TOPIC: Ag processing and presentation Lecture #12

TEACHING OBJECTIVES:

1. To compare and contrast Ag recognized by the TCR and BCR
2. To describe the pathways involved in processing endogenous and exogenous antigens
3. To discuss self MHC restriction in APCs
4. To compare and contrast presentation of conventional and superantigens
5. To discuss the role of positive and negative selection in the thymus in generation of self MHC restricted T cells

REQUIRED READING:

Male, *et al*. Immunology, 7th Ed., Cpt 7 and pp 40-48.

ANTIGEN PROCESS AND PRESENTATION

1) Comparison of BCR and TCR

a) 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; 5) small chemicals (haptens).

b) 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 cytotoxic T cells recognize only those peptides associated with class I MHC molecules.

2) Ag processing and presentation

a) 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 expression of the peptide-MHC molecules at the cell surface 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.

b) MHC class I pathway - 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 beta$_2$ microglobulin occurs in the endoplasmic reticulum. Within the endoplasmic reticulum, the MHC class I heavy chain, beta$_2$microglobulin and peptide form a stable complex that is transported to the cell surface.

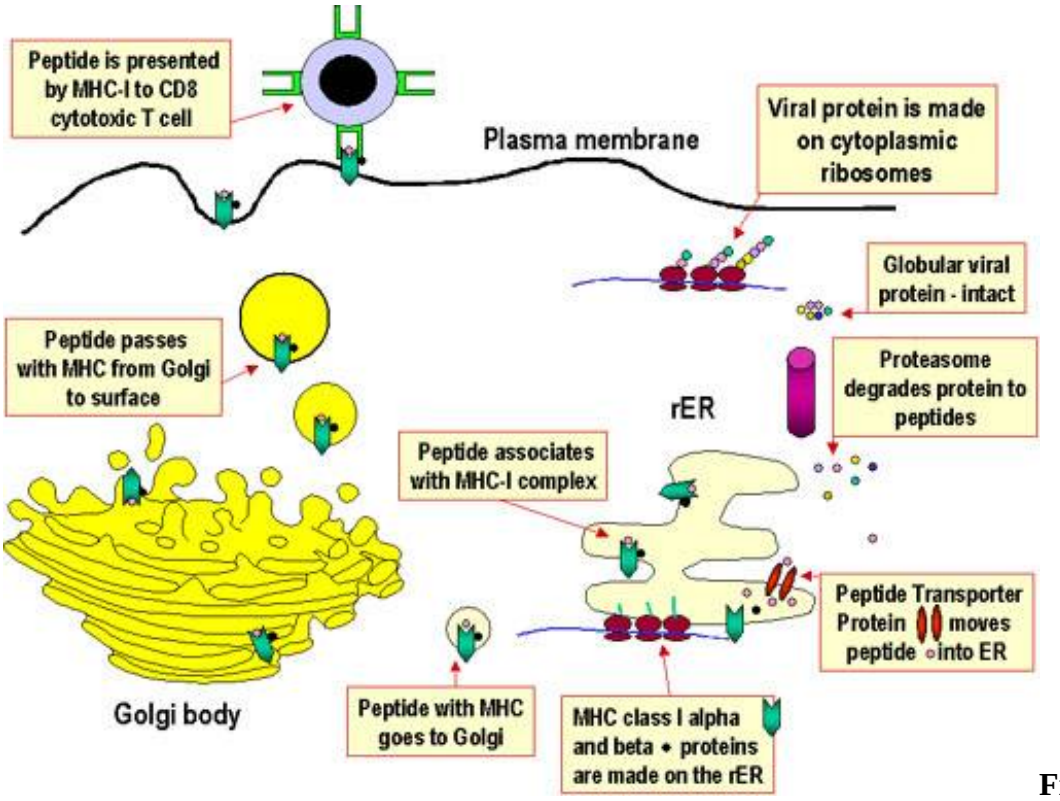

Fig 1.

c) MHC class II pathway - Whereas all nucleated cells express class I MHC, only a limited group of cells express class II 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.

