COURSE: Medical Microbiology (MBIM 650/720)

Lecture 13

TOPIC: Cell Interactions in Specific Immune Response

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TEACHING OBJECTIVES: Helper T cell-B cell interactions for antibody formation against hapten-conjugated proteins and complex proteins
Thymus-independent antigens
Properties and functions of cytokines

SUGGESTED READING: Roitt, Brostoff, Male, 6th Edition, Mosby, 2001 Chapter 8, pp. 132-136; Chapter 7, pp. 119-129

I. HELPER T CELL-B CELL INTERACTIONS IN ANTIBODY FORMATION

A. Hapten-carrier effect

Historically one of the major findings was that T cells and B cells are required in order to produce antibody to a complex protein. A major contribution to our understanding of this process came from studies on the formation of anti-hapten antibodies.

Recall that a hapten injected by itself cannot elicit an antibody response. **Rather antibodies against haptens require that the hapten be conjugated to a protein (sometimes termed a carrier).**

These studies with hapten-carrier established that:

1. Th cells recognize the carrier, and B cells recognize hapten.

2. There must be cooperation between **hapten-specific B cells** and **protein (carrier)-specific helper T cells**.

3. Interaction between the hapten-specific B cell and the carrier-specific helper T cell are **class II self MHC-restricted**. The helper T cell cooperates only with B cells that express class II MHC molecules recognized as self by the T cells.

B. B cells as antigen presenting cells

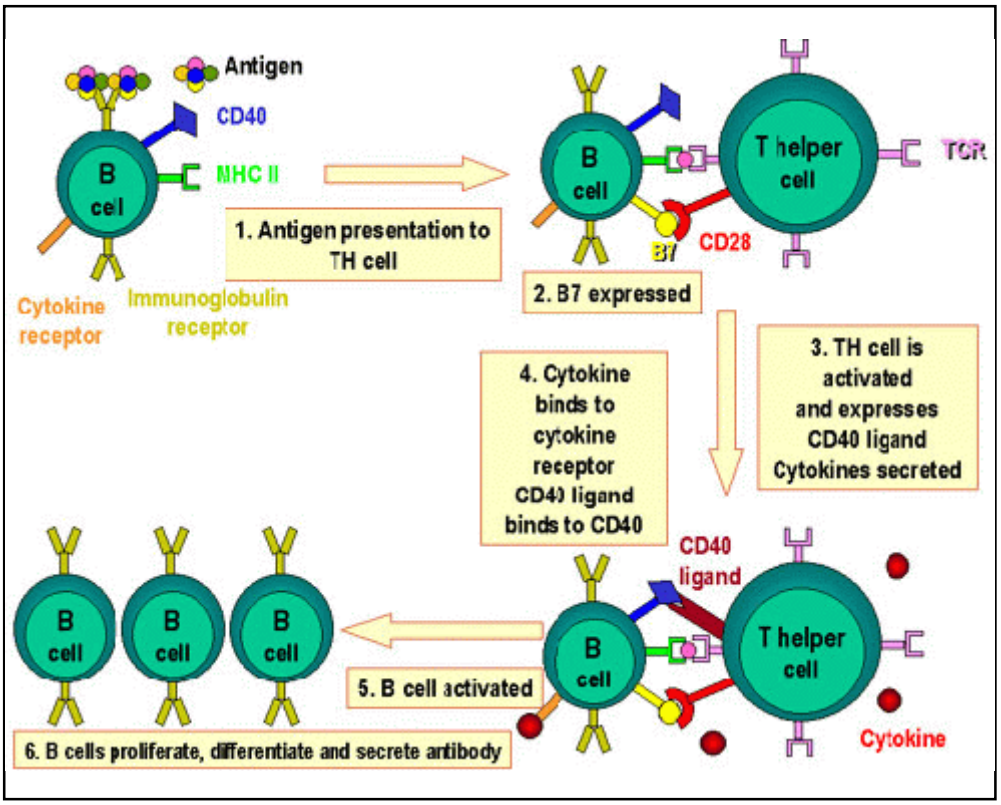


Figure 1: Molecules involved in the interactions of B and TH cells Antigen is processed by B cell. Co-stimulators are expressed. The processed antigen peptide is presented in association with MHC class II antigens. The T cell recognizes the peptide along with the MHC antigen and the co-stimulators. The T cell expresses CD40 ligand. The latter binds to CD40 antigen on the B cell and the B cells divide and differentiate. Antibodies are produced by the B cell.

