

TOPIC: Immunoregulation

FACULTY: Dr. William Bowers

Phone: 733-3275 e-mail: wbowers@med.sc.edu

TEACHING OBJECTIVES: Subpopulations of helper T cells: Th1 and Th2
Cytokines and class (isotype) switching
Cytokine activation of macrophages and functions
Maturation and mechanism of killing by cytotoxic T lymphocytes (CTL)
Characteristics of killing mechanisms of other cytotoxic cells
Immunoregulatory processes

SUGGESTED READING: Roitt, Brostoff, Male, 6th Edition, Mosby, 2001 Chapter 9, pp.157-161; chapter 10 pp. 163-171

I. CENTRAL ROLE OF HELPER T CELLS IN CELL-MEDIATED IMMUNITY

After a helper T cell recognizes specific antigen, it can initiate several key immune processes.

1. Selection of effector mechanisms.
2. Induction of proliferation in appropriate effector cell types.
3. Enhancement of functional activities of phagocytes and other effector cells.

These activities are depicted in Figure 1.

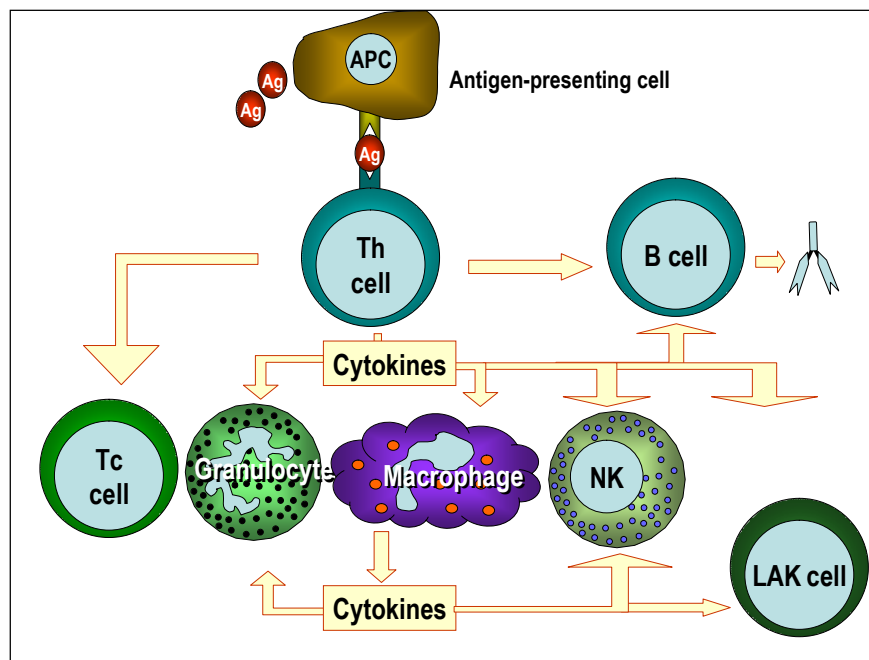


Figure 1: Th cells are at the center of cell-mediated immunity. The antigen-presenting cells present antigen to the T helper (Th) cell. The Th cell recognizes specific epitopes which are selected as target epitopes. Appropriate effector mechanisms are now determined. For example, Th cells help the B cells to make antibody and also activate other cells. The activation signals produced by Th cells are cytokines (lymphokines) but similar cytokines made by macrophages and other cells also participate in this process.

II. SUBPOPULATIONS OF HELPER T CELLS: Th1 AND Th2

When a naive $CD4^+$ T cell (Th cell) responds to antigen in secondary lymphoid tissues, it is capable of differentiating into an inflammatory Th1 cell or a helper Th2 cell, which release distinctive patterns of cytokines, as shown in Figure 2.

