.

The experimental systems demonstrating self MHC restriction for APC-helper T cell interactions and for class I MHC-cytotoxic T cell interactions are shown in Figures 3 and 4, respectively.

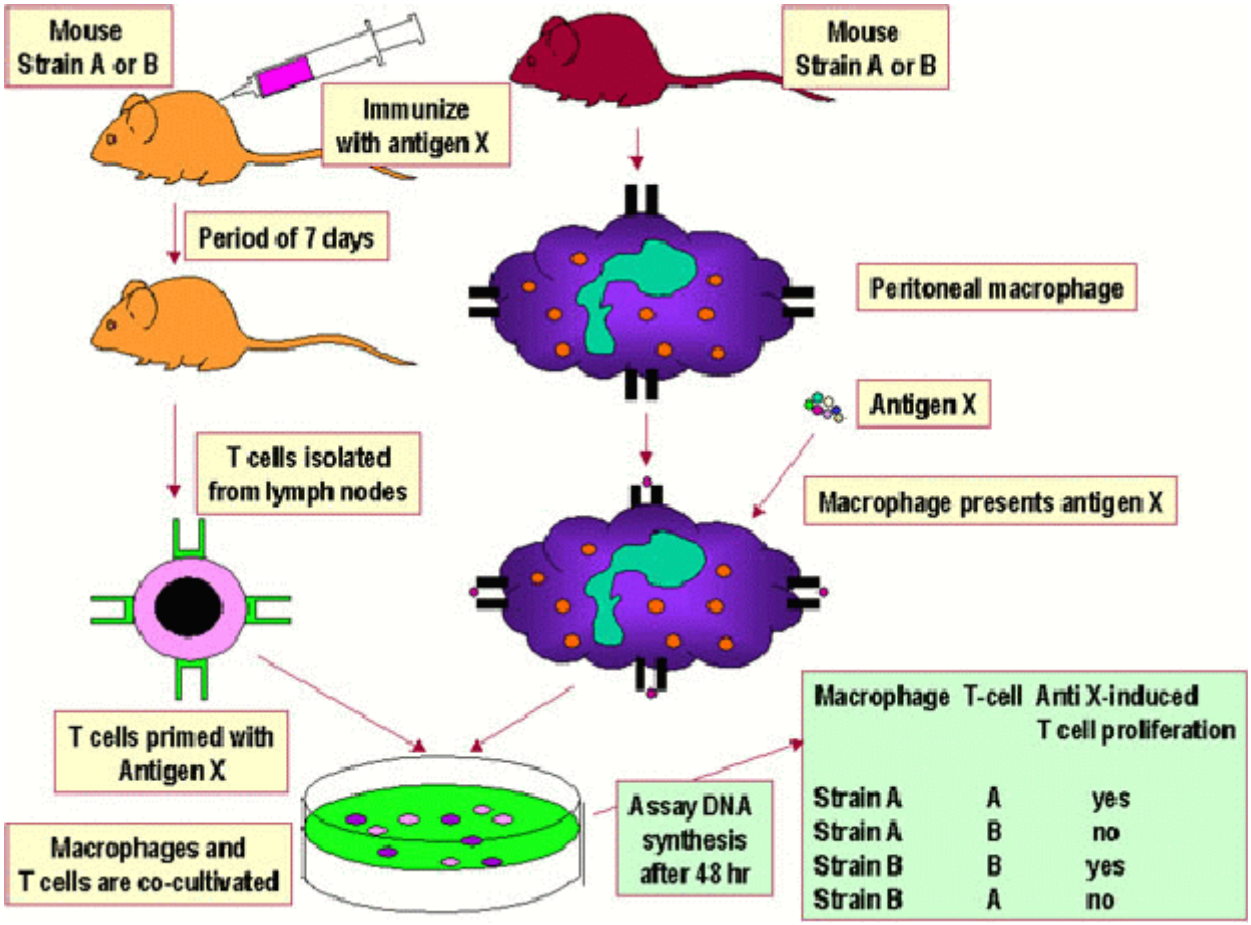

Figure 3: T cells from strain A or strain B mouse primed with antigen X proliferate in response to that antigen only in the being there of strain A or strain B antigen_presenting cells (macrophages in this figure)

