

CELL MEDIATED IMMUNITY

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History. The immune system is a remarkable mosaic of anti-infective strategies. The word “immunity” (L: immunis, “free of”) was originally used in the context of being free of the burden of taxes or military conscription. The application of this term today, in a medical context, refers to ability of the human body to remain “free of” disease. The history of immunology dates back over 100 years if you consider Louis Pasteur as the “Father of immunology”. However, the concept of cellular immunology began in the late 1960’s with the discovery by Jacques Miller that the surgical removal of the thymus from infant mice resulted in a deficiency in “T Lymphocytes” (T cells) named after the organ in which they differentiate. Largely as a consequence of this finding, the immune system has been separated into two branches: (i) humoral immunity, for which the protective function of immunization could be found in cell-free serum and (ii) cellular immunity, for which the protective function of immunization was associated with cells. Cell mediated immunity protects the body using a repertoire of differentiated cell types as described in Table 1.

Table 1. Cells involved with the Immune Response

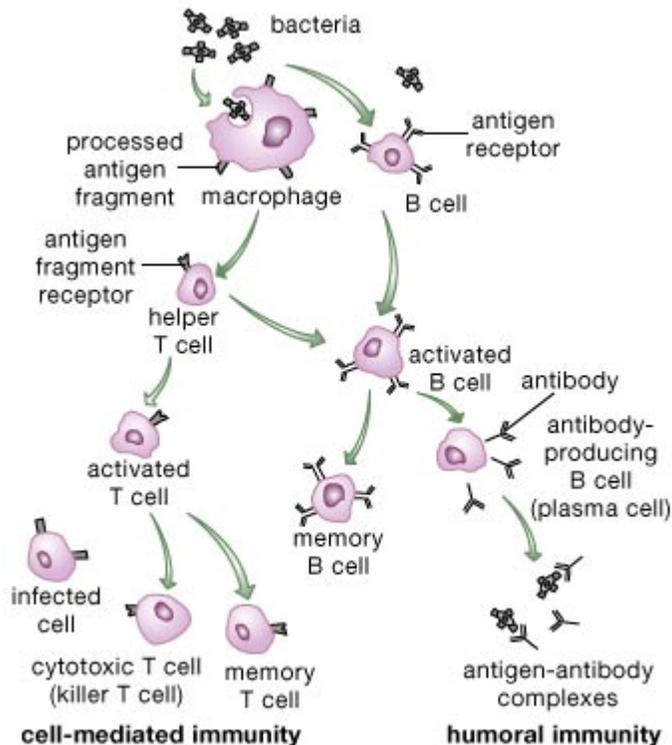
1. **Antigen-specific cytotoxic T-lymphocytes:** induce apoptosis in body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens.
2. **Macrophages and Natural Killer Cells:** Destroy cells harboring intracellular pathogens;
3. **Stimulating cells:** Epithelial and dendritic cells also present antigens or secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses.

Function. Cell mediated immunity is directed to viruses, bacteria, fungi, and parasites (collectively referred to as microbes) that survive within human host cells or toward processed parts of the infecting microbe as displayed on the surface of antigen presenting cells. In general this process is most effective in removing virus- or bacteria-infected cells, but also participates in defending against fungi, protozoa, cancers. Cell mediated immunity also plays a major role in transplant rejection. The specific cells involved in immunologic reactions are summarized in Table 2.

Table 2. Specific Cells Involved in Cellular Immunity

Designation	Cell Types	Functions
T helper cells	CD4 ⁺ T lymphocytes	Secretion of cytokines
Antigen Presenting cells	Mononuclear phagocytes, dendritic cells, B cells, langerhans cells,	Processing & presentation of foreign antigens to T _h lymphocytes
Activated Macrophages	Differentiated & stimulated monocytes	Killing of microbes & tumor cells; stimulation of inflammation; production of growth factors & cytokines
T killer cells	CD8 ⁺ T lymphocytes	Lysis of cells that are virally infected or associated with allografts and tumors
Natural Killer (NK) cells	Large granular lymphocytes	Non-specific killing of tumor cells & some infected cells
Killer (K) cells	NK cells with Fc receptors	Antibody-dependent cell killing

The process of antigen recognition (Fig. 1) is initiated by an antigen presenting cell (APC) (typically a macrophage, but not limited to this cell type) that has digested protein antigens into small (ca. 9-residue) peptides and present them on the major histocompatibility (MHC) surface protein. These MHC:antigen complexes are recognized by T cells that express surface receptors (T cell receptor, TCR) to initiate the stimulation of immunologic activity.



