

Overview of the human immune system

A general overview of the immune system is provided so that an appreciation can be gained of how biological molecules from certain mushrooms may modulate the immune response and tackle cancer cells. Immunology is the study of the methods by which the body defends itself from infectious agents and other foreign substances in its environment. The immune system is a remarkably adaptive defence system that has evolved in humans to protect against invading pathogenic microorganisms and evidence is accumulating that the immune system can provide protection against some tumours (Wood, 2001). An infectious organism that causes a disease is called a pathogen and the individual (person or animal) that is infected by a pathogen is called the host. There are thousands of components to the immune system and it would appear that the immune system is far more complicated than necessary for achieving what is, on the surface, a simple task of eliminating a pathogenic organism or abnormal 'self' cells. However there are a number of reasons for this complexity, including the desirability of eliminating pathogens without causing damage to the host.

Getting rid of a pathogen or dead host cells is theoretically easy, but eliminating these without damaging the host is much more complicated. The immune system must be able to distinguish between pathogens or abnormal cells and healthy host cells so that it can direct its destructive powers towards their elimination. As a consequence of this dynamic complexity, the immune system is able to generate a tremendous variety of cells and molecules capable of specifically recognising and eliminating an apparently limitless variety of foreign invaders, in addition to the recognition and destruction of abnormal cells. Furthermore, these host cells and molecules act together in an exquisitely adaptable dynamic manner.

Functionally, an immune response can be divided into the interrelated activities of recognition and response. The immune system is remarkably specific as it is able to recognise subtle chemical differences that distinguish foreign or 'non-self' cells from healthy self-cells. At the same time, the system is able to discriminate between foreign

molecules and the body's own cells and proteins. Once a foreign protein, microorganism (e.g., bacterium, fungus or virus) or abnormal cell is recognised, the immune system enlists the participation of a variety of cells and molecules to mount an appropriate effector response to eliminate or neutralise them. Later exposure to the same foreign organism (e.g., a virus that may have the potential to transform normal healthy cells into tumour cells) induces a memory response, characterised by a heightened immune reactivity, which serves to eliminate the microbial pathogen, prevent disease and protect against the development of some tumour cells (Wood, 2001).

Immunity - the state of protection from infectious disease, has both non-specific and specific components. Innate, or non-specific immunity refers to the basic resistance to disease that an individual is born with. Acquired or specific immunity requires activity of a functional immune system, involving cells called lymphocytes and their products. Innate defence mechanisms provide the first line of host defence against invading microbial pathogens and also provides protection against some tumour cells until an acquired immune response develops. In general, most of the foreign molecules or microbial cells encountered by a healthy individual are readily cleared within a few days by non-specific defence mechanisms without enlisting a specific immune response. When the non-specific defences fail to eliminate foreign invaders or abnormal cells, a specific or humoral immune response is then enlisted. Because immunity was shown to be mediated by molecules known as antibodies that were contained in body fluids (known in earlier times as *humors*), it was known as humoral immunity (Wood, 2001). An antibody is a protein or immunoglobulin that recognises a particular epitope or site on an antigen, which is any substance that binds specifically to an antibody or T-lymphocyte receptor, and facilitates clearance of that antigen. The other arm of the specific immune response is cell-mediated immunity or CMI. CMI response refers to host defences that are mediated by antigen-specific T lymphocyte cells (i.e., leukocytes) and various non-specific cells of the immune system. It protects against intracellular bacteria, viruses and cancer and is responsible for graft rejection. Acquired immunity does not operate independently of innate immunity; rather, the

specific immune response supplements and augments the non-specific defence mechanisms, producing a more effective total response (Wood, 2001).

Innate (non-specific) immunity

Innate immunity can be envisioned as comprising four types of defensive barriers: anatomic, physiologic, endocytic and phagocytic, and inflammatory. Tissue damage and infection induce leakage of vascular fluid, containing serum with antimicrobial activity, and influx of phagocytic cells into the affected area. While physical and anatomic barriers, such as skin and the surface of mucous membranes, prevent the entry of pathogenic microorganisms and are the body's first line of defence, this component of innate immunity will not be developed any further as it has no bearing on immuno-modulation or anti-tumour responses. The physiologic barriers that contribute to innate immunity include elevated temperature (e.g., fever), pH (e.g., acidity produced in stomach and within macrophages), oxygen tension, and various soluble factors (Kuby, 1997). Among these soluble proteins are lysozyme (a hydrolytic enzyme found in mucous secretions that kills bacteria), interferons (INF) and other cytokines (chemical messengers), and complement (plasma proteins that participate in a controlled enzymatic cascade which results in damage to the membranes of pathogenic organisms or abnormal cells, either destroying or facilitating their clearance), markedly influence immunomodulation and regulation, in addition to the prevention of some tumour cells (Kuby, 1997).