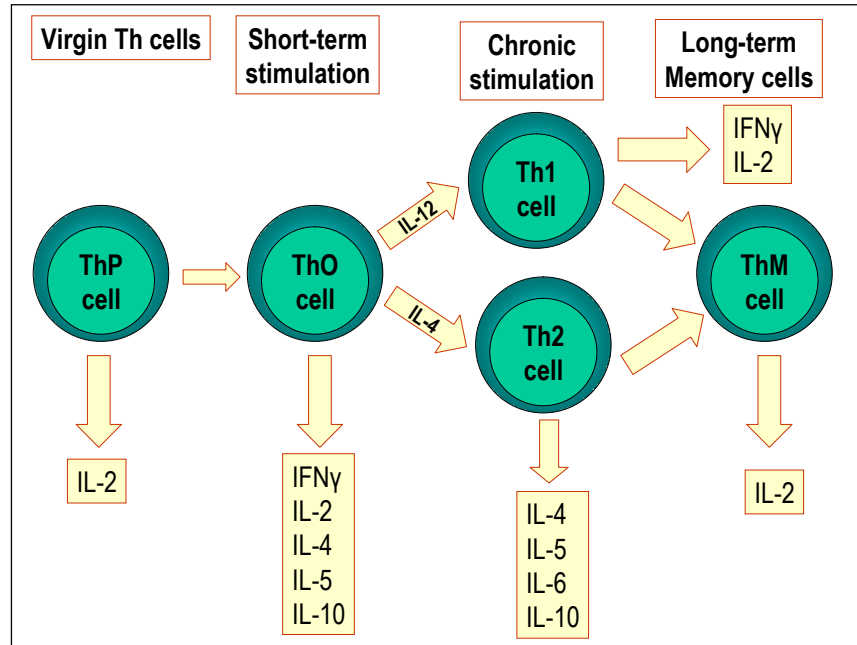


Figure 2: Differentiation of murine Th cells. Mouse Th cells differentiate into subsets that synthesize different patterns of lymphokines. This also occurs in humans.

Functionally these subpopulations, when activated, affect different cells, as shown in Figure 3

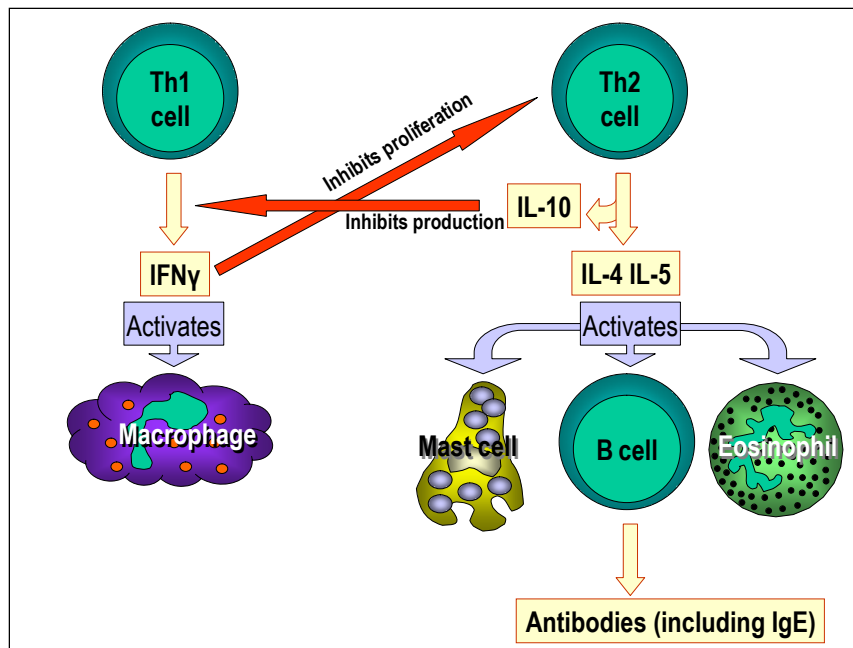


Figure 3: Selection of effector mechanisms by Th1 and Th2 cells. In addition to determining various effector pathways by virtue of their lymphokine production, Th1 cells switch off Th2 cells and vice versa.

Th1 cells produce IFN-gamma that activates macrophages.

Th2 cells produce IL-4 and IL-5 that increases production of eosinophils and mast cells and enhances production of antibody, especially IgE.

Equally important, each subpopulation can exert inhibitory influences on the other:

IFN gamma produced by Th1 cells inhibits proliferation of Th2 cells.