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Figure 1. Interaction of cells of the immune cells. In this figure a microbe, in this case a bacterium is processed by an antigen presenting cells, in this case a macrophage. The microbe can induce a T cell immune response by directly stimulating a B cell, however this response is amplified upon T cell activation. Likewise, T cell activation gives rise to cytotoxic T cells and memory cells of the immune system.

express; these proteins are designated CD4 (cluster of differentiation 4) and CD8 (cluster of differentiation 8). CD4 is expressed on the surface of T helper cells, macrophages, and dendritic cells. CD4 is the co-receptor for the T cell receptor (TCR). It amplifies the signal generated by the TCR for stimulating a T cell. CD8 serves as a co-receptor for the TCR. It is predominantly expressed on the surface of Cytotoxic T cells, but can also be found on natural killer cells. CD8 facilitates the interaction of these cells and the target cell intimately juxtaposing these cells during antigen-specific

T-helper 1 (Th₁) vs. T-helper 2 (Th₂).

Within the category of T-cell responses, it should be appreciated that not all T-cells respond the same. It is generally accepted that Th₁ cells drive the type-1 pathway (cellular immunity) to fight viruses and other intracellular pathogens, eliminate cancerous cells, and stimulate delayed-type hypersensitivity (DTH) skin reactions. Th₂ cells drive the type-2 pathway (humoral immunity) and upregulate antibody production to fight extracellular organisms. For example, Th₂ dominance is associated with tolerance of xenografts and of the fetus during pregnancy whereas Th₁ dominance is associated the response to gram negative sepsis.

CD4 vs. CD8 T-cells. T-cells are divided into two general subsets depending on the proteins that they

activation. Different infections initiate different ratios of CD4:CD8 cells, which in turn influence the type of immune response that is stimulated by an infection.

Cytokines. Immunologic recognition is communicated to other immunologic cells by the secretion of cytokines. Cytokines are a family of signaling molecules that include several interleukins (IL-1 through 18), growth and colony-stimulating factors, ciliary neurotrophic factors, interferons, and several other molecules that exhibit pleiotropic effects on cell differentiation, tissue development and homeostasis. Cytokines communicate among cells in the immune system through binding to specific receptors on target cells. Their biological actions vary widely depending upon the type of cell presenting that antigen and the target tissue involved. They have both proliferative and anti-proliferative properties. As a consequence they regulate the synthesis of acute phase proteins following tissue injury, trauma, inflammation, and sepsis (Table 3).

Table 3. Selected Immune Cytokines and Their Activities:

(Taken from: <http://microvet.arizona.edu/courses/MIC419/Tutorials/cytokines.html>)

Cytokine	Producing Cell	Target Cell	Function
GM-CSF	Th cells	progenitor cells	growth and differentiation of monocytes and DC
IL-1 α IL-1 β	monocytes macrophages B cells DC	Th cells	co-stimulation
		B cells	maturation and proliferation
		NK cells	activation
		various	inflammation, acute phase response, fever
IL-2	Th ₁ cells	activated T and B cells, NK cells	growth, proliferation, activation
IL-3	Th cells NK cells	stem cells	growth and differentiation
		mast cells	growth and histamine release
IL-4	Th ₂ cells	activated B cells	proliferation and differentiation IgG ₁ and IgE synthesis
		macrophages	MHC Class II
		T cells	proliferation
IL-5	Th ₂ cells	activated B cells	proliferation and differentiation IgA synthesis
IL-6	monocytes macrophages Th ₂ cells stromal cells	activated B cells	differentiation into plasma cells
		plasma cells	antibody secretion
		stem cells	differentiation
		various	acute phase response
IL-7	marrow stroma thymus stroma	stem cells	differentiation into progenitor B and T cells
IL-8	macrophages endothelial cells	neutrophils	chemotaxis
IL-10	Th ₂ cells	macrophages	cytokine production
		B cells	activation

IL-12	macrophages B cells	activated Tc cells	differentiation into CTL (with IL-2)
		NK cells	activation
IFN- α	Leukocytes	various	viral replication MHC I expression
IFN- β	fibroblasts	various	viral replication MHC I expression
IFN- γ	Th ₁ cells, Tc cells, NK cells	various	Viral replication
		macrophages	MHC expression
		activated B cells	Ig class switch to IgG _{2a}
		Th ₂ cells	proliferation
		macrophages	pathogen elimination
MIP-1 α	macrophages	monocytes, T cells	chemotaxis
MIP-1 β	Lymphocytes	monocytes, T cells	chemotaxis
TGF- β	T cells, monocytes	monocytes, macrophages	chemotaxis
		activated macrophages	IL-1 synthesis
		activated B cells	IgA synthesis
		various	proliferation
TNF α	macrophages, mast cells, NK cells	macrophages	CAM and cytokine expression
		tumor cells	cell death
TNF- β	Th ₁ and Tc cells	phagocytes	phagocytosis, NO production
		tumor cells	cell death

CTL: cytotoxic T lymphocytes; DC: dendritic cells; GM-CSF: Granulocyte-Monocyte Colony Stimulating Factor; IL: interleukin; IFN: Interferon; TGF: Tumor Growth Factor; TNF: Tumor Necrosis Factor; CAM: Cell Adhesion Molecule; NO: Nitric Oxide.

