COURSE: Medical Microbiology, MBIM 650 – Fall 2009

TOPIC: Cell-cell interactions in immune responses Lecture #13

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TEACHING OBJECTIVES:

1. To discuss the central role of Th cells in immune responses
2. To describe the cell-cell interactions which occur in 1) Ab responses to T-dependent Ag, 2) generation of CTL, and 3) activation of macrophage and NK cells
3. To discuss responses to T-independent Ag
4. To discuss the mechanisms of killing by CTL and macrophages

REQUIRED READING:

Male, *et al*. Immunology, 7th Ed., Cpt 8 and 10.

KEY WORDS:

Th1 cells, Th2 cells, Th17 cells, Hapten-carrier model, CD28, CD40, CD40 ligand, CD80, CD86, B1 cells, B2 cells, CTL, Fas ligand, Perforin, Granzymes, Caspases, IFN-γ, Activation.

CELL-CELL INTERACTIONS IN IMMUNE RESPONSES

1) Central role of Th cells in immune response

a) As depicted in Figure 1, after Th cells recognize specific antigen presented by an APC, they can initiate several key immune processes. These include: 1) selection of appropriate effector mechanisms (e.g., B cell activation or CTL generation); 2) induction of proliferation of appropriate effector cells and 3) enhancement of the functional activities of other cells (e.g., granulocytes, macrophages, NK cells).

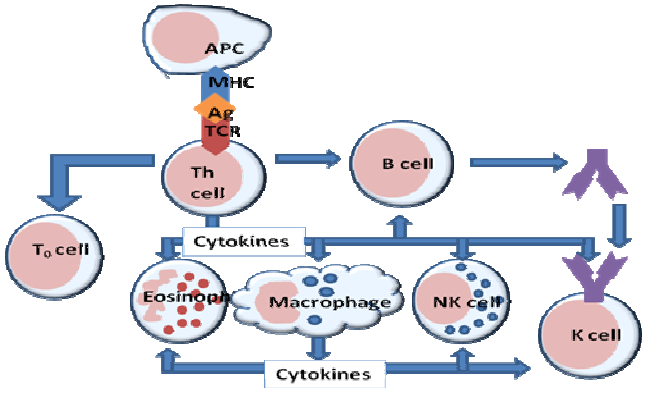


Fig 1.

b) There are four subpopulations of Th cells, Th0, Th1, Th2, and Th17 cells. When naïve Th0 cells encounters Ag in secondary lymphoid tissues, they are capable of differentiating into inflammatory Th1 cells, helper Th2 cells, or pathogenic Th17 cells, which are distinguished by the cytokines they produce (Figure 2). Whether a Th0 cells becomes a Th1, Th2, or Th17 cell depends upon the cytokines in the environment, which is influenced by Ag. For example some antigens stimulate IL-4 production which favors the generation of Th2 cells while other antigens stimulate IL-12 production, which favors the generation of Th1 cells. Th1, Th2, and Th17 cells affect different cells and influence the type of immune response, as shown in Figure 3 for Th1 and Th2.

i) Cytokines produced by Th1 cells activate macrophages and participate in the generation of CTL cells, resulting in a cell-mediated immune response.

ii) In contrast cytokines produced by Th2 cells help to activate B cells, resulting in antibody production. In addition, Th2 cytokines also activate granulocytes.

iii) A relatively recent discovery, Th17 cells (designated as such by their production of IL-17) differentiate (in humans) in response to IL-1, IL-6, and IL-23 (TGF-β is important for Th17 differentiation in mice, although not in humans). IL-17 enhances the severity of some autoimmune diseases including MS, inflammatory bowel disease, and rheumatoid arthritis.