Cytokines: the chemical messengers

The term cytokine covers a variety of small proteins less than 20 kDa (usually) that serve a hormone-like function in enabling cells to communicate with each other (Wood, 2001). There are many cytokines and they can be divided into families (Table 2). The main families of cytokines are the interleukins (ILs), colony-stimulating factors (CSF), interferons (INFs), tumour necrosis factors (TNFs), chemokines and growth factors. The functions of cytokines will be described in detail at the appropriate times

when particular immunological mechanisms are being explained. Cells in the body are never exposed to single cytokines – they will be exposed to a number of different cytokines, probably produced by a number of different cell types (Wood, 2001). Different cytokines can either act cooperatively in promoting a response, or act antagonistically in inhibiting each other's actions (Kuby, 1997, Wood, 2001).

Table 2. Cytokine families*

Family	Members	Comments
Interleukin (IL)	IL-1 to IL-22	Different IL have different functions and are secreted by different cells
Interferon (IFN)	IFN α	Leucocyte IFN. Inhibits viral replication
	IFN β	Fibroblast IFN. Inhibits viral replication
	IFN γ	Secreted by T lymphocytes and NK cells. Many immunoregulatory functions
Tumour necrosis Factor (TNF)	TNF α	Secreted by monocytes and other cells. Factor activates macrophages and endothelial cells
	TNF β	Secreted by T cells. Similar activity to TNF α
Colony-stimulating Factors (CSF)	G-CSF, M-CSF, GM-CSF and others	Originally identified by ability to make bone-marrow cells differentiate into particular cell type, e.g. neutrophil. Also have effects on mature cells of same lineage, e.g. monocytes, macrophages and neutrophils
Chemokine	MCP, Ecotaxin and others	Very important in controlling the migration of cells between and within tissues. Also influence function of many cells

* Source: Wood, 2001

Macrophages and phagocytosis

Other important components of innate immunity are phagocytic cells (macrophages, neutrophils) and other lymphocytes such as natural killer (NK) cells that do not require activation but can lyse certain infected or abnormal cells. Macrophages are large leukocytes (any blood cell that is not an erythrocyte; white blood cell) derived from monocytes that functions in phagocytosis, antigen processing and presentation, secretion of cytokines, and antibody-dependent cell-mediated cytotoxicity (ADCC). While explained in more detail later, ADCC is a cell-mediated reaction in which non-specific cytotoxic cells that express Fc receptors, such as neutrophils, macrophages, NK cells, recognise bound antibody on a target cell and subsequently causes lysis (destruction) of the target cell. Phagocytosis is a process by which certain cells (phagocytes) engulf microorganisms, other cells, and foreign particles (Figure 1).

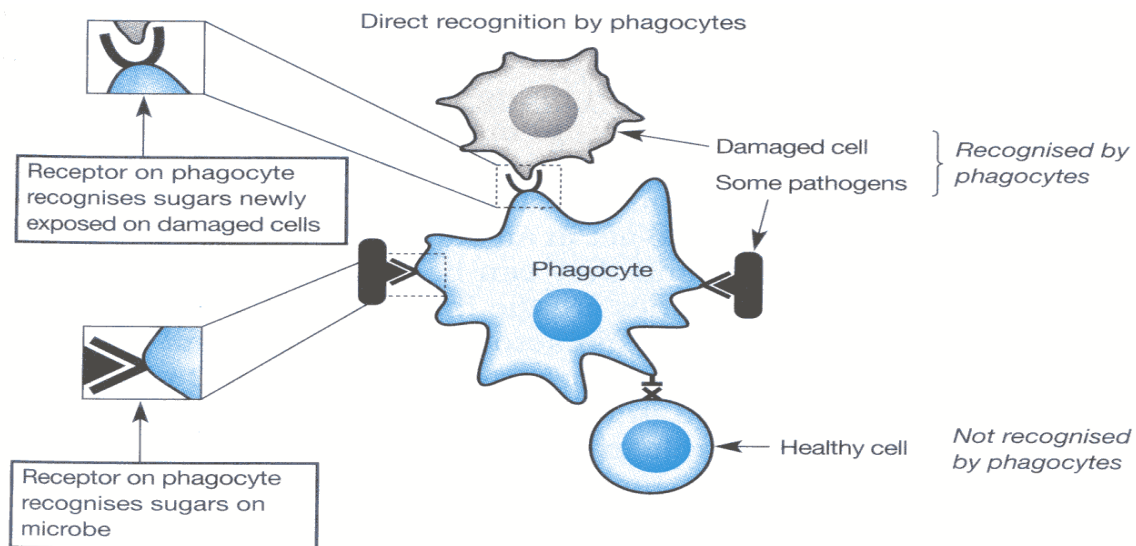


Figure 1. Recognition by phagocytes. Phagocytes must distinguish microbes and dead host cells from healthy host cells so that healthy host cells are not phagocytosed. Phagocytes have receptors on their surface that recognise sugars present on microbes or sugars that are newly expressed on dead or damaged host cells. These sugars are not present on healthy host cells and therefore the host cells are not phagocytosed (Source: Wood, 2001).