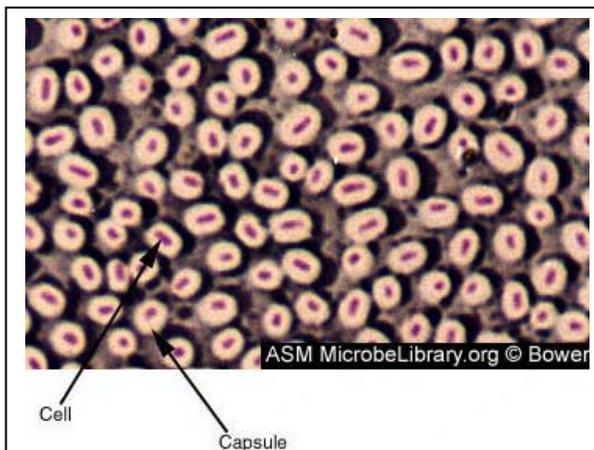
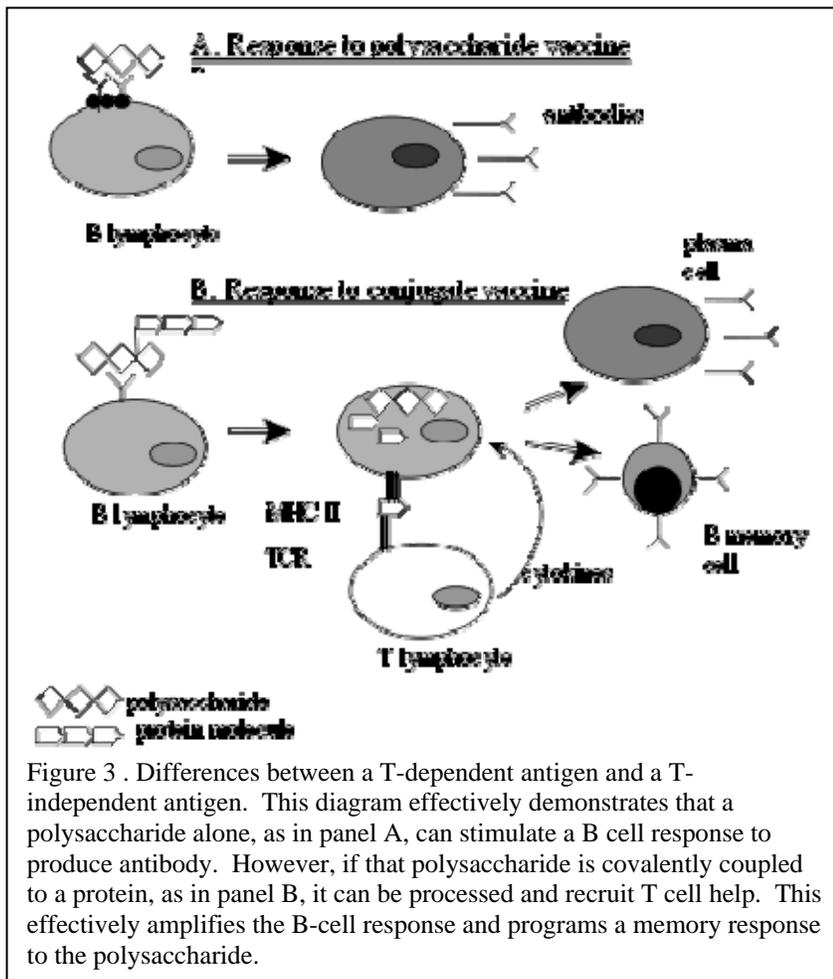


Figure 2. Capsule-producing bacillus-shaped bacteria. The capsule is composed of polysaccharides and in a few cases, polyaminoacids. Capsules have a role in adherence, virulence, protection, securing nutrients, and cell-to-cell recognition. Capsules vary in thickness and can easily be 2 times the volume of the organism. In a capsule stain, the background is stained grayish blue and the cells are stained red. The capsule is unstained and appears as a halo around the cell. (© Judy Bowen Buckman Laboratories International, Inc. Memphis, Tennessee, USA and [The MicrobeLibrary.org](http://TheMicrobeLibrary.org)).