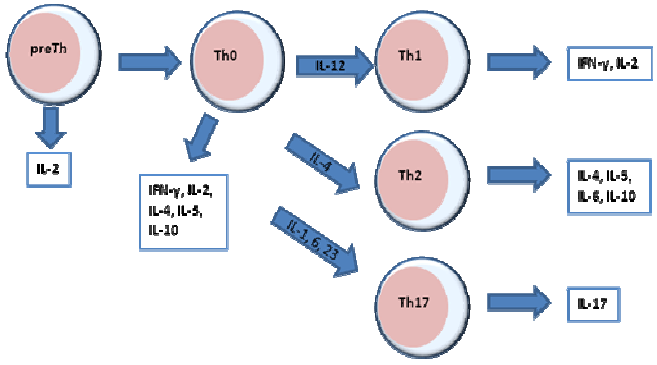


Figure 2.

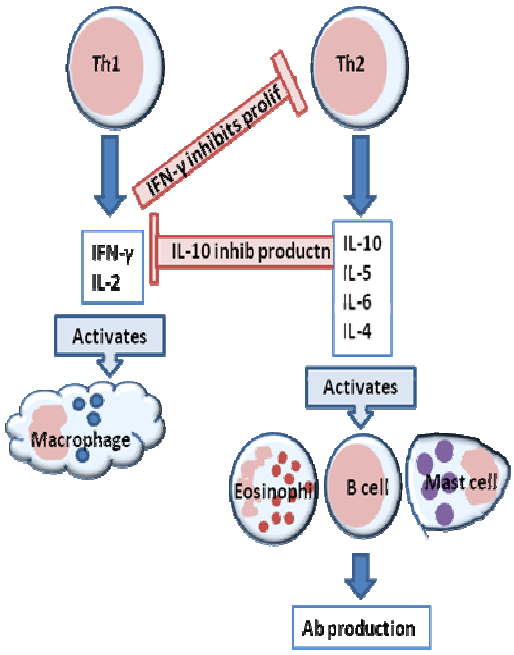


Figure 3.

c) Equally important, each subpopulation can exert inhibitory influences on the other. IFN-γ produced by Th1 cells inhibits proliferation of Th2 and differentiation of Th17 cells and IL-10 produced by Th2 cells inhibits production of IFN-γ by Th1 cells. In addition, although not shown, IL-4 inhibits production of Th1 and differentiation of Th17 cells.

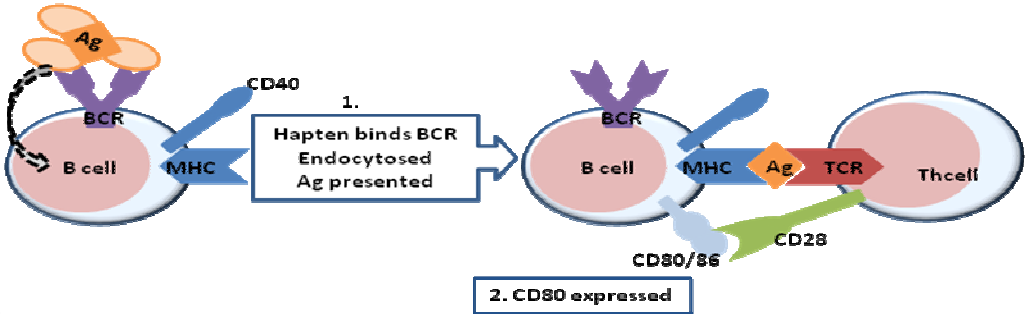
Thus, the immune response is directed to the type of response that is required to deal with the pathogen encountered – cell-mediated responses for intracellular pathogens or antibody responses for extracellular pathogens.

