

An overview of the immune system

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We are continually exposed to organisms that are inhaled, swallowed, or inhabit our skin and mucous membranes. Whether these organisms penetrate and cause disease is a result of both the pathogenicity of the organism (the virulence factors at its disposal) and the integrity of host defence mechanisms. The immune system is an interactive network of lymphoid organs, cells, humoral factors, and cytokines. The essential function of the immune system in host defence is best illustrated when it goes wrong; underactivity resulting in the severe infections and tumours of immunodeficiency, overactivity in allergic and autoimmune disease. In this review we have covered the normal function of the immune system in recognising, repelling, and eradicating pathogens and other foreign molecules.

Immunity is divided into two parts determined by the speed and specificity of the reaction. These are named the innate and the adaptive responses, although in practice there is much interaction between them. The term innate immunity is sometimes used to include physical, chemical, and microbiological barriers, but more usually encompasses the elements of the immune system (neutrophils, monocytes, macrophages, complement, cytokines, and acute phase proteins) which provide immediate host defence. The highly conserved nature of the response, which is seen in even the simplest animals, confirms its importance in survival.¹ Adaptive immunity is the hallmark of the immune system of higher animals. This response consists of antigen-specific reactions through T lymphocytes and B lymphocytes. Whereas the innate response is rapid but sometimes damages normal tissues through lack of specificity, the adaptive response is precise, but takes several days or weeks to develop. The adaptive response has memory, so that subsequent exposure leads to a more vigorous and rapid response, but this is not immediate.^{2,3}

The innate response

Neutrophil recruitment

A central feature of the innate reaction is recruitment and activation of neutrophils at the site of infection to eradicate pathogens.⁴ The same process occurring inappropriately leads to the inflammation of connective tissue diseases, vasculitis, and the systemic inflammatory response syndrome. There is intense interest in the mechanisms underlying the process for the development of new anti-inflammatory therapies.^{5,6}

During the very early stages of infection or tissue damage, there is release of cytokines from activated macrophages. Two of these, granulocyte and granulocyte-macrophage colony stimulating factors, stimulate division of myeloid precursors in the bone marrow, releasing millions of cells into the circulation and causing a characteristic neutrophil leucocytosis. Neutrophils, like most cells involved in immune responses, are not static

within a particular compartment, but are mobile cells that travel round the body. They normally flow freely in the blood as the circulating pool, or roll along the vascular endothelium as the marginating pool. To home to a site of infection, neutrophils use a multistep process involving proinflammatory mediators, adhesion molecules, chemoattractants, and chemokines. Although most work was initially done within the neutrophil system, it is now clear that all leucocytes, including lymphocytes, use this mechanism of localisation.⁷ The recruited neutrophils phagocytose organisms by making pseudopodia (projections of cytoplasmic membrane) which form a membrane-bound vesicle (phagosome) around the particle. This fuses with neutrophil cytoplasmic granules to form the phagolysosome. In this protected compartment killing of the organism occurs by a combination of two mechanisms. The oxygen-dependent response or respiratory burst, involves the sequential reduction of oxygen by an NADPH oxidase leading to production of toxic oxygen metabolites, such as hydrogen peroxide, hydroxyl radicals, and singlet oxygen. The oxygen-independent response, uses the highly toxic cationic proteins and enzymes (eg, myeloperoxidase and lysozyme) contained within the neutrophil cytoplasmic granules.⁸ Ingestion and killing of organisms is 100-fold more effective if the particle is first opsonised with specific antibody or complement (C'). These molecules bind to neutrophil Fc and C' receptors, increasing adhesion between particle and phagocyte and priming the cell for activation. Some encapsulated organisms, such as pneumococcus and haemophilus are not susceptible to neutrophil phagocytosis unless first coated with antibody. This explains why individuals with antibody deficiency are so susceptible to this type of infection, despite normal neutrophil numbers and function.

Complement

The complement system has several important functions in innate immunity and consists of at least 20 serum glycoproteins, some being regulatory. These are activated in a cascade sequence, with amplification stages. This means that activation of a single molecule will lead to thousands of molecules being generated. There are three pathways of complement activation that can be driven by the presence of a foreign substance (figure 1), the classical by antigen-antibody reactions, the alternative by polysaccharides from yeasts, and gram negative bacteria. The more recently identified mannan binding lectin pathway⁹ feeds into the classical sequence by activating it

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