Cell mediated immunity by antigen type. Depending on the composition of the antigenic target, a different strategy is employed by the cell mediated immune system:

Encapsulated Bacteria. Many bacteria produce a capsule (also known as an exopolysaccharide, glycocalyx, or slime layer) that coats the surface bacteria (Figure 2). Because bacterial capsules are nearly always carbohydrate (with the notable exception of the *Bacillus anthracis* polyglutamic acid capsule), they lack the ability to be presented on an MHC molecule and consequently stimulate an active T-cell response. In other words these surface structures

are poorly immunogenic. The result is that bacteria expressing capsular polysaccharides do not produce a vigorous antibody response and are not able to be phagocytized by macrophages. This is particularly true in the very young (where the cellular immune system is immature), the



immunocompromised (where the cellular immune system is functioning poorly), and the very old (where the cellular immune system has been functionally exhausted).

Carbohydrate antigens such as capsules are therefore referred to as “T-independent antigens” because they induce an immune response independent of the T cell arm of the comprehensive host immune response. Perhaps the best example of this is the current formulation of the Hib vaccine. *Haemophilus influenzae* type b (Hib) is a common respiratory pathogen that causes invasive diseases such as pneumonia, bacteremia, and meningitis among

others, particularly in infants. In the 1980s this organism was responsible for the majority of infantile meningitis cases in the United States at an incidence of roughly 20 cases per 100,000 in the population. The “type b” component of the Hib name refers to a particular capsule comprised of polyribosylphosphate (PRP) that is by itself is poorly immunogenic. In order to render this PRP capsule immunogenic, the capsular polysaccharide is covalently attached (conjugated) to an immunogenic protein (referred to as a carrier protein) (Figure 3). This form of the vaccine (immunogen) is referred to as the “capsule-conjugate-carrier”, or a “conjugate vaccine”. The key to this strategy is to establish an effective antibody response that recognizes the capsular structure on the surface of a bacterium, allowing the antibody Fab regions to recognize the capsule and the Fc portion of the antibody to be bound by the Fc receptors on the macrophage as demonstrated in Figure 4. The end result of this is opsonization and the destruction of the bacterium.

The success of this approach is clearly demonstrated in Figure 5 demonstrating the precipitous decline in *H. influenzae* associated meningitis cases upon broad distribution of the Hib vaccine. Arguably, this is among the most tangible successes realized over the past 4 decades of “molecular

medicine”. It also exemplifies our ability to “engineer” productive immune responses through the cellular immune system.

Intracellular bacteria and viruses. Intracellular pathogens such as invasive bacteria and viruses subvert the humoral immune system by existing within the plasma membrane of the host cell. Cell-mediated immunity is critical to removing these pathogens and is most easily demonstrated by the process of eliminating viral infection. Viruses have both extracellular and