Natural Killer (NK) cells

Another population of cells that form part of the innate immune system are natural killer cells (NK) cells. NK cells are large, granular lymphocytes that are capable of lysing or killing infected or tumour cells without overt antigenic stimulation (recruiting specific immune response). NK cells osmotically lyse target cells and induce apoptotic cell death. Apoptosis is known as programmed cell death that is characterised by morphologic changes including nuclear fragmentation, blebbing, and release of apoptotic bodies, which are phagocytosed. In contrast to necrosis, it does not result in damage to surrounding cells (Kuby, 1997). NK cells lack the T lymphocyte receptor for antigen recognition. Another important role for NK cells is in the inflammatory response (discussed in more detail later). NK cells enter sites of inflammation where they can be stimulated by a cytokine called IL-12 that is produced by activated macrophages. The NK cells are stimulated by IL-12 to produce IFN- γ that is a powerful activator of macrophages (Wood, 2001). The cellular origin of natural killer cells is unknown.

Complement system

Target cells can also be destroyed through the activation of complement which is a complex series of interrelated proteins present in normal serum. Components of the complement system (i.e., activated components C3a, C3b through to C9) mediate and amplify immune reactions. Following the release of chemotactic factors and histamine C3a this induces considerable inflammation and tissue damage at the sites of reactions with antibodies. Residual C3b component bound to the antigen-antibody complexes attaches to C3b receptors present on macrophages and thus acts as an opsonin, promoting enhanced phagocytosis. Where antibody has reacted with the surface of virus-infected or transformed cells, the complement system is activated to form a membrane attack complex resulting in cell lysis. The latter processes are known as antibody-dependent cellular cytotoxicity (ADCC). As with the effector response to unwanted or 'non-self' antigen-presenting cells, a well-orchestrated *in vivo* system regulates the overproduction of specialised B and T lymphocytes (discussed in more

depth later). For example, transforming growth factor (TGF)- β inhibits B and T cell proliferation; INF- γ inhibits IL-4 activation of B cells; and IL-4 / IL-10 inhibit INF- γ activation of macrophages.

Inflammatory and acute phase responses

Usually there are not enough macrophages or monocytes present in tissue to phagocytose and remove all invading pathogens and therefore the tissue macrophages must initiate a response that will bring additional phagocytes, together with a variety of host proteins (cytokines) and cells (lymphocytes), to the site of infection from the bloodstream (Kuby, 1997). This response is known as the inflammatory response and in addition to removing pathogens it also eliminates dead or abnormal host cells. Figure 2 illustrates the four main events occurring in an inflammatory response that are:

1. **Vasodilation** – causes increased blood flow to the area, increasing the supply of cells and factors
2. **Activation of endothelial cells** – lining the blood vessels makes them more 'sticky' to white blood cells so that the blood cells can adhere more strongly to the endothelium
3. **Increased vascular permeability** – makes it easier for cells and proteins to pass through the blood vessel walls and enter the tissue
4. **Chemotactic factors** are produced – these are molecules that attract cells into the tissue from the blood (Wood, 2001).

The first stage of the inflammatory response is recognition of the pathogen and activation of tissue macrophages that on stimulation, produce a number of factors including prostaglandins (small biologically active lipid molecules), platelet-activating factor (PAF) and cytokines (of particular importance are interleukin-2 and IL-8, and tumour necrosis factor- α or TNF- α).