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 protein findings, the mechanism is thought to be the following: the hapten is recognized by the Ig receptor, the hapten-carrier brought into the B cell, processed, and peptide fragments of the carrier protein presented to a helper T cell. 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 1 summarizes the B cell-T cell interaction. Note that there are multiple signals delivered to the B cells in this model of TH 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 **costimulatory** 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.

C. Extension of this model to complex protein antigens (T-dependent antigens)

The same mechanism described above can cover all multideterminant complex protein antigens that require helper T cells. These antigens are referred to as **thymus-dependent antigens**. If one determinant is recognized by B cells (analogous to the hapten) and the same or different determinant is recognized by the helper T cells (analogous to the carrier), the same model applies. This is shown in Figure 2.

D. B cells in secondary responses

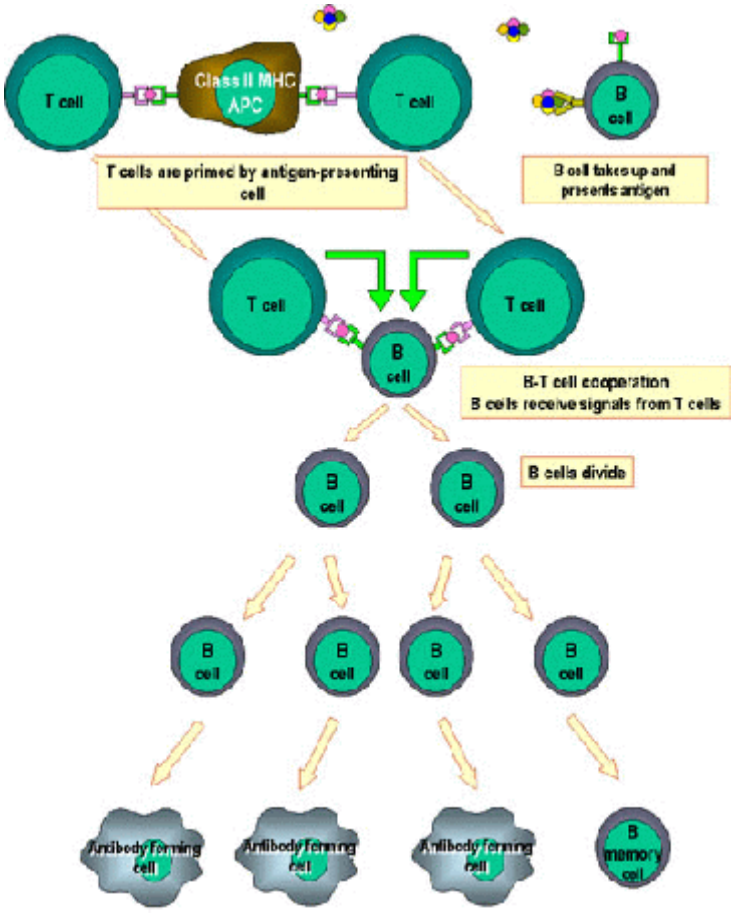


Figure 2: Cooperation of cells in the immune response Antigen-presenting cells (e.g. dendritic cells) present processed antigen to virgin T cells, thereby priming them. B cells also process the antigen and present it to the T cells. They then receive signals from the T cells that cause them to divide and differentiate. Some B cells form antibody-forming cells while a few form B memory cells.

As a consequence of a primary response, many memory B cells are created. These carry a high-affinity receptor, Ig, which allows them to bind and present antigen at much lower concentrations than is required for macrophages or dendritic cells.

II. THYMUS-INDEPENDENT ANTIGENS

The **thymus-independent antigens** (T-independent antigens) are those that produce normal antibody responses in **athymic** (thymus-less or nude) mice, i.e. under conditions where T cells are absent. T-independent antigens have the following properties:

1. activate B cells at high concentrations, i.e. are **polyclonal B cell activators** (antigens like lipopolysaccharide, LPS, sometimes termed **B cell** mitogens).