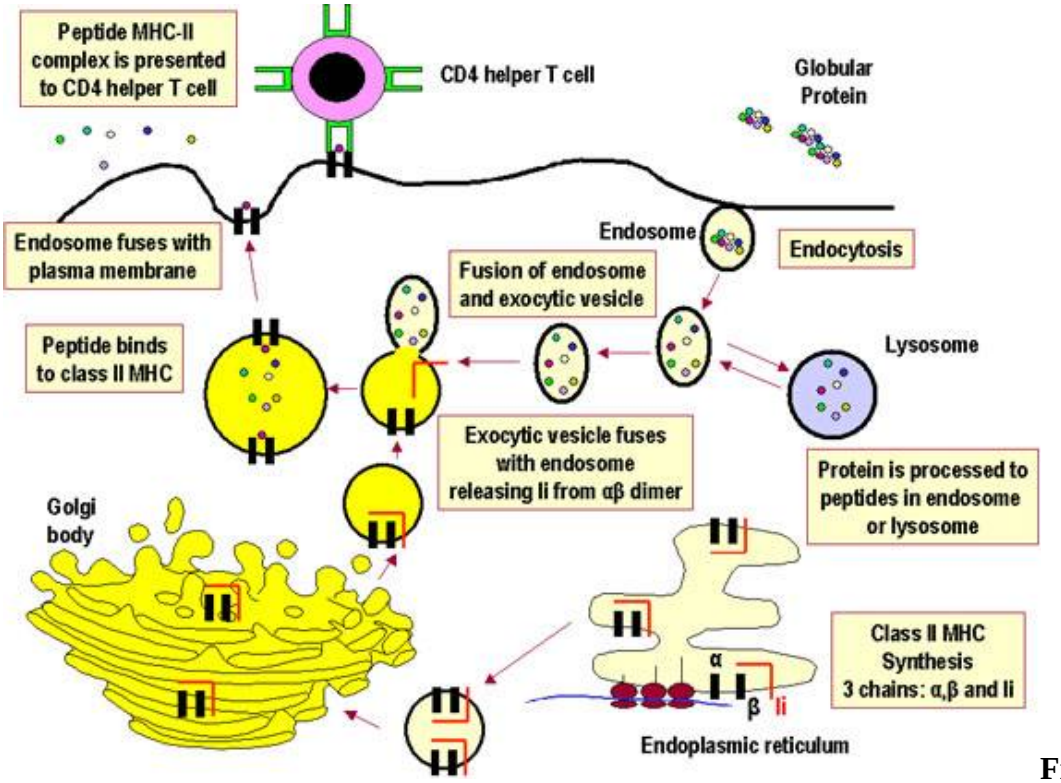

Fig 2.

d) Important aspects of Ag processing - One way of rationalizing the development of two different pathways is that each ultimately stimulates the population of T cells 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 MHC class I. By killing these infected cells, CTL 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 MHC class II 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. Fragments of self, as well as non-self, proteins associate with MHC molecules of both classes and are expressed at the cell surface. Which protein fragments bind is a function of the chemical nature of the groove for that specific MHC molecule.

3) Self MHC restriction

a) 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. CTL 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.

4) Ag presenting cells (APCs)

a) The three main types of antigen presenting cells are dendritic cells, macrophages and B cells, although other cells, that express class II MHC molecules, (*e.g.,* thymic epithelial cells) can act as antigen presenting cells in some cases. Dendritic cells, which are found in skin and other tissues, ingest antigens by pinocytosis and transport antigens to the lymph nodes and spleen. In the lymph nodes and spleen they are found predominantly in the T cells areas. Dendritic cells are the most effective antigen presenting cells and can present antigens to naïve (virgin) T cells. Furthermore, they can present internalized antigens in association with either class I or class II MHC molecules (cross presentation), although the predominant pathway for internalized antigen is the class II pathway. The second type of antigen presenting cell is the macrophage. These cells ingest antigen by phagocytosis or pinocytosis. Macrophages are not as effective in presenting antigen to naïve T cells but they are very good in activating memory T cells. The third type of antigen presenting cell is the B cell. These cells bind antigen via their surface Ig and ingest antigens by pinocytosis. Like macrophages these cells are not as effective as dendrite cells in presenting antigen to naïve T cells. B cells are very effective in presenting antigen to memory T cells, especially when the antigen concentration is low because surface Ig on the B cells binds antigen with a high affinity.