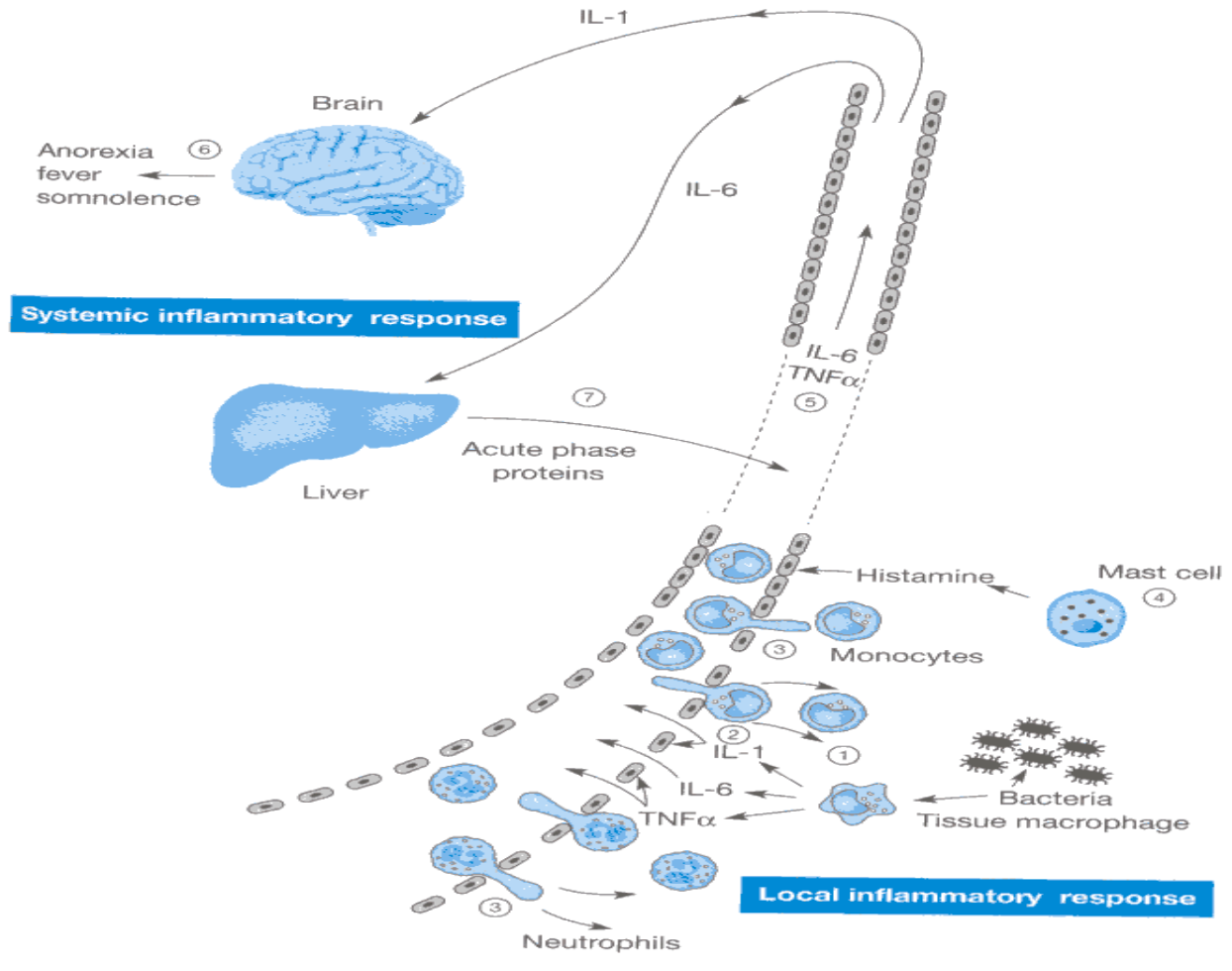


Figure 2. Inflammatory responses. *Inflammatory responses can be local or systemic. 1, tissue macrophages recognise microbial products. 2, macrophages release cytokines and other inflammatory mediators (IL-1, TNF- α) that cause vasodilation, increased vascular permeability and have chemotactic effects on monocytes and neutrophils. 3, Monocytes and neutrophils are recruited to the site and there is accumulation of plasma fluid and proteins at the site, causing oedema or swelling. 4, Inflammatory mediators can activate mast cells to release further mediators that amplify the response. 5, if the local production of cytokines is high enough, the cytokines travel in the blood and affect other organs. 6, IL-1 affects the brain causing fever. 7, IL-6 stimulates hepatocytes to produce acute phase proteins (source: Wood, 2001).*

These cytokines act directly on the endothelium to increase vascular permeability and PAF also causes platelets to release histamine (another agent that increases vascular permeability). IL-1 and TNF- α activate endothelial cells lining the blood vessels

at the site of infection that causes these cells to express surface molecules that neutrophils in the bloodstream can bind to, enabling the neutrophils to leave the bloodstream and enter the tissue. Neutrophils (also promoted by IL-8) and macrophages eliminate pathogens by the process of phagocytosis (Wood, 2001).