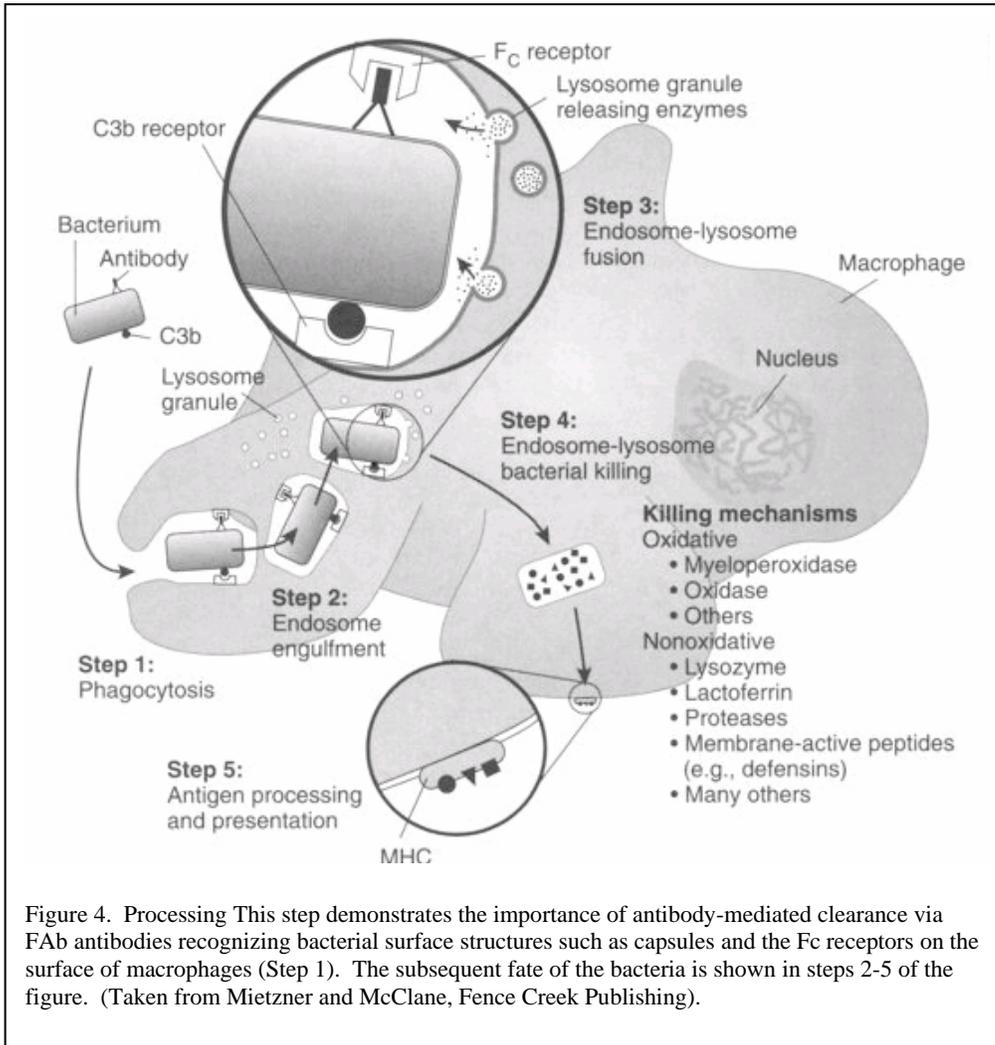


Figure 4. Processing This step demonstrates the importance of antibody-mediated clearance via FAb antibodies recognizing bacterial surface structures such as capsules and the Fc receptors on the surface of macrophages (Step 1). The subsequent fate of the bacteria is shown in steps 2-5 of the figure. (Taken from Mietzner and McClane, Fence Creek Publishing).

intracellular phases. An antigen-presenting cell (APC) may take up virus in two ways—through phagocytosis and through direct viral infection. Viruses that are phagocytized are fused with vesicles containing digestive enzymes. Viral peptides are then processed, bound to class II MHC molecules, and presented on the APC cell surface to CD4 T-cells. The T-cells recognize the antigen-MHC complex via the TCR, stabilized by the CD4 receptor. Th cells differentiate and are

Haemophilus influenzae type b (Hib) Disease – Reported Cases, Canada, 1979-2000*

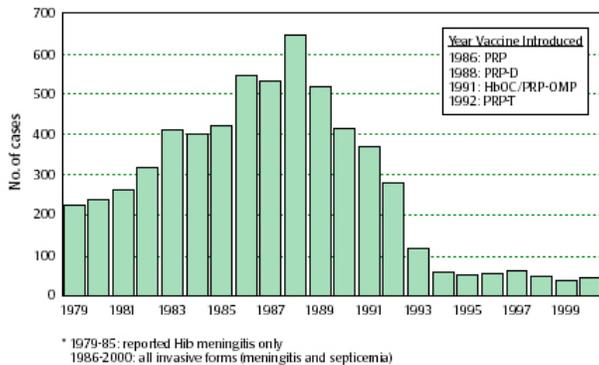
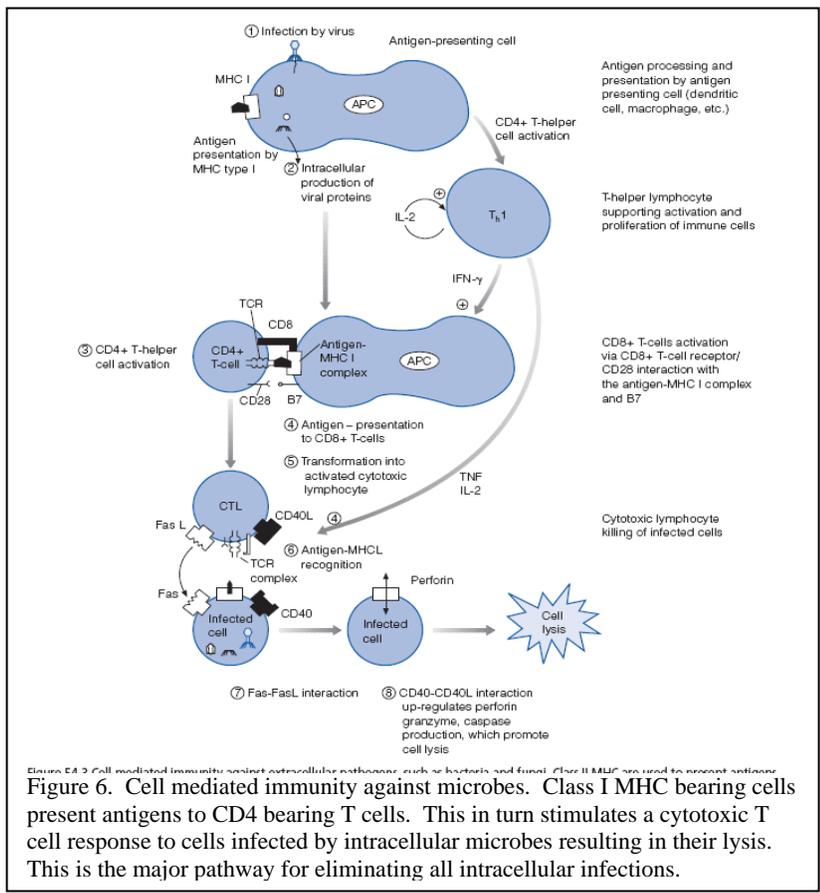