2. are large **polymeric** molecules with repeating antigenic determinants.

3. are particularly resistant to degradation

4. Some antigens activate **both** immature and mature B cells; other antigens activate only mature B cells and are thus not especially effective in infants where B cells are mostly immature.

5. Responses to several T-independent antigens are dominated by $CD5^+$ B cells, described below.

Unlike the thymus-dependent antigens, the thymus-independent antigens:

1. do **not** produce isotype **switching** (IgM is almost exclusively produced)

2. do **not** demonstrate **affinity maturation** (in which antibodies of progressively higher affinity are produced)

3. do not show secondary responses (no memory B cells).

The thymus-independent antigen pathway is important because humoral immunity is the major mechanism of defense against many harmful bacteria that have polysaccharides in their cell wall. Individuals with depressed T cell systems can still resist these types of bacterial infections.

III. $CD5^+$ B CELLS

$CD5^+$ B cells (sometimes referred to as **B-1** cells) form a population that is distinct from conventional B cells (sometimes referred to as B-2 cells). They have the following characteristics:

1. are the **first** B cells to appear in ontogeny

2. express surface IgM, but **little** or **no IgD**

3. produce immunoglobulins, mainly **IgM**, from unmutated or minimally mutated germline genes

4. produce antibodies of **low avidity** that are **polyreactive** (i.e., bind multiple different antigens, mainly bacterial polysaccharides and double stranded DNA)

5. contribute most of the IgM found in **adult** serum
6. do **not** develop into memory cells

7. are **self-renewing** in adults (i.e., do not continue to arise from a stem cell in the bone marrow as do conventional B cells)

8. reside in peripheral tissues and are the predominant lymphocyte in the **peritoneal cavity**.

The following table contrasts $CD5^+$ B cells with conventional B cells.

Table 1 - COMPARISON OF PROPERTIES OF $CD5^+$ B CELLS AND CONVENTIONAL B CELLS

Properties	$CD5^+$ B cells	Conventional B Cells
Ontogeny	Early	Late
Renewal	Self Renewal	Replaced from bone marrow
Production of Immunoglobulin	High	Low
Isotypes secreted	IgM>>IgG	IgG>IgM
Bind multiple different ligands	Yes	No

Adapted from Janeway and Travers, Immunobiology

Figure 3

As shown in Figure 4, tumors can arise from $CD5^+$ B cells and conventional B cells at various stages in their development.