2) Cell-cell interactions in Ab responses to exogenous T-dependent Ag

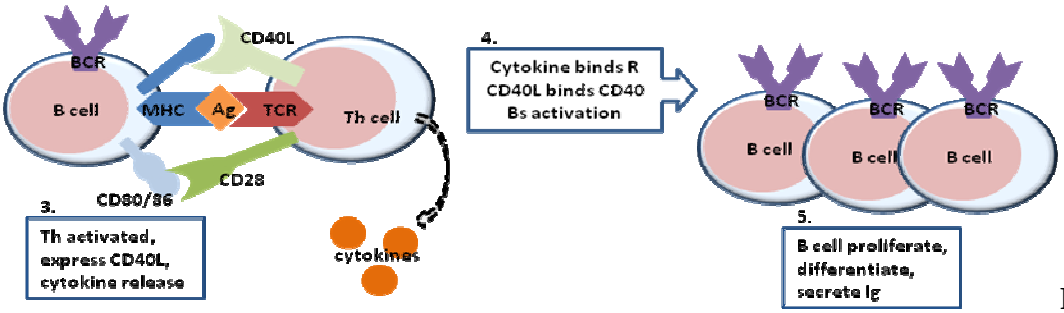
a) Hapten-carrier model:

i) Historically one of the major findings in immunology was that both T cells and B cells were required for antibody production to a complex protein. A major contribution to our understanding of this process came from studies on the formation of anti-hapten antibodies. Studies with hapten-carrier conjugates established that: 1) Th2 cells recognized the carrier determinants and B cells recognized haptenic determinants; 2) interactions between hapten-specific B cells and carrier-specific Th cells was self MHC restricted; and 3) B cells can function both in antigen recognition and in antigen presentation.

ii) B cells occupy a unique position in immune responses because they express immunoglobulin (Ig) and class II MHC molecules on their cell surface. They therefore are capable of producing antibody having the same specificity as that expressed by their immunoglobulin receptor; in addition they can function as an antigen presenting cell. In terms of the hapten-carrier conjugate model, the mechanism is thought to be the following: the hapten is recognized by the Ig receptor, the hapten-carrier is brought into the B cell, processed, and peptide fragments of the carrier protein are presented to a helper T cell (Figure 4A). Activation of the T cell results in the production of cytokines that enable the hapten-specific B cell to become activated to produce soluble anti-hapten antibodies (Figure 4 B).



A.



B.

Figure 4.

iii) Note that there are multiple signals delivered to the B cells in this model of Th2 cell-B cell interaction. As was the case for activation of T cells where the signal derived from the TCR recognition of a peptide-MHC molecule was by itself insufficient for T cell activation, so too for the B cell. Binding of an antigen to the immunoglobulin receptor delivers one signal to the B cell, but that is insufficient. Second signals delivered by co-stimulatory molecules are required; the most important of these is CD40L on the T cell that binds to CD40 on the B cell to initiate delivery of a second signal.

b) Cell-cell interactions in primary Ab response

i) B cells are not the best antigen presenting cell in a primary antibody response; dendritic cells or macrophages are more efficient. Nevertheless, with some minor modifications the hapten-carrier model of cell-cell interactions described above also applies to interactions in a primary antibody response (Figure 5). In a primary response the Th2 cell first encounters antigen presented by dendritic cells or macrophages. The "primed" Th2 cell can then interact with B cells that have encountered antigen and are presenting antigenic peptides in association with class II MHC molecules. The B cells still requires two signals for activation – one signal is the binding of antigen to the surface Ig and the second signal comes from CD40/CD40 ligand engagement during Th2/B cell-cell interaction. In addition, cytokines produced by the Th2 cells help B cells proliferate and differentiate into antibody secreting plasma cells.