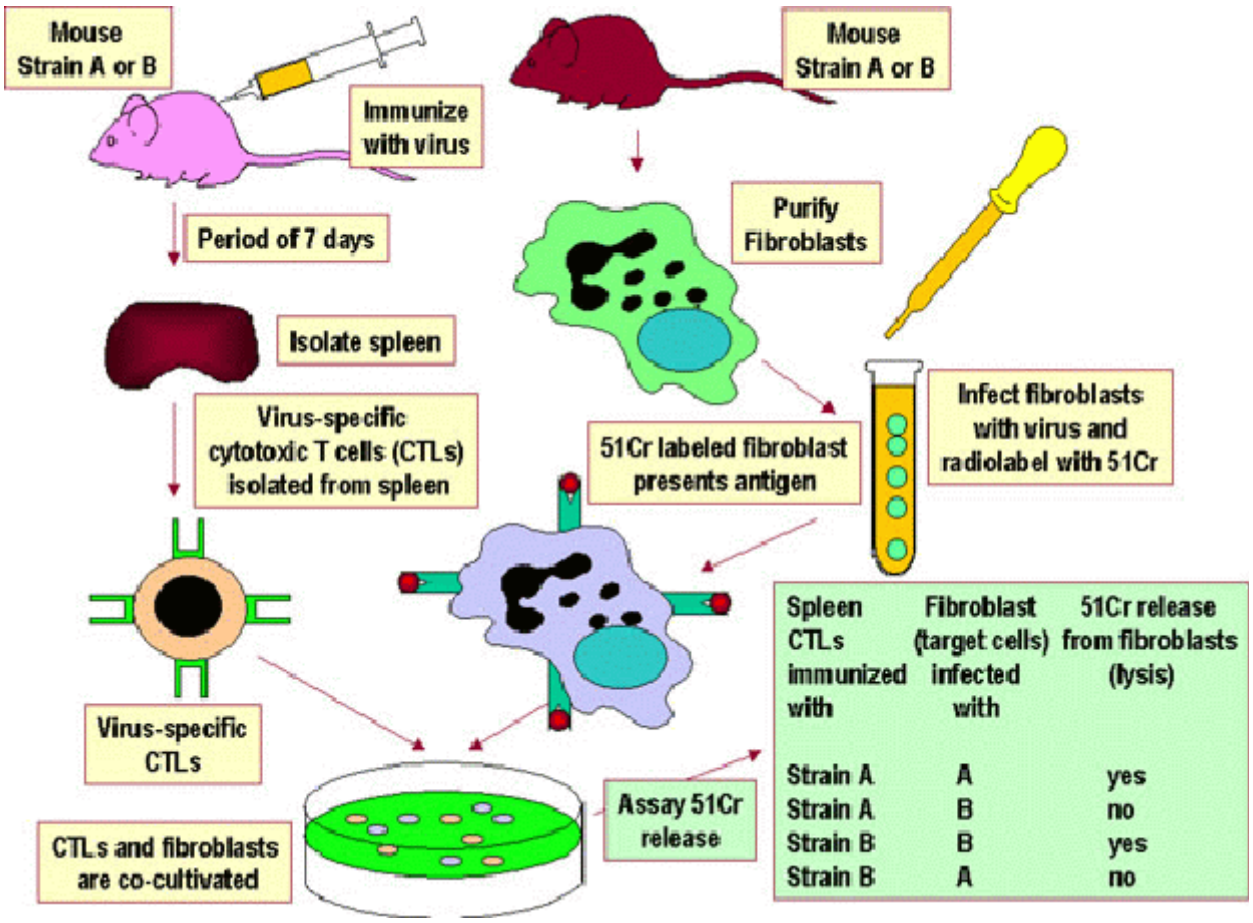

Figure 4: Virus-specific CTLs from a strain A or strain B mouse lyse only syngeneic target cells infected with a specific virus. The CTLs do not lyse uninfected target cells and are not alloreactive. Further analysis has shown that the CTLs and target cells must come from animals that share class I MHC alleles in order for the target to present viral antigens to the CTLs.