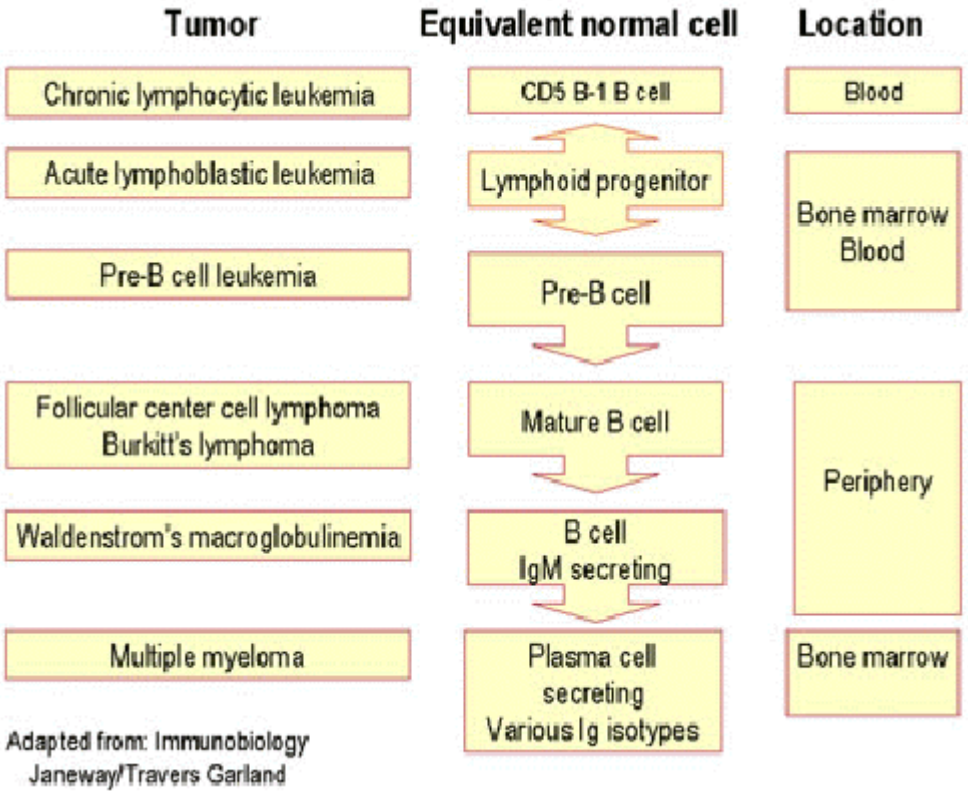


Figure 4: Origin of B cell tumors. These tumors arise as clonal outgrowths from normal B cells at different developmental stages. The tumor cells behave in a similar manner to their normal equivalent and go to similar parts of the body.

IV. CYTOKINES

Cytokines are a diverse group of non-antibody proteins released by cells that act as intercellular mediators, especially in immune processes.

A. Cytokines are clinically important as biological response modifiers. Terms in the literature:

1. **Monokines** - produced by mononuclear phagocytes
2. **Lymphokine**s - produced by activated T cells, primarily helper T cells
3. **Interleukins** - name given to many cytokines, abbreviated as IL and given a number

B. Properties

1. Produced by cells involved in both natural and specific immunity
2. Mediate and regulate immune and inflammatory responses
3. Secretion is brief and limited

   a. Cytokines are not stored as pre-formed molecules
   b. Synthesis is initiated by new short-lived gene transcription
   c. mRNA is short-lived
   d. This results in production of cytokine as needed

4. Many individual cytokines are produced by many cell types and act on many cell types (they are **pleiotropic**)

5. In many cases cytokines have similar actions (they are redundant). Redundancy is due to the following: Receptors for cytokines are heterodimers (sometimes heterotrimers) that can be grouped into families in which one subunit is common to all members of a given family. Some examples are shown in Figure 5.

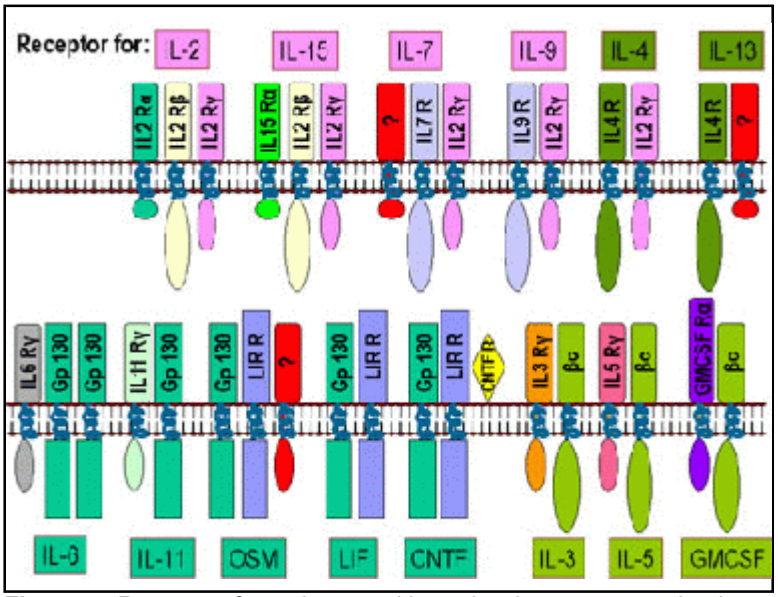


Figure 5: Receptors for various cytokines showing common subunits

Since the subunit common to all members of the family functions in binding cytokine and in signal transduction, a receptor for one cytokine can often respond to another cytokine in the same family. Thus, an individual lacking IL-2, for example, is not adversely affected because other cytokines (IL-15, IL-7, IL-9, etc.) assume its function. Similarly, a mutation in a cytokine receptor subunit other than the one in common often has little effect. On the other hand, a mutation in the common subunit has profound effects. Again, as an example, mutation in the gene for the IL-2Rgamma subunit causes human X-linked severe combined immunodeficiency (XSCID) characterized by a complete or nearly complete T cell defect.