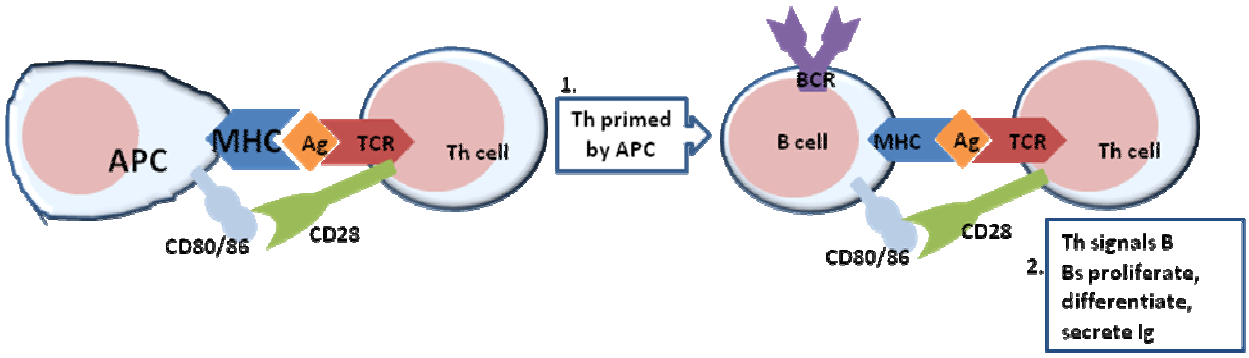


Fig 5.

c) Cell-cell interactions in secondary Ab response

i) As a consequence of a primary response, many memory T and B cells are produced. Memory B cells have a high affinity Ig receptor (due to affinity maturation), which allows them to bind and present antigen at much lower concentrations than that required for macrophages or dendritic cells. In addition, memory T cells are more easily activated than naïve T cells. Thus, B/Th cell interactions are sufficient to generate secondary antibody responses. It is not necessary (although it can occur) to "prime" memory Th cells with antigen presented by dendritic cells or macrophages.

ii) Cytokines and class switching: cytokines produced by activated Th2 cells not only stimulate proliferation and differentiation of B cells, they also help regulate the class

of Ab produced. Different cytokines influence the switch to different classes of Ab with different effort functions (Table 1). In this way the antibody response is tailored to suit the pathogen encountered (e.g. IgE antibodies for parasitic worm infections).

Table 1.

Cytokine	IgG1	IgG2a	IgG2b	IgG3	IgA	IgE	IgM
IL-4	↑	↓		↓		↑	↓
IL-5					↑		
IFN-γ	↓	↑		↑		↓	↓
TGF-β			↑	↓	↑		↓

3) Cell-cell interactions in Ab responses to exogenous T-<u>independent</u> Ag

   a) Antibody responses to T-independent antigens do NOT require cell-cell interactions. The polymeric nature of these antigens allows for cross-linking of antigen receptors on B cells resulting in activation. No secondary responses, affinity maturation or class switching occurs. Responses to T-independent antigens are due to the activation of a subpopulation of B cells called CD5+ B cells (also called B1 cells), which distinguishes them from conventional B cells that are CD5- (also called B2 cells).

   b) CD5+ cells are the first B cells to appear in ontogeny. They express surface IgM but little or no IgD and they produce primarily IgM antibodies from minimally somatically mutated germ line genes. Antibodies produced by these cells are of low affinity and are often polyreactive (bind multiple antigens). Most of the IgM in serum is derived from CD5+ B cells. CD5+ B cells do not give rise to memory cells. An important characteristic of these cells is that they are self-renewing, unlike conventional B cells which must be replaced from the bone marrow. CD5+ B cells are found in peripheral tissues and are the predominant B cell in the peritoneal cavity. B1 cells are a major defense against many bacterial pathogens that characteristically have polysaccharides in their cell walls. The importance of these cells in immunity is illustrated by the fact that many individuals with T cell defects are still able to resist many bacterial pathogens

4) Cell-cell interactions in cell-mediated immune response: generation of CTL in response to exogenous Ag in cytosol

   a) Cytotoxic T lymphocytes are not fully mature when they exit the thymus. They have a functional TCR that recognizes antigen, but they cannot lyse a target cell. They must differentiate into fully functional effector CTL cells. Cytotoxic cells differentiate from a "pre-CTL" in response to two signals: specific Ag in the context of MHC class I on a stimulator cell, and cytokines produced by Th1 cells (especially IL-2 and IFN-γ) (Fig 6).