IV. ROLE OF THYMUS

A. Functions

1. Site of T cell maturation
2. Determines specificity of the TCR expressed on the T cells released to periphery

B. $CD4^-$ $CD8^-$ cells from bone marrow mature in the thymus to become either $CD4^+$ (helper) or $CD8^+$ (cytolytic) T cells

C. Functional considerations of T cells to be released into the periphery

1. **Functional T cells** in the periphery have to recognize **foreign antigens associated with self MHC**, because APC or target cells present foreign antigen associated with self MHC.

2. Therefore, an individual does not need functional T cells in the periphery that recognize antigen (self or **foreign**) associated with **foreign** MHC.

3. An individual especially does **not** want functional T cells in the periphery that can recognize **self** antigens associated with **self** MHC because they could lead to damage of healthy, normal tissues.

D. As a result of genetic events occurring in immature T cells within the thymus, TCR of all specificities are produced. Processes in the thymus determine which TCR specificities are retained. There are two sequential steps shown in Figure 5.

1.

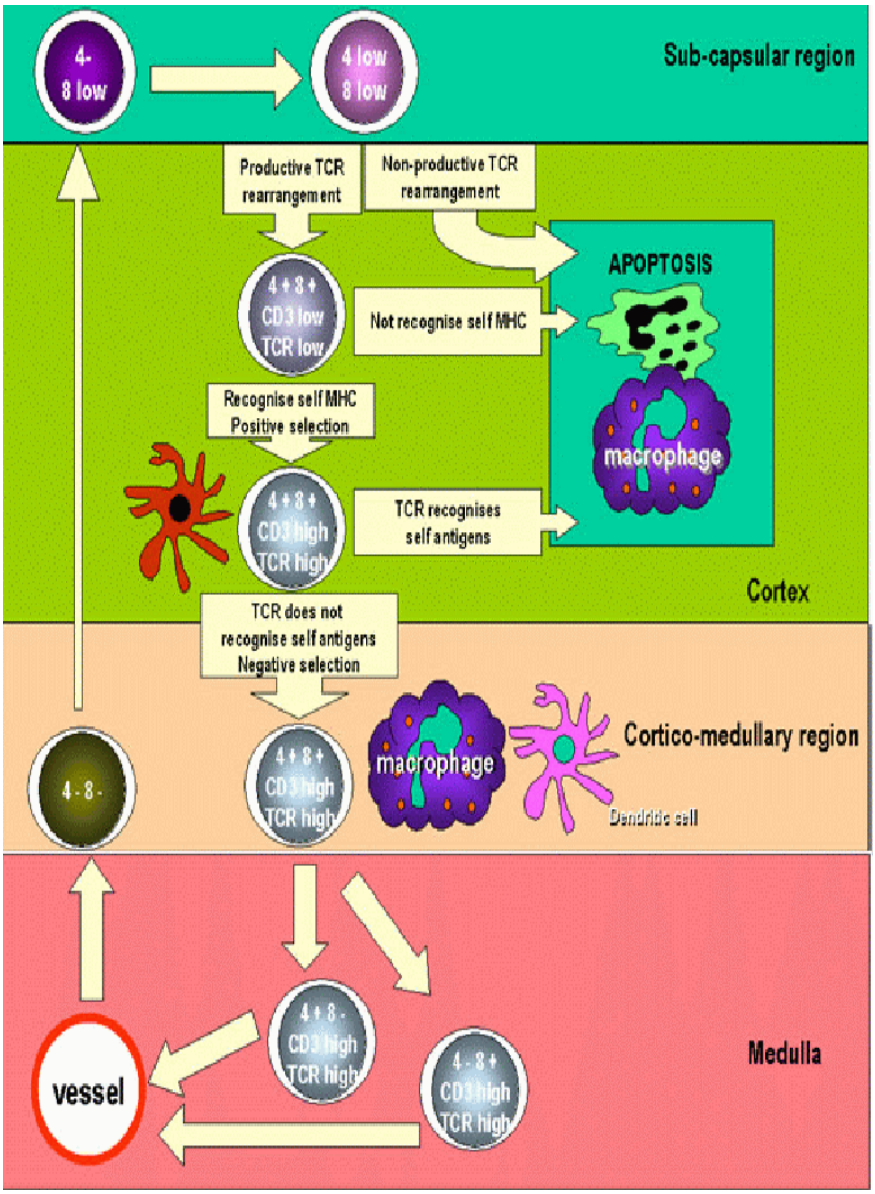

Figure 5: Prethymic T cells enter the thymus rudiment and proliferate as large lymphoblasts in the sub-capsular region of the thymus. The lymphoblasts replicate resulting in a pool of cells that differentiate. Here the cells become CD8 and CD4 positive but expression is low. TCR genes are also rearranged in these cells and the products may also be expressed on the cell surface at low levels. As the cells mature, they move into the cortex where they adhere to cortical epithelial cells which are long and branched, providing a large surface area to interact with other cells. TCRs on the surfaces of thymocytes interact with the MHC molecules on the epithelial cells leading to positive selection. The cells that are not selected