Figure 5. Decrease in cases of Hib disease as a function of the distribution of the conjugated Hib vaccine in Canada. These data parallel data in other countries that the Hib conjugate vaccine has been in broad distribution.

activated to secrete cytokines, depending on the nature of the pathogen to be eliminated. T-helper cells universally secrete IL-2 upon activation, which stimulates proliferation of many immune cells including B-cells, CTL, and Th cells. Th cells differentiate into two types: Th₁-cells and Th₂-cells. Th₁-cell differentiation is stimulated by APC-secreted IL-12. Th₁-cells primarily promote immunity against small extracellular pathogens such as viruses and bacteria, eliminated through phagocytosis and cell killing. Both CD40L and the IFN- γ promote B-cell isotype switching toward IgG production. IFN- γ activates macrophage and antigen presentation by upregulating their oxidative killing machinery and MHC expression, respectively. Th₁-cells also secrete TNF, which activates neutrophil phagocytosis and CTL-mediated elimination of virally infected cells. This antigen-MHC I complex specifically



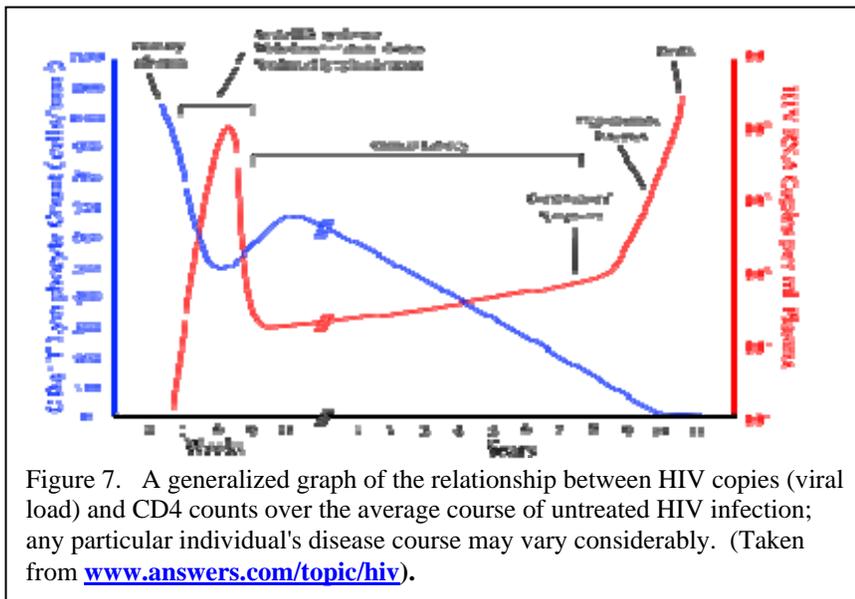
activate CD8⁺ T-lymphocytes, which are then stimulated to differentiate into cytotoxic lymphocytes. One important molecular interaction includes the upregulated CD40L/CD40 receptors. The CD40L-CD40 interaction activates CTLs to produce several cell-killing mediators. Fas ligand (FasL) surface expression is up regulated, which binds to Fas on infected cells and thereby induces cell apoptosis. The CD40L-CD40 interaction also promotes the production of perforin, granzymes, and caspases that kill cells by inserting into the plasma membrane of the infected cell, forming a pore that leads to cell lysis.

CTLs recognize virus-infected cells by their TCRs binding to class I MHC molecules, presenting the specific viral peptide to which they are sensitized. This activates the cytotoxic machinery described above. Finally, the T-helper cell's CD40L also binds CD40 on B-cells, promoting activation and antibody production. Immunologic memory to a pathogen is established through the differentiation of a subpopulation of activated Th-, B-, and CD8⁺ T-cells into their own respective memory cells.