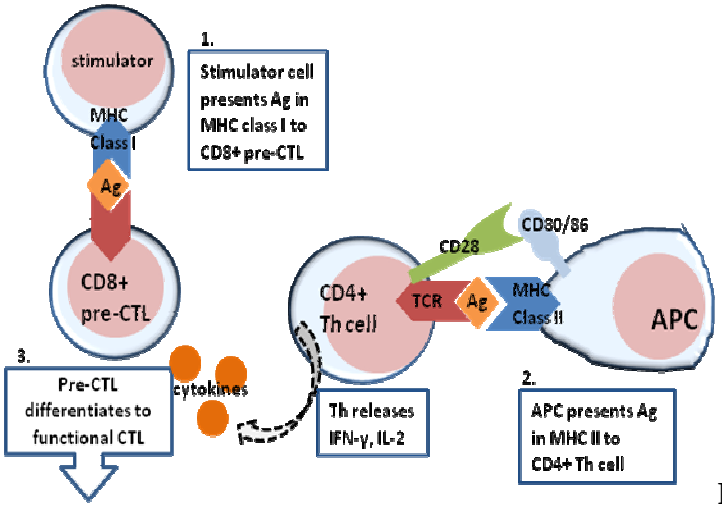


Figure 6.

b) Features of CTL-mediated lysis- CTL killing is Ag-specific. To be killed by a CTL, the target cell must bear the same class I MHC-associated Ag that triggered pre-CTL differentiation. CTL killing requires cell contact. CTL are triggered to kill when they recognize the target Ag associated with a cell surface MHC molecule. Adjacent cells lacking the appropriate target MHC-Ag are not affected. CTLs are not injured when they lyse target cells; therefore, each CTL is capable of killing sequentially numerous target cells.

c) Mechanisms of CTL killing - CTLs utilize several mechanisms to kill target cells, some of which require direct cell-cell contact and others that result from the production of certain cytokines. In all cases death of the target cells is a result of apoptosis.

i) Fas- and TNF-mediated killing (Figure 7): Once generated CTLs express Fas ligand on their surface, which binds to Fas receptors on target cells. In addition, TNF-α secreted by CTLs can bind to TNF receptors on target cells. The Fas and TNF receptors are a closely related family of receptors, which when they encounter their ligands, form trimers of the receptors. These receptors also contain death domains in the cytoplasmic portion of the receptor, which after trimerization can activate caspases that induce apoptosis in the target cell.

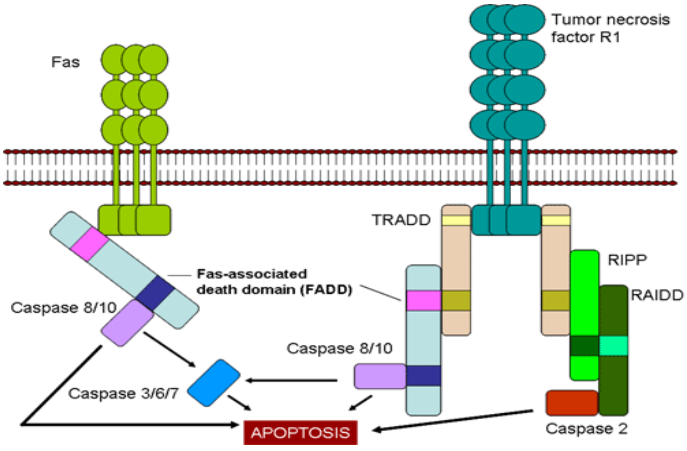


Figure 7.

ii) Granule-mediated killing (Figure 8): Fully differentiated CTLs have numerous granules that contain perforin and granzymes. Upon contact with target cells, perforin is released and it polymerizes to form channels in the target cell membrane. Granzymes, which are serine proteases, enter the target cell through the channels and activate caspases and nucleases in the target cell resulting in apoptosis.