6. Often influence the synthesis of other cytokines

    a. They can produce cascades, or enhance or suppress production of other cytokines
    b. They exert positive or negative regulatory mechanisms for immune and inflammatory responses

7. Often influence the action of other cytokines. Effects can be:

    a. **antagonistic**
    b. **additive**
    c. greater than additive (**synergistic**)

8. Bind to specific receptors on target cells with high affinity. Compare with antigen binding to antibody or peptide binding to a MHC molecule which both show much lower binding affinities.

9. Cells that can respond to a cytokine are:

    a. same cell that secreted cytokine: **autocrine**
    b. a nearby cell: **paracrine**
    c. a distant cell reached through the circulation: **endocrine**

10. Cellular responses to cytokines are generally slow (hours), require new mRNA and protein synthesis

C. Can be grouped

1. Mediators and Regulators of Natural Immunity

    Tumor Necrosis Factor (TNF)
    Interleukin-1 (IL-1)
    Chemokines
    Interleukin-10 (IL-10)
    Interferon-((IFN-()

2. Mediators and regulators of specific immunity

    Interleukin-2 (IL-2)
    Interleukin-4 (IL-4)
    Interleukin-5 (IL-5)
    Interleukin-10 (IL-10)

Interferon-((IFN-()

3. Stimulators of Hematopoiesis

Interleukin -3 (IL-3)
Colony-Stimulating Factors (CSFs)

D. Functions of Selected Cytokines: Mediators and Regulators of Natural Immunity

1. Tumor Necrosis Factor (TNF) also called TNF-(

a) produced by activated macrophages
b) most important mediator of acute inflammation in response to Gram-negative bacteria and other infectious microbes
c) mediates the recruitment of polymorphonuclear leukocytes (PMN) and monocytes to site of infection

(1) stimulates endothelial cells to express new adhesion molecules that make the cell surface "sticky" for PMN and monocytes
(2) stimulates endothelial cells and macrophages to produce **chemokines** that induce leukocyte chemotaxis and recruitment

d) acts on hypothalamus to produce **fever**
e) promotes production of acute phase proteins by the liver

2. Interleukin-1

a) produced by activated macrophages
b) effects are similar to those of TNF

3. Chemokines

a) contraction of **chemo**tactic cyto**kine**s
b) large family of substances (>50) produced by many different leukocytes and tissue cells
c) recruit leukocytes to sites of infection
d) play a role in lymphocyte trafficking

4. Interleukin-10
a) produced by activated macrophages
b) acts as an inhibitor of activated macrophages by blocking production of TNF

E. Functions of Selected Cytokines: Mediators and Regulators of Specific Immunity

1. Interleukin-2

a) produced mainly by helper T cells ($CD4^+$); less by cytoxic T cells ($CD8^+$)
b) main function is to promote T cell division and to increase production of other cytokines
c) other functions shown in Figure 6
d) autocrine functions on T cell proliferation depicted in Figure 7