5) Presentation of superAg

a) Superantigens are antigens that can polyclonally activate T cells (see lecture on antigens) to produce large quantities of cytokines that can have pathological effects. These antigens must be presented to T cells in association with class II MHC molecules but the antigen does not need to be processed. In the case of a superantigen the intact protein binds to class II MHC molecules and to one or more V_β regions of the TCR. The antigen is not bound to the peptide binding groove of the MHC molecule or to the antigen binding site of the TCR. Thus, any T cell that uses a particular V_β in its TCR will be activated by a superantigen, resulting in the activation of a large numbers of T cells. Each superantigen will bind to a different set of V_β regions.

6) Thymic education

a) Both Th and CTL cells are self-MHC restricted. In addition, T cells do not normally recognize self antigens. How are self MHC restricted T cells generated and why are self reacting T cells not produced? Random VDJ rearrangements in T cells would be expected to generate some T cells that can recognize non-self MHC and some T cells that can recognize self antigens. It is the role of the thymus to ensure that the only T cells that get to the periphery are self-MHC restricted and unable to react with self antigen.

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

b) As a result of random VDJ recombination events occurring in immature T cells within the thymus, TCRs of all specificities are produced. Processes in the thymus determine which TCR specificities are retained. There are two sequential steps shown in Figure 3. 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, undergo apoptosis. Thus, T cells having a TCR that recognizes self MHC survive. Next, T cells with the ability to bind to self MHC molecules associated with self molecules expressed by thymic epithelial cells, 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. 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).