Other cell types and biochemical pathways can also be activated during an inflammatory response that can result in the accumulation and activation of granulocytes and monocytes resulting in the removal of pathogenic microorganisms by phagocytosis. Activation of the complement and clotting systems is also important for the inflammatory response. The complement system is made up of a number of different plasma proteins that participate in a controlled enzymatic cascade that results in damage to membranes of pathogenic organisms or abnormal cells, either destroying or facilitating their clearance. The roles of activated complement components in eliminating pathogens and abnormal cells will be addressed later. The clotting system leads to the cleavage of fibrinogen to generate fibrin threads that form blood clots and fibrinopeptides which are chemotactic for phagocytes (Wood, 2001).

If the pathogen is not eliminated the continued recruitment and stimulation of macrophages will lead to a rise in the concentration of macrophage-derived cytokines in the plasma (Wood, 2001). These cytokines can affect organs such as the brain and liver, that causes a systemic response known as an acute phase response. Of particular importance is the production of a series of proteins called acute phase proteins (APPs) such as, fibrinogen (involved in the clotting and generation of fibrinopeptides), heptoglobulin (binds iron whereby limiting bacterial growth), complement component C3 (its cleavage to C3a - activates mast cells that contain large granules of histamine, heparin and proteolytic enzymes (protein attacking), and C3b - helps phagocytes recognise pathogens), and proteins such as C-reactive and mannose binding proteins that target specific receptors on invading microorganisms facilitating their elimination by phagocytosis. Because the cells and proteins of the inflammatory and acute phase responses are pre-existing, they provide an immediate response to tissue damage and infection (Wood, 2001).

Acquired (non-specific) immunity

Acquired, or specific, immunity reflects the presence of a functional immune system that is capable of specifically recognising and selectively eliminating foreign microorganisms and molecules (i.e. foreign antigens). Unlike innate immune responses, acquired immune responses are adaptive and display the following characteristics:

1. **Antigenic specificity** – permits the immune response to distinguish subtle differences among antigens. Antibodies can differentiate between two molecules that differ by a single amino acid (building block of proteins).
2. **Diversity** – it is capable of generating tremendous diversity in its recognition molecules, allow it to specifically recognise billions of uniquely different structures on foreign antigens.
3. **Immunologic memory** – once the immune system has recognised and responded to an antigen, a second encounter with the same antigen induces a heightened state of immune reactivity.
4. **Self/nonself recognition** - the immune system normally responds only to foreign antigens indicating that it is capable of self/nonself recognition. The ability of the immune system to distinguish self from nonself and respond only to nonself-molecules is essential, for the outcome of an appropriate response to self-molecules can be a fatal autoimmune disease (Kuby, 1997).

Acquired immunity does not occur independently of innate immunity. The phagocytic cells (NK cells, neutrophils, macrophages) crucial for non-specific immunity are intimately involved in the activation of the specific immune response. Conversely, various soluble factors produced during a specific immune response, have been shown to augment the activity of these phagocytic cells. Thus, through the carefully orchestrated interplay of acquired and innate immunity, the two systems work in tandem to eliminate a foreign invader or abnormal cells (Kuby, 1997). Generation of an effective immune response involves two major groups of cells: lymphocytes and antigen-presenting cells (APCs). Lymphocytes are one of the many types of white blood

cells produced in the bone marrow during the process known as hematopoiesis. There are three general classes of cells produced from hematopoietic stem cells, (1) red blood cells (erythrocytes) that are responsible for oxygen transport, (2) platelets that are responsible for the control of bleeding, and (3) white blood cells (lymphocytes), the vast majority of which are involved in host immunity. Lymphocytes leave the bone marrow, circulate in the blood and lymph system, and reside in various lymphoid organs (Kuby, 1997). Lymphocytes possess antigen-binding cell-surface receptors, mediate the defining immunologic attributes of specificity, diversity, memory, and self/nonself recognition. There are two major populations of lymphocytes – B lymphocytes (B cells) and T lymphocytes (T cells) (Kuby, 1997).

B lymphocytes

B lymphocytes mature within the bone marrow and leave the marrow expressing a unique antigen-binding receptor on their membrane. The B cell receptor is a membrane-bound antibody molecule. Upon activation, B cells specific for the antigen (usually foreign) proliferate and become antibody secreting or plasma cells. Antibodies are complex molecules (glycoproteins) that have the property of combining specifically to the antigen that induced its formation. The resulting antibodies bind to the invading pathogen, marking it for destruction by killer T-lymphocytes by a process called antibody dependent cell cytotoxicity (ADCC). Antibodies also mark cells for phagocytosis by neutrophils and other phagocytic cells by a process called opsonisation. Most of the daughter cells produced by B cell activation die within a few weeks but a proportion of them recirculate in the body for many years as memory cells. If they are reintroduced to the same antigen that elicited an initial response, they rapidly become reactivated and produce antigen-specific antibody. This function provides the basis for vaccination. It is estimated that a single antibody secreting or plasma cell can produce more than 2000 molecules of antibody per second (Kuby, 1997). Secreted antibodies are the major effector molecules of humoral immunity.