are subject to apoptosis and are phagocytosed by macrophages. As the thymocytes migrate further into the cortex of the thymus, the expression of CD3, CD4, CD8 and TCR increases. TCRs with self-reactivity are deleted because of contact with autoantigens presented by dendritic cells and macrophages. This leads to negative selection. Cells that express CD4 or CD8 appear and migrate to the periphery by specialized vessels in the cortico-medullar region.

1. First, T cells with the ability to bind to **self MHC molecules** expressed by cortical thymic epithelial cells are **retained**. This is known as **positive selection**. Those that do not bind, die. Thus, T cells having a TCR that recognizes self MHC survive.

2. Next, T cells with the ability to bind to **self MHC molecules associated with self molecules** expressed by dendritic cells and macrophages are **killed**. This is known as **negative selection**. Those that do not bind are **retained**. As a result of these two steps, T cells having a TCR that recognizes self MHC and foreign antigen survive.

3. Each T cell that survives positive and negative selection in the thymus and is released into the periphery **retains its specific T cell receptor (TCR)**.

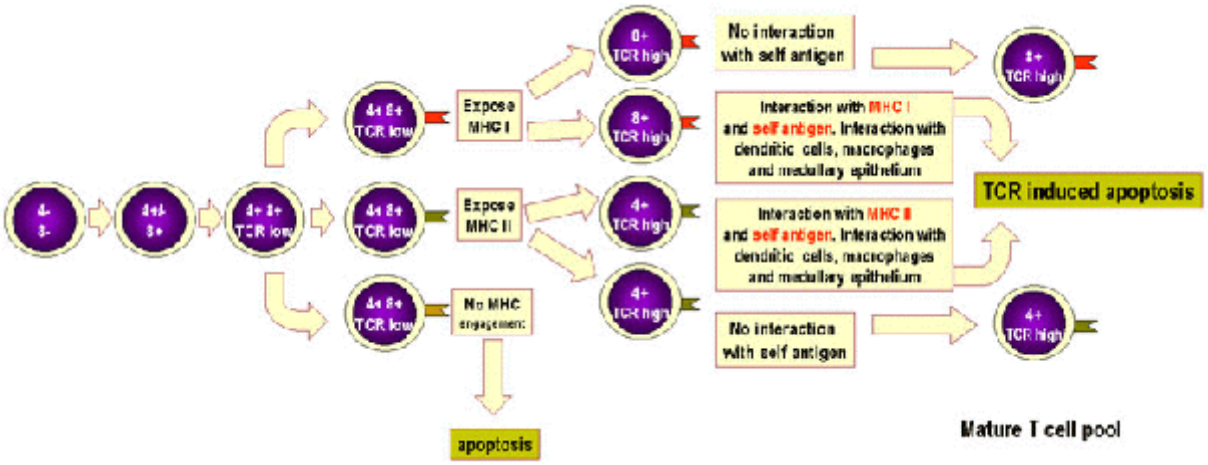

Figure 6: $CD4^-$ $CD8^-$ precursor thymocytes become double positive, $CD4^+$ $CD8^+$ cells expressing low levels of the alpha and beta chains of the T cell receptor (TCR). Positive selection for interaction with self MHC-I or MHC-II molecules occurs in the cortical epithelium. The majority of the cells are unselected and undergo apoptosis. The cells that remain either interact with MHC-I and lose their CD4 antigen or interact with MHC-II and lose their CD8 antigen. Autoreactive cells are then removed as a result of their interaction with self antigen peptides that are presented by cells in the corticomedullary junction and the medulla of the thymus.