IL-10 produced by Th2 cells inhibits production of IFN gamma and, although not shown, IL-4 inhibits the production of Th1 cells

III. CENTRAL ROLE OF MACROPHAGES IN NATURAL AND SPECIFIC IMMUNITY

Macrophages play a central role in the immune system. As shown in Figure 4, macrophages are involved in:

1. initial defense
2. antigen presentation
3. effector functions

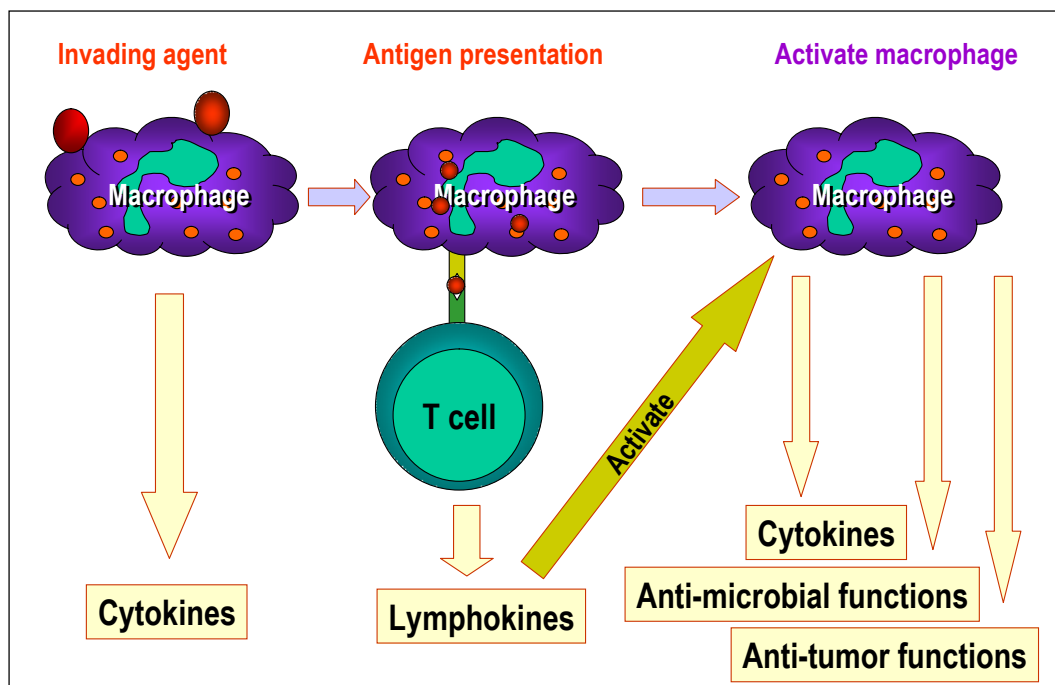


Figure 4: Macrophages play a central role in the immune system before T and B-cell immunity starts. Macrophages process antigens and present them to T cells which then release lymphokines which activate the macrophages to perform various other functions including the production of more cytokines.

Figure 5 presents a more detailed picture of the roles played by macrophages in immunity and inflammation. These are:

1. inflammation and fever
2. lymphocyte activation
3. tissue reorganization
4. tissue damage
5. microbicidal activity
6. tumoricidal activity

<p>Inflammation - Fever Production of: IL-6, TNF alpha, IL-1 – act as pyrogen</p>	<p>Damage to tissues Hydrolases, Hydrogen peroxide production Complement C3a TNF alpha production</p>
<p>Immunity Selection of lymphocytes to be activated: IL-12 results in TH1 activation IL-4 results in TH2 activation Activation of lymphocytes: Production of IL-1 Processing and presentation of antigen</p>	<p>Antimicrobial action O2 –dependent production of: hydrogen peroxide superoxide, hydroxyl radical hypochlorous acid O2-independent prodn of: acid hydrolases cationic proteins lysozyme</p>
<p>Reorganization of tissues, Secretion of a variety of factors: Degradative enzymes (elastase, hyaluronidase, collagenase) Fibroblast stimulation factors Stimulation of angiogenesis</p>	<p>Anti-tumor activity Toxic factors Hydrogen peroxide Complement C3a Proteases Arginase Nitric oxide TNF alpha</p>

Figure 5

A number of these functions are performed by **activated macrophages**. **Macrophage activation** can be defined as quantitative alterations in the expression of various gene products that endow the activated macrophage to perform some function that cannot be performed by the resting monocyte.