T lymphocytes

T-lymphocytes (T cells) also arise from hematopoietic stem cells in the bone marrow. Unlike B cells, which mature within bone marrow, T cells migrate to the thymus gland to mature. During its maturation within the thymus, the T cell comes to express a unique antigen-binding receptor on its membrane, called the T cell receptor (TCR). Unlike membrane bound antibodies on B cells, which can recognise antigen alone, TCRs can only recognise antigen that is associated with cell membrane proteins known as major histocompatibility complex (MHC) molecules (Kuby, 1997). When a naïve T cell encounters antigen associated with a MHC molecule on a cell, the T cell proliferates (clones) and differentiates into memory T cells and various effector T cells.

There are two well-defined subpopulations of T cells: T helper (T_H) and T cytotoxic (T_C) cells. T helper and T cytotoxic cells can be distinguished from one another by the presence of either membrane glycoproteins CD4+ or CD8+ on their surfaces. T cells displaying CD4+ generally function as T_H cells, whereas those displaying CD8+ function as T_C cells. After a T_H cell recognises and interacts with an antigen-MHC II molecule complex, the cell is activated and becomes an effector cell that secretes various cytokines. These secreted cytokines play an important role in activating B cells, T_C cells, macrophages, and various other T cells, and initiate the delayed type hypersensitivity (DTH) response. The DTH reaction promotes local inflammation resulting in the recruitment of more lymphocytes and activated macrophages (i.e., converted monocytes from the bloodstream) to target cells. Under the influence of T_H -derived cytokines, a T_C cell that recognises an antigen-MHC I molecule complex proliferates and differentiates into an effector cell called a cytotoxic T lymphocyte (CTL). In contrast to the T_H cell, the CTL generally does not secrete many cytokines and instead exhibits cytotoxic activity (Kuby, 1997). The CTL has a vital function in monitoring the cells of the body and eliminating any that display antigen, such as infected or tumour cells. Figure 3 illustrates key cellular interactions involved in induction of acquired immune responses.