V. SUPERANTIGENS

1. Name given to proteins produced by many pathogens, including bacteria, mycoplasma and viruses that are capable of stimulating large numbers of T cells

2. Most important are the bacterial toxins, especially staphylococcal enterotoxins, each of which can bind to the **variable region of the $ chain (V$) of the T cell receptor (TCR)**

3. Activation of T cells also requires that the superantigen bind to class **II MHC molecules on the surface of antigen presenting cells (APC)**; however they are not processed and presented by APC. Rather superantigens function as intact molecules.

4. Extent of T cell stimulation is a function of the frequency of T cells bearing V beta that can bind a specific superantigen. Each superantigen can bind one or a few of the different V beta regions, of which there are 20-50 in human, so a superantigen can activate large numbers of T cells.

5. Pathology due to large amounts of cytokines: produced by such a high frequency of activated T cells causing effects such as "septic shock" or "endotoxin shock".

The differences between conventional antigens and superantigens are shown in Figure 7.

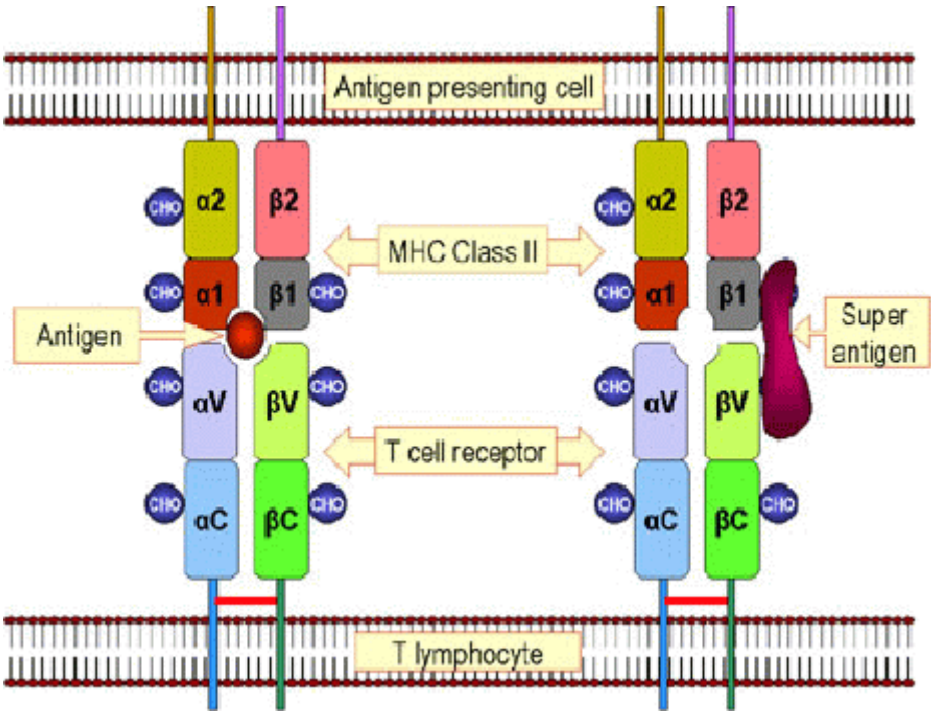

Figure 7: Differences between antigen and superantigen. Antigenic peptides are processed within the cell and presented on the cell surface in association with class II MHC molecules. They then trigger the T-cell receptor on a T lymphocyte. Superantigens are not processed but bind to the class II MHC protein and to the V beta chain of the T cell receptor. A given superantigen activates a distinct class of T cells that express a certain V beta chain.

Note: In the case of MHC II-TCR interaction with a normally processed peptide, recognition of the peptide on the MHC molecule requires V alpha, J alpha, V beta, D beta and J beta segments of the TCR. Such an interaction occurs at low frequency. In the case of MHC II-TCR interaction with an unprocessed superantigen, only a given V beta region is recognized. This clearly would occur at a much higher frequency.