Macrophage activation is the most important function of **Th1 cells**, which release **IFN gamma**, one of two signals required to activate a macrophage. **Lipopolysaccharide (LPS)** from bacteria can deliver the second signal, as well as **TNF alpha**. See Figure 6.

One example of the importance of macrophage activation by Th1 cells is the following: **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.

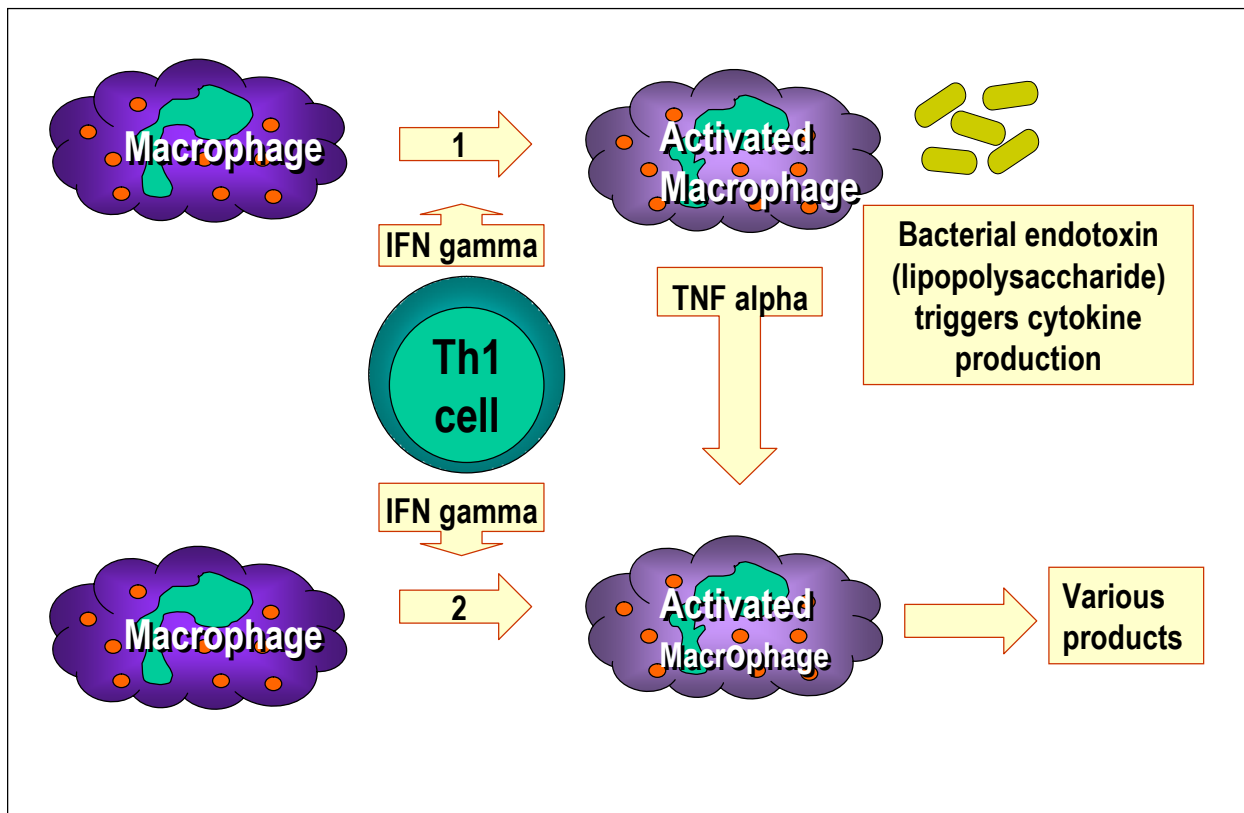


Figure 6: Macrophage activation results from the interaction of multiple cytokines and other factors. In pathway 1, macrophages are activated by interferon gamma and interaction with bacterial components. An example of such a triggering component is bacterial lipopolysaccharide. In pathway 2 macrophages are activated by a combination of interferon gamma and TNF alpha.