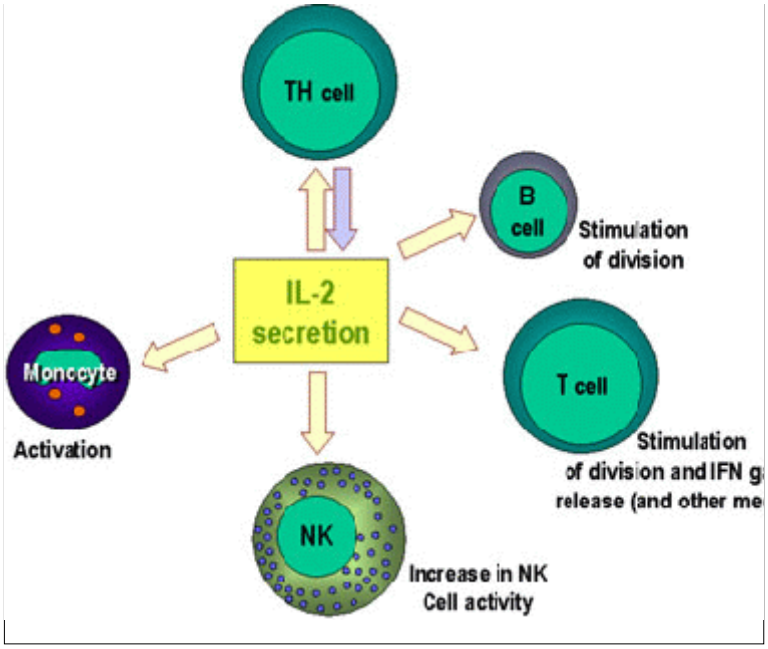


Figure 6: Immunoregulatory actions of interleukin-2

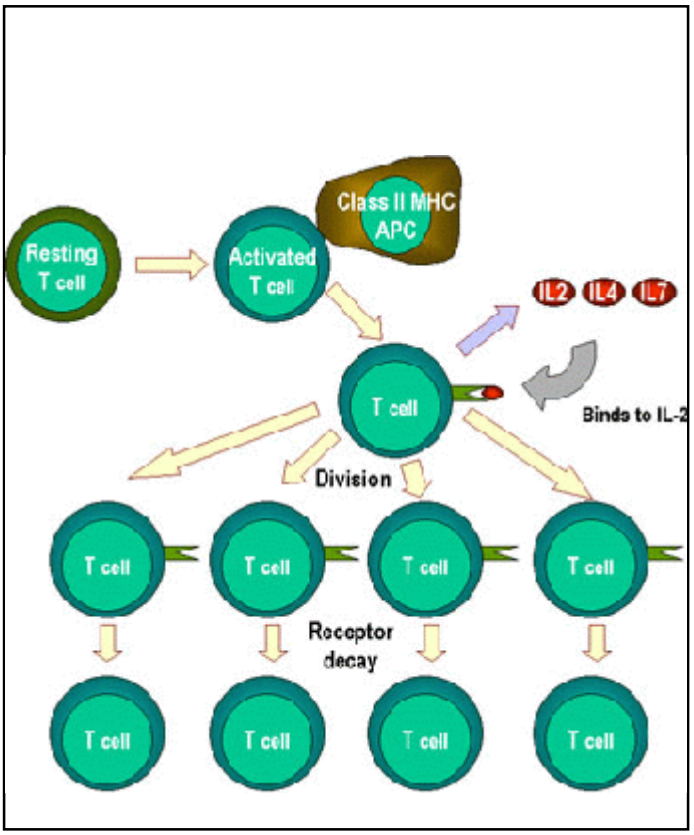


Figure 7: T cell proliferation and cytokines. When T cells are resting, they do not make cytokines such as interleukins 2, 4 or 7. Nor do they express large amounts of their receptors. There are no IL-2 receptors. Activation of T cells results in the formation of high affinity IL-2 receptors and induction of the synthesis and secretion of IL-2 and Il-4. These bind to their receptors and the cells proliferate. When stimulation by interleukins declines (e.g. when antigen stimulation declines), receptors decay and the proliferative phase is at an end. Note: stimulation by the cytokines can be **paracrine** or **autocrine**.

2. Interleukin-4

   a) produced mainly by TH2 subpopulation of helper T cells ($CD4^+$). RECALL that TH2 cells are required for antibody production by B cells
   b) stimulates immunoglobulin class switching to the IgE isotype. (IgE is involved in eosinophil-mediated elimination of helminths and arthropods.)
   c) stimulates development of TH2 cells from naive $CD4^+$ T cells
   d) promotes growth of differentiated TH2 cells

3. Interleukin-5

   a) produced mainly by TH2 subpopulation of helper T cells ($CD4^+$)
   b) promotes growth and differentiation of eosinophils
   c) activates mature eosinophils

      IL-4 and IL-5 function together.
      IgE opsonizes helminths that then bind to eosinophils which upon activation kill the helminth.

4. Interferons (IFN)

   Three groups: IFN-", IFN-$, IFN-(

   a) IFN-"
      (1) 20 variants produced by leukocytes in response to viruses

   b) IFN-$
      (1) a single protein produced by fibroblasts and other cells in response to viruses

   Both IFN-" and IFN $ inhibit viral replication and increase expression of class I MHC on cells

   c) IFN-(

      (1) produced by TH1 subpopulation of helper T cells ($CD4^+$), cytotoxic T cells ($CD8^+$), and NK cells.