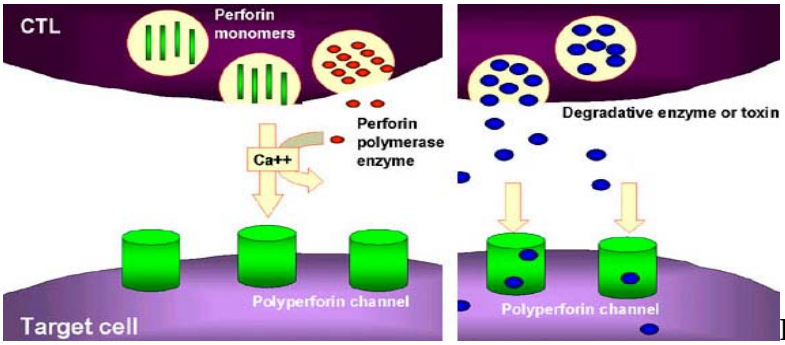


Figure 8.

5) Cell-cell interactions in cell-mediated immune response: activation of macrophages in response to endogenous Ag in vesicles

a) Macrophages play a central role in the immune system. They are involved in 1) the initial defense against pathogens as part of the innate immune system, 2) Ag presentation to Th cells, and 3) various effector functions (*e.g.*, cytokine production, bactericidal and tumoricidal activities) (Figure 9). Indeed macrophages play an important role not only in immunity but also in reorganization of tissues. However, because of their potent activities, macrophage can also do damage to tissues.

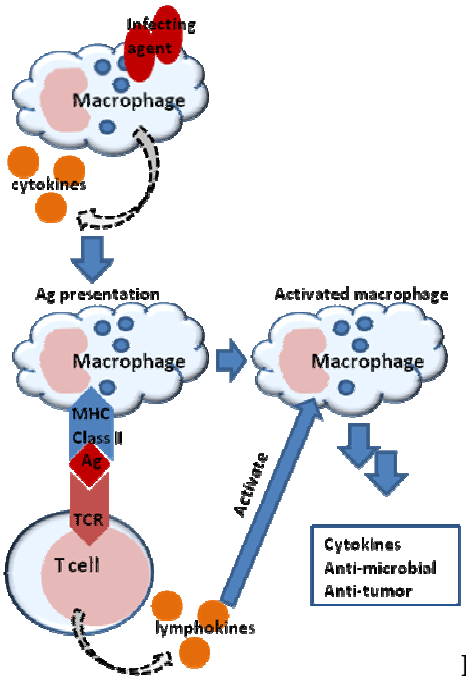


Figure 9.

b) Many of these macrophage functions can only be performed by activated macrophages. Macrophage activation can be defined as quantitative alterations in the expression of various gene products that enable the activated macrophage to perform some function that cannot be performed by the resting macrophage.

c) Macrophage activation is an important function of Th1 cells. When Th1 cells get activated by an APC such as a macrophage, they release IFN-γ, which is one of two signals required to activate a macrophage. Lipopolysaccharide (LPS) from bacteria or TNF-α produced by macrophages exposed to bacterial products deliver the second signal.

d) As discussed in the lecture on the innate immune response (lecture 1), effector mechanisms employed by macrophages include production of 1) TNF-α, which can induce apoptosis, 2) nitric oxide and other reactive nitrogen intermediates, 3) reactive oxygen intermediates, and 4) cationic proteins and hydrolytic enzymes.

e) Macrophage activation by Th1 cells is very important in protection against many different pathogens. For example, *Pneumocystis carinii*, an extracellular pathogen, is controlled in normal individuals by activated macrophages; it is, however, a common cause of death in AIDS patients because they are deficient in Th1 cells. Similarly, *Mycobacterium tuberculosis*, an intracellular pathogen that resides in vesicles, is not efficiently killed by macrophages unless they are activated; hence this infection is a problem in AIDS patients.