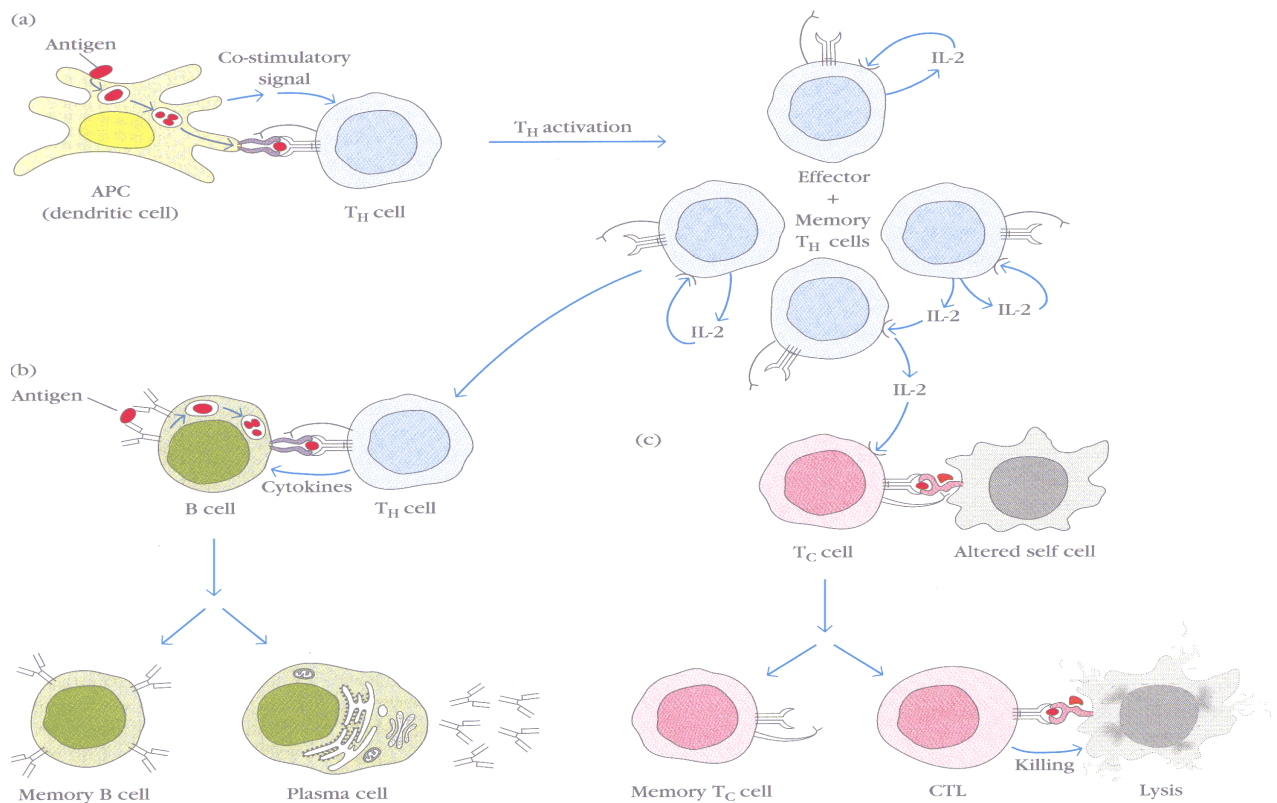


Figure 3. Cellular interactions involved in induction of the specific immune responses. Activation and proliferation of T_H cells (a) is required for generation of a humoral response (b) and a cell-mediated response to altered self-cells (c). APC = antigen-presenting cell; Ag = antigen (Source: Kuby, 1997).

Thus, acquired immunity is composed of activated CD4 (T_H) and CD8 (T_C) cellular responses. Furthermore, T_H cells recognise foreign proteins or antigens that have been processed through an exogenous pathway by antigen-presenting cells such as dendritic cells in lymph nodes, macrophages or B cells expressing major histocompatibility complex (MHC) class II molecules (Fig. 4). This MHC II mediated-recognition of foreign antigens causes T_H cells to become activated, whereupon differentiation occurs into functional subsets termed T helper 1 or (T_H1)-type and T helper 2 or (T_H2)-type cells. Activation of T_H cells is central to cellular immunity and is facilitated through the action of IL-1 and INF- γ secreted by antigen-presenting cells.