IV. CYTOTOXIC T LYMPHOCYTES (CTL, T_c)

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 to become “armed”.

A. Differentiation of T_c

They differentiate from a "pre-T_c" in response to two signals: 1) **specific antigen** associated with class I MHC, and 2) **cytokines**, especially IL-2, and IFN-gamma. This is shown in Figure 7.

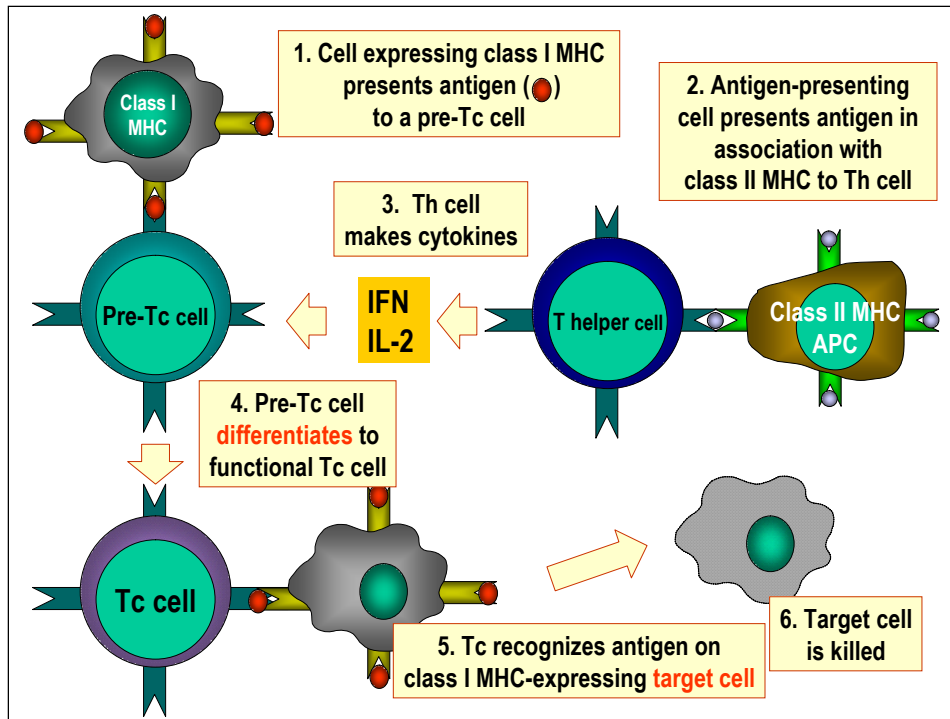


Figure 7: Tc cells must differentiate in response to antigen. In order to differentiate into functional cytotoxic T lymphocytes, pre-CD8⁺ Tc must receive two different signals. First, they must recognize antigen presented by class I MHC-expressing cells (the stimulator cells) and, second, they must be stimulated by cytokines. IL-2, interferon-gamma and others are made by CD4⁺ helper T cells as a result of their interaction with class II MHC-expressing antigen presenting cells. As a result of these two signals, the pre-Tc differentiates into an active Tc that can then lyse target cells that bear the same antigen. Adapted from Abbas, et.al. *Cellular and Molecular Immunology*, 3rd Ed., p. 292.

B. Features of Tc-mediated lysis

1. Tc killing is **antigen-specific**. To be killed by a Tc, the target cell must bear the same class I MHC-associated antigen that triggered pre- Tc differentiation.
2. Tc killing requires **cell contact**. Tc are triggered to kill when they recognize the target antigen associated with a cell surface MHC molecule. Adjacent cells lacking the appropriate target MHC-antigen are not affected.
3. Tc are **not** injured when they lyse target cells. Each Tc is capable of killing sequentially numerous target cells.