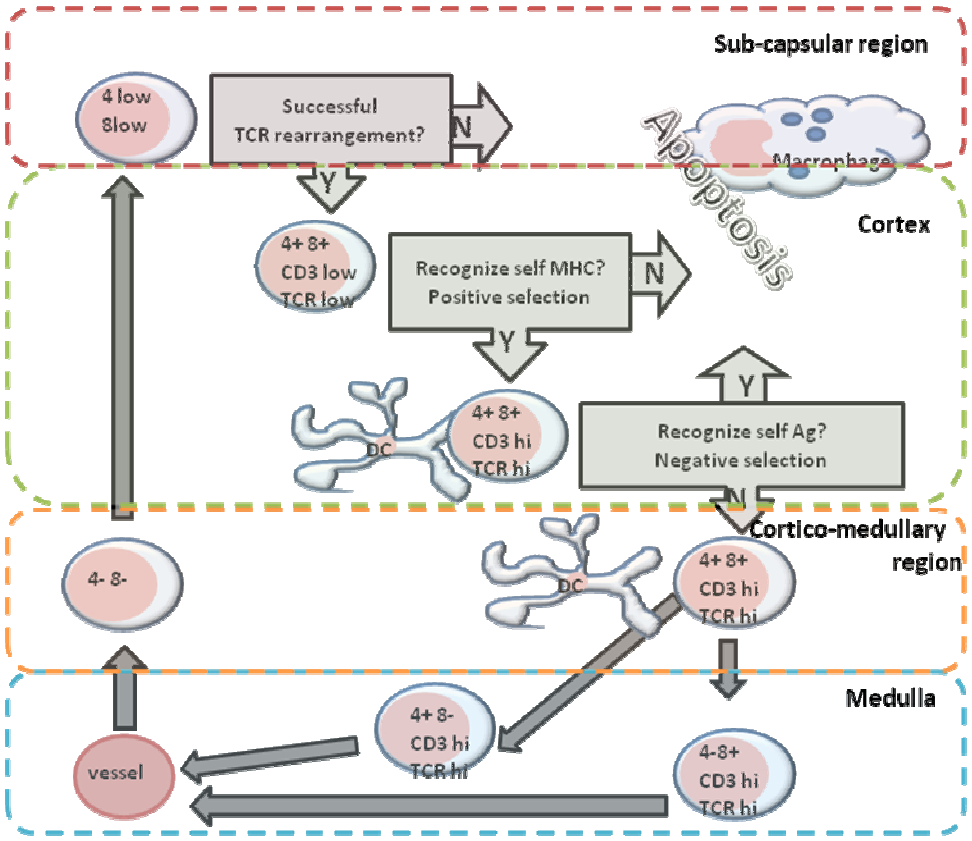

Figure 3.

c) While positive and negative selection is occurring in the thymus the immature T cells are also expressing CD4 or CD8 antigens on their surface. Initially the pre-T cell that enters the thymus is CD4-CD8-. In the thymus it becomes CD4+CD8+ and as positive and

negative selection proceed a cell becomes either a CD4+ or CD8+ cell. The commitment to become either a CD4+ or CD8+ cells depends on which class of MHC molecule the cell encounters. If a CD4+CD8+ cell is presented with a class I molecule it will down regulate CD4 and become a CD8+ cell. If a cell is presented with a class II MHC molecule it will down regulate CD8 and become a CD4+ cell (Figure 4).

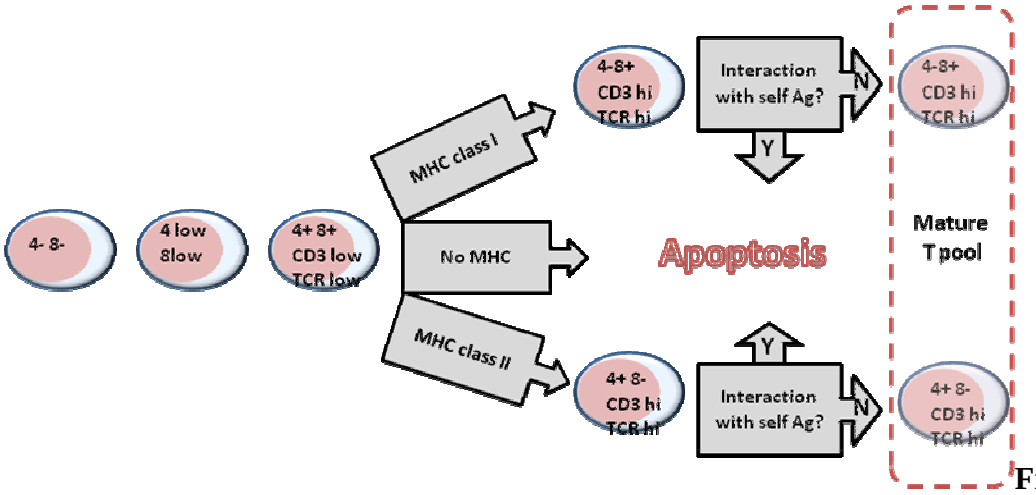

Figure 4.

7) Negative selection in the periphery

a) Positive and negative selection in the thymus is not a 100% efficient process. In addition, not all self antigens may be expressed in the thymus. Thus some self reactive T cells may get to the periphery. Thus, there are additional mechanisms that are designed to eliminate self reactive T cells in the periphery. These will be discussed in the tolerance lecture.

8) B cell selection

a) Since B cells are not MHC-restricted there is no need for positive selection of B cells. However, negative selection (i.e., elimination of self-reactive clones) of B cells is required. This occurs during B cell development in the bone marrow. However, negative selection of B cells is not a critical as for T cells since, in most instances, B cells require T cell help in order to become activated. Thus, if a self reactive B cell does get to the periphery it will not be activated due to lack of T cell help