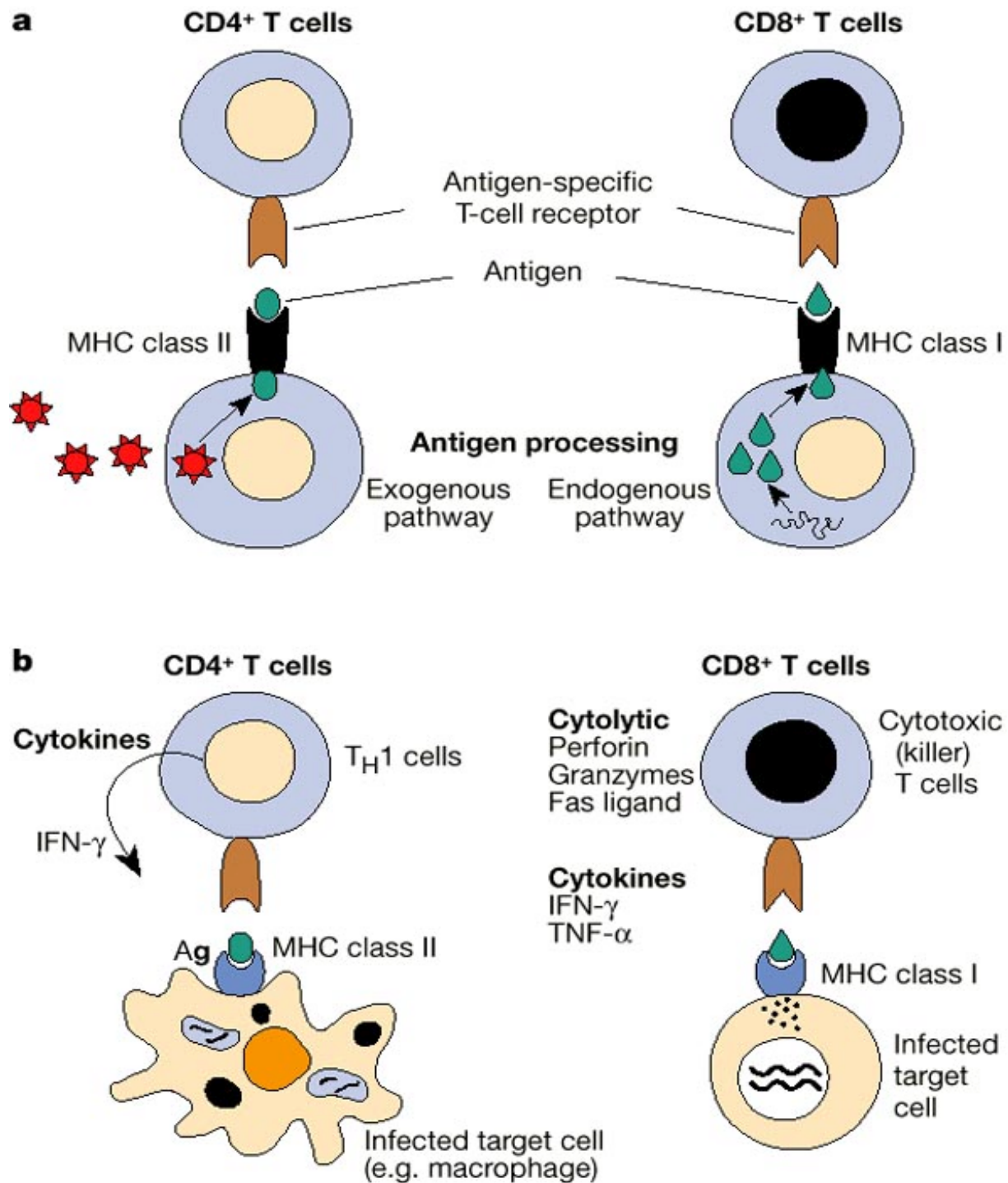


Figure 4. Mechanism of T-cell activation and effector function. a Mechanism of antigen (Ag) processing and recognition by T cells. **b** Effector function of TH1 and CD8⁺ T cells. (Source, Seder and Hill, 2000).

Cytokines such as INF- γ and certain interleukins (including IL-2, IL-4, IL-5, IL-8, IL-10 and IL-12) assist T_H cells in the activation, proliferation and clonal expansion of effector lymphocytes such as NK cells, T_C cells and B cells. Additional factors produced by antigen-presenting cells, e.g., IL-1 and IL-6 act as co-stimulators of T cell activation.

Cytotoxic T lymphocytes (termed T_C cells) recognise antigens that are processed through an endogenous pathway and presented by infected or transformed cells expressing MHC I class molecules (Fig. 4). T_C cells also mediate their effector function through the production of cytokines such as INF- γ and tumour necrosis factor (TNF)- α and/or through a direct cytotoxic mechanism. The mechanism of cytotoxic killing can be mediated by the release of granule contents such as perforin and granzyme from T_C cells resulting in irreparable pore formation in the cell membrane and apoptosis (i.e., programmed cell death). In addition, T_C cells can destroy cells by a process of Fas-mediated lysis.

Antigen-presenting cells (APCs)

Activation of both humoral (antibody-generating) and cell-mediated (T-lymphocytes) branches of the immune system requires cytokines produced by T_H cells (Kuby, 1997). It is essential that activation of T_H cells be carefully regulated as an inappropriate T_H-cell response to self components can have fatal autoimmune consequences. To ensure carefully regulated activation of T_H cells, they only recognise antigen that is displayed together with class MHC II molecules on the surface of antigen-presenting cells (APCs). These specialised cells, which include macrophages, B lymphocytes, and dendritic cells, are distinguished by two properties: (1) they express class II MHC molecules on their membrane, and (2) they are able to deliver a co-stimulatory signal that is necessary for T_H-cell activation (Kuby, 1997). Dendritic cells are professional antigen-presenting cells that have long membrane processes. They are found in the lymph nodes, and thymus (follicular and interdigitating dendritic cells); skin (Langerhans cells); and other tissues (interstitial dendritic cells) (Kuby, 1997). Indeed, dendritic cell ability to prime naïve CD4⁺ or T_H cells is a unique and critical function both *in vitro* and *in vivo*.

In the presence of soluble antigen, T_H cells primed by dendritic cells can interact with B cells and stimulate antigen-specific antibody production. Dendritic cells are equally important in priming CD8⁺ or TC cells. Interestingly, dendritic cells can directly induce cytotoxic T_C cell proliferation with help from T_H cells. It remains to be determined if the unique ability of dendritic cells to prime T lymphocytes results from the expression of unique dendritic cells, or if it results from the high density of molecules involved in dendritic cell (DC)/Tcell interactions. However, a crucial factor for sustaining this DC/T cell interaction is the interaction of co-stimulatory molecules on dendritic cells (CD40, CD83, CD86) and their ligands (i.e., any molecule recognised by a receptor) on the T cells (Young and Steinman, 1990).

Therefore, as antigen-presenting cells (APC) they can also elicit a local rapid reaction or cascade of events that triggers the specific-immune responses. While APCs can be simply described as any cell that alters the immune system to respond to foreign invaders and cancer cells by presenting non-self molecules (or antigens) that are associated with these infected or abnormal cells. Specifically, APCs are any cells that can process and present antigenic peptides (usually foreign) in association with class II MHC molecules (heterodimeric membrane proteins that function in antigen presentation to T_H cells) on the surface of antigen-presenting cells or altered self-cells.

[for references, please see Chapter 6]