C. Steps in Tc-mediated lysis (These are indicated in Figure 8)

1. Recognition of antigen
2. Activation
3. Delivery of lethal hit
4. Release of Tc from target cell]
5. Death of target cell

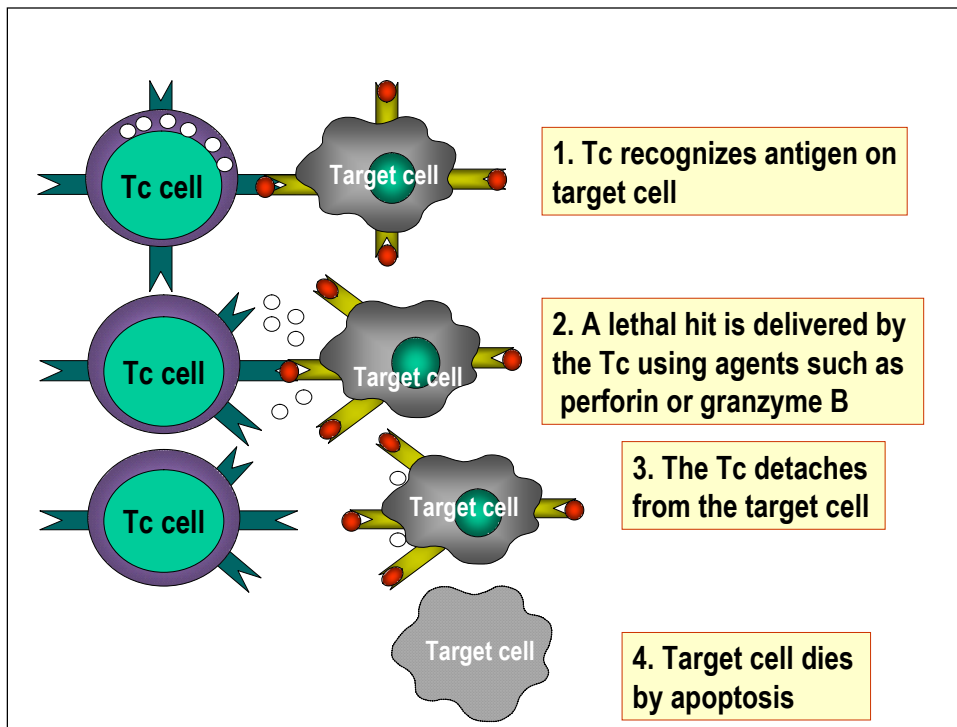


Figure 8: Steps in cytotoxic T cell (CTL)-mediated lysis of a target cell. Step 1 also requires interaction of specific molecules on the CTL cell (e.g. CD8) with their specific ligands on the target cell.

D. Mechanism of Tc killing

Upon contact with target cells, **perforin** in Tc granules is released and polymerizes to form a channel in the target cell membrane. **Granzymes**, which are serine proteases, enter the target cell through the channel, activate caspases and nucleases that lead to apoptosis of target cell. (Figure 9).