      RECALL that TH1 cells are involved in the elimination of pathogens residing intracellularly in vesicular compartments.

      (2) functions in both **natural** and **specific** immunity

      natural: enhances the microbicidal function of macrophages through formation of nitric oxide and reactive oxygen intermediates (ROI)

      specific:stimulates the expression of class I and class II MHC molecules and costimulatory molecules on antigen presenting cells

      promotes the differentiation of naive helper T cells into TH1cells

      activates polymorphonuclear leukocytes (PMN) and cytotoxic T cells an increases the cytotoxicity of NK cells.

These functions are shown in Figure 8.

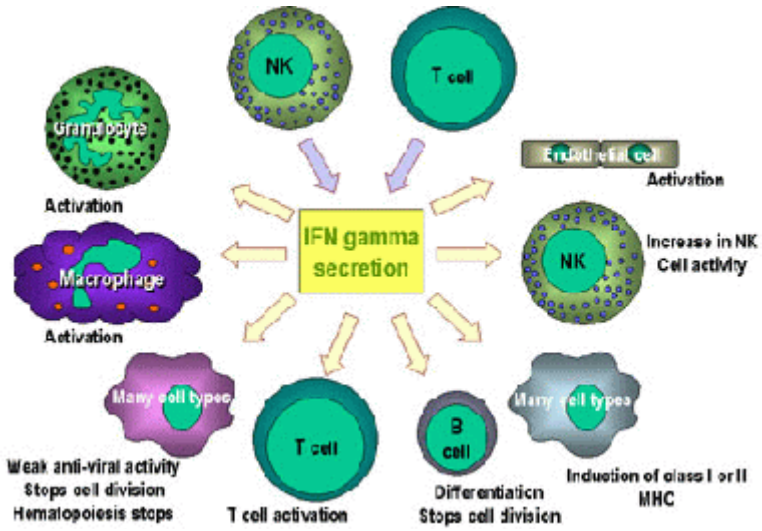


Figure 8: Immunoregulatory actions of interferon gamma on the immune system. Note the anti-proliferation and antiviral activities are weaker than those of IFN alpha and IFN beta. IFN gamma is the most potent of the three at macrophage activation and in inducing class II MHC expression.

5. Transforming Growth Factor (TGF-$)

    a) an **inhibitory** cytokine produced by T cells, macrophages, and many other cell types.
    b) inhibits proliferation and differentiation of T cells
    c) inhibits activation of macrophages
    d) acts on PMN and endothelial cells to block the effects of proinflammatory cytokines

F. Functions of Selected Cytokines: Stimulators of Hematopoiesis

1. Interleukin-3

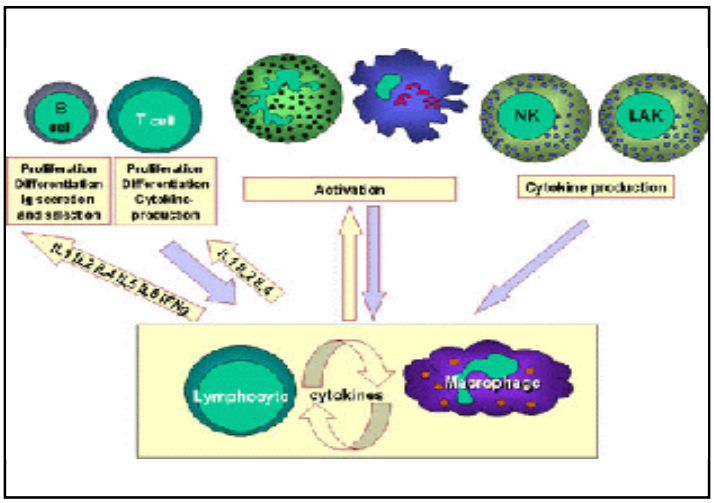
    a) produced by helper T cells
    b) promotes growth and differentiation of bone marrow progenitors