Parasites. Th₂-cells are differentiated toward eliminating large extracellular pathogens, such as parasites. Th₂-cell differentiation is driven by IL-4, secreted by mast cells and basophils after a parasite encounter. Th₂-cells in turn secrete more cytokines like IL-4 itself, which promotes B-cell

isotype switching to IgE, the primary anti-parasitic antibody. IL-5 promotes eosinophil function, the primary parasite killer cell. Eosinophils recognize the Fc portion of IgE via Fc-receptors, which activate the extracellular release of major basic protein and other enzymes that attack the parasite's cell membrane.

Human Immunodeficiency Virus (HIV) infection. One infection that optimizes the loss of a functional cellular immune system is HIV, a retrovirus, meaning that it must convert its RNA genome to DNA using a reverse transcriptase (RT). The virus is transmitted through the exchange of body fluids, most commonly sexual intercourse or the sharing of intravenous needle drug use. Less common is transmission by blood product transfusions, transplantation of infected organs, or inadvertent needle sticks by health care workers. Currently in the United States the number of HIV infected individuals is on the order of 10 per 100,000 in the population. The probability of infection is a function of both the number of infective HIV virions in the body fluid which contacts the host as well as the number of cells

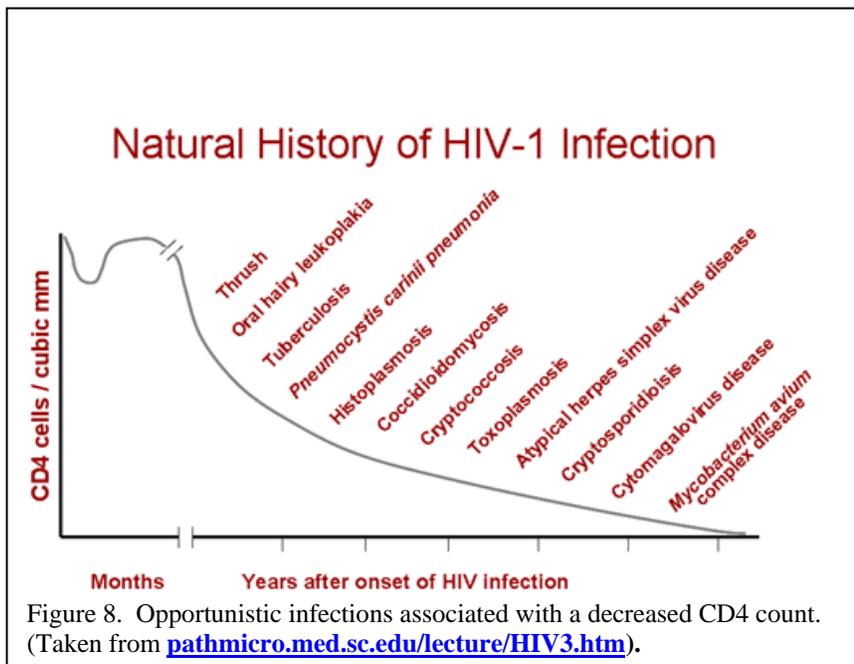


available at the site of contact that have appropriate CD4 receptors. For example, individuals with concurrent sexually transmitted infections such as gonorrhea or chlamydia (that recruit inflammatory cells to the site of infection) are more susceptible to HIV infection. Cells with CD4 receptors at the site of HIV entry become infected followed by aggressive viral

infection. The infected cells release which spread to cells in the local area. Some HIV-infected cells are carried from the site of infection to the regional lymph nodes where other immune system cells become infected. Although CD4⁺ T cells are the virus' main target, other immune system cells with CD4 molecules on their surfaces are infected as well. Long-lived monocytes and macrophages harbor large quantities of the virus without being killed. Normal immune processes may activate these cells, resulting in the production of new HIV virions. Systemically, increased production of a variety of cytokines such as TNF and IL-6 activate CD4⁺ T lymphocytes render cells more susceptible to HIV infection. Activation allows uninfected cells to be more easily infected and increases replication of HIV-infected cells. While certain cytokines are secreted in higher quantities during HIV infection, other cytokines may be secreted in decreased amounts—leading to a state of general lymphokine dysregulation. Current evidence suggests that billions of CD4⁺ T cells are destroyed

every day as a result of HIV infection, eventually overwhelming the immune system's regenerative capacity.