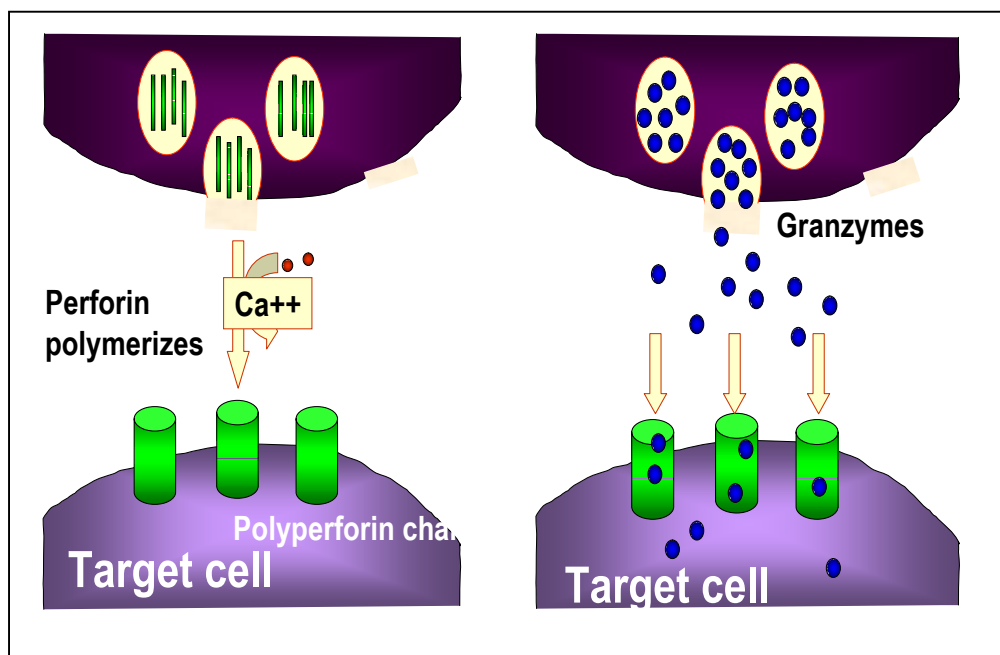


Figure 9

V. OTHER CYTOLYTIC CELLS

A. Natural killer (**NK**) cells

1. Characteristics

- a. Derived from bone marrow
- b. **Lack most markers for T and B cells** (no TCR or CD3)
- c. Do **not** undergo thymic maturation
- d. Express **CD56**, a specific NK marker
- e. Express a low-affinity receptor for Fc portion of IgG, called **FcRIII (CD16)**, also expressed on granulocytes and macrophages
- f. Cytokines, especially **IL-2**, promote further differentiation into lymphokine-activated killer (**LAK**) cells

2. Effector mechanisms

- a. Similar to CTL
- b. **Not MHC-restricted**
- c. Kill a variety of virus-infected cells and tumor cells, but not all. Susceptibility to killing by NK cells is inversely correlated to expression of class I MHC. Killer inhibitory receptors (KIRs) on human NK cells that recognize class I MHC prevent killing. Some virally-infected cells and tumors down-regulate the expression of class I MHC and they can be killed by NK cells. Because such class I MHC-deficient cells might escape being killed by cytotoxic T cells, NK cells afford another type of protection to the host
- d. IgG-coated target cells recognized by **CD16** are killed by antibody-dependent cell mediated cytotoxicity, (**ADCC**)
- e. **LAK** kill broader range of cells - including some normal cells - than NK cells
- f. LAK cells predominate in lesions in graft-vs-host disease in recipients of bone marrow transplants

VI. IMMUNOREGULATION

An immune response has the following features:

1. Its magnitude is determined by a balance between lymphocyte activation and tolerance induced by an antigen.
2. Its nature is determined by the specificities and functional classes of lymphocytes that are activated by that antigen.
3. Regulatory mechanisms may act at the recognition, activation, and effector phases of an immune response.

Some of the factors that regulate immune responses are given in Figure 10.

Figure 10 - Factors that determine the nature and magnitude of immune responses		
	Factors that favor:	
	stimulation of immune response	inhibition of immune response
Cognitive phase		
Lymphocyte repertoire	Diversity of lymphocyte receptors for foreign antigens	Absence of MHC molecules capable of binding specific antigenic determinants
Antigen presentation	Presence of MHC molecules capable of binding processed antigen	Absence of MHC molecules capable of binding certain antigenic determinants
Induction and activation phase		
Features of Antigen		
Nature	Immunogenic forms	Tolerogenic forms
Amount	Optimal doses vary for different antigens	High doses favor tolerance
Portal of entry	Subcutaneous, intradermal	Intravenous, oral
Adjuvants	Recruitment and activation of accessory cells, induction of co-stimulators	Antigens with adjuvants are non-immunogenic or tolerogenic
Accessory cells	Presence of co-stimulators from T cells	Absence of co-stimulators
Antigen-specific T cells	Helper T cells	Suppressor T cells
Anti-idiotypic immune responses	Stimulatory or inhibitory	Stimulatory or inhibitory
Antibodies	Enhance antigen uptake and presentation by macrophages	Antibody feedback
Cytokines	Positive amplification loops	Antagonistic effects of various cytokines. Immunosuppressive effects
Adapted from Abbas et al. Cellular and Molecular Immunology. Second edition, 1994, p. 209		