2. Colony-Stimulating Factors (CSFs)

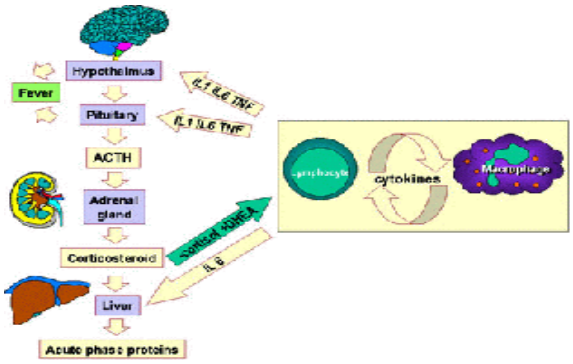
    a) produced by T cells, macrophages, endothelial cells, fibroblasts
    b) granulocyte-macrophage colony-stimulating factor (GM-CSF) promotes growth and differentiation of bone marrow progenitors
    c) macrophage colony-stimulating factor (M-CSF) is involved in the development and function of monocytes/macrophages
    d) granulocyte colony-stimulatory factor (G-CSF) stimulates the production of PMN

G. Cytokine Network

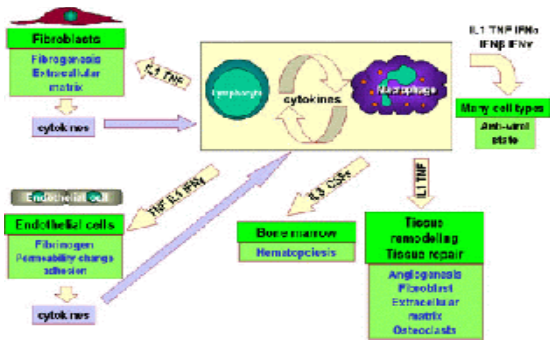
Although the focus has been on the production and action of cytokines on cells of the immune system, it is important to understand that many of them have effects on other cells and organ systems. A schematic diagram showing some of the interactions in the **cytokine network** is presented in Figure 9.



Cytokine network. Communication between lymphocytes and macrophages and other components of the immune system



Cytokine network. Communication between lymphocytes and macrophages and the hypothalamus, adrenals and the liver.



Cytokine network. Communication between lymphocytes and macrophages and other cells and tissues

Figure 9

Table 2 - FEATURES OF CYTOKINES			
Cytokine	**Cell Source**	**Cell Target**	**Primary Effects**
IL-1	Monocytes Macrophages Fibroblasts Epithelial cells Endothelial cells Astrocytes	T cells; B cells Endothelial cells Hypothalamus Liver	Costimulatory molecule Activation (inflammation) Fever Acute phase reactants
IL-2	T cells; NK cells	T cells B cells Monocytes	Growth Growth Activation
IL-3	T cells	Bone marrow progenitors	Growth and differentiation
IL-4	T cells	Naive T cells T cells B cells	Differentiation into a T_H 2 cell Growth Activation and growth; Isotype switching to IgE
IL-5	T cells	B cells Eosinophils	Growth and activation
IL-6	T cells; Macrophages; Fibroblasts	T cells; B cells Mature B cells Liver	Costimulatory molecule Growth (in humans) Acute phase reactants
IL-8 family	Macrophages; Epithelial cells; Platelets	Neutrophils	Activation and chemotaxis
IL-10	T cells (T_H2)	Macrophages T cells	Inhibits APC activity Inhibits cytokine production
IL-12	Macrophages; NK cells	Naive T cells	Differentiation into a T_H 1 cell
IFN-gamma	T cells; NK cells	Monocytes Endothelial cells Many tissue cells; especially macrophages	Activation Activation Increased class I and II MHC
TGF-beta	T cells; Macrophages	T cells Macrophages	Inhibits activation and growth Inhibits activation
GM-CSF	T cells; Macrophages; Endothelial cells, Fibroblasts	Bone marrow progenitors	Growth and differentiation
TNF-alpha	Macrophages; T cells	Similar to IL-1	Similar to IL-1

IL = interleukin GM-CSF = granulocyte-macrophage colony stimulating factor
IFN = interferon TNF = tumor necrosis factor
TGF = transforming growth factor