Primary HIV infection is followed by a burst of viremia in which virus is easily detected in peripheral blood in mononuclear cells and plasma. The number of CD4⁺ T cells in the bloodstream decreases by 20 to 40 percent. Two to four weeks after exposure to the virus, up to 70 percent of HIV-infected persons suffer flu-like symptoms related to the acute infection. The burst is followed by replication at a lower level when, the patient's immune system fights back to dramatically reduce HIV levels with killer T cells (CD8⁺ T cells), which attack and kill infected cells that are producing virus, and B-cell-produced antibodies (Fig. 7). A patient's CD4⁺ T cell count may rebound to near its original level. An infected individual may remain free of HIV-related symptoms, often for years, despite low-level HIV replication in the lymphoid organs and ongoing immune system destruction. During this latency period, the immune system is sufficiently intact to prevent most infections. In addition, HIV can mutate very easily; the HIV RT makes many mistakes while making DNA copies from the viral RNA. As a consequence, many variants of HIV develop in an individual (known as antigenic variation), some of which may escape immune attack by antibodies or killer T cells. Due to antigenic variation, antibodies



formed against HIV are not protective and an infective state can persist despite the presence of even high antibody titers. Chronic immune system activation during HIV disease may also result in a massive stimulation of B cells, impairing the ability of these cells to make antibodies against other pathogens. When a significant number of CD4 lymphocytes have been destroyed and when production of new ones cannot

match destruction. Patients exhibit fatigue, long-lasting fever (>1 month) and weight loss. The failure of the immune system progresses from HIV-positive to a clinical diagnosis of AIDS.

Without treatment, HIV-infected individuals may live up to an average of 8 or 10 years after initial infection (before development of the clinical symptoms of AIDS). Most AIDS-defining conditions are marked by a CD4⁺ T count of less than 200 cells mL³ blood and the appearance of 1 or more opportunistic infections or cancers, including Kaposi's sarcoma, *Pneumocystis carinii* pneumonia, and

Mycobacterium avium complex. Opportunistic infections are caused by microbes that usually do not cause illness in healthy people (Figure 8). These infections are often severe and sometimes fatal because the immune system is destroyed by HIV infection that the body cannot contain them.

Immune Reconstitution Syndrome. Immune reconstitution, or the reversal of HIV-related immune system decline, is one of the primary goals of highly active antiretroviral therapy (HAART). Reconstitution involves an increase in functional CD4 cells to guide the immune response against HIV, resulting in the suppression of viral load. However, immune reconstitution may trigger an inflammatory reaction in some people soon after they begin anti-HIV therapy and show signs of immunological improvement. Known as immune reconstitution syndrome (IRS), this set of symptoms often resembles an AIDS-defining illness or other condition seen in people with HIV. While in most cases the symptoms of IRS resolve after a few weeks, the syndrome may be severe or mistaken for true disease progression, and should be properly diagnosed and treated. Reports in the medical literature described HIV positive individuals that appeared to develop a spectrum of illnesses after they had started and responded to HAART. Remarkably, the individuals in these cases developed conditions associated with poor immune system function at a time when their immune function was actually improving. The "paradoxical reactions" seen in HIV positive individuals in whom the immune system was restored involved inflammatory responses to pathogens that were either latent or controlled by drug treatment when the immune system was seriously weakened. The inflammation therefore did not signal a reactivation or worsening of a disease, but rather a protective process initiated by the body. As the immune system continues to improve, the IRS inflammation usually resolved, though often accompanied by some form of treatment.

IRS is associated with a variety of latent or subclinical infections, many of which are more commonly seen in people with very low CD4 cell counts. IRS is perhaps most typically associated with mycobacterial infections (such as *M. avium* complex) and herpesvirus infections (such as shingles

Table 4. Disease and Pathogens Associated with IRS
<i>Cryptococcus neoformans</i>
cytomegalovirus (CMV)
Hansen's disease (leprosy)
hepatitis B virus (HBV)
hepatitis C virus (HCV)
herpes simplex virus (HSV)
herpes zoster (shingles)
<i>Histoplasma capsulatum</i>
human papillomavirus (HPV)
Kaposi's sarcoma (KS)
<i>Mycobacterium avium</i> complex (MAC)
<i>Pneumocystis carinii</i> pneumonia (PCP)

and cytomegalovirus). For example, someone previously responding to treatment for tuberculosis would be at risk for a new or worsening fever, new effusions (escape of fluid), new or worsening lymphadenopathy (enlarged lymph glands), and other uncharacteristic reactions, rather than progression of the lung disease itself. Other common syndromes are listed in Table 4.

Summary. Clearly, the health of any individual living in this “microbial world” requires a comprehensive immune system. In this chapter, it is hoped that the reader appreciates that one critical arm of the immune system is the cellular response. This arm can be engineered to enhance a response (such as that seen for the Hib vaccine), maintain immunity from microbes that we are constantly exposed to, and if specifically targeted for destruction by a pathogen (such as by HIV infection), can lead to fatal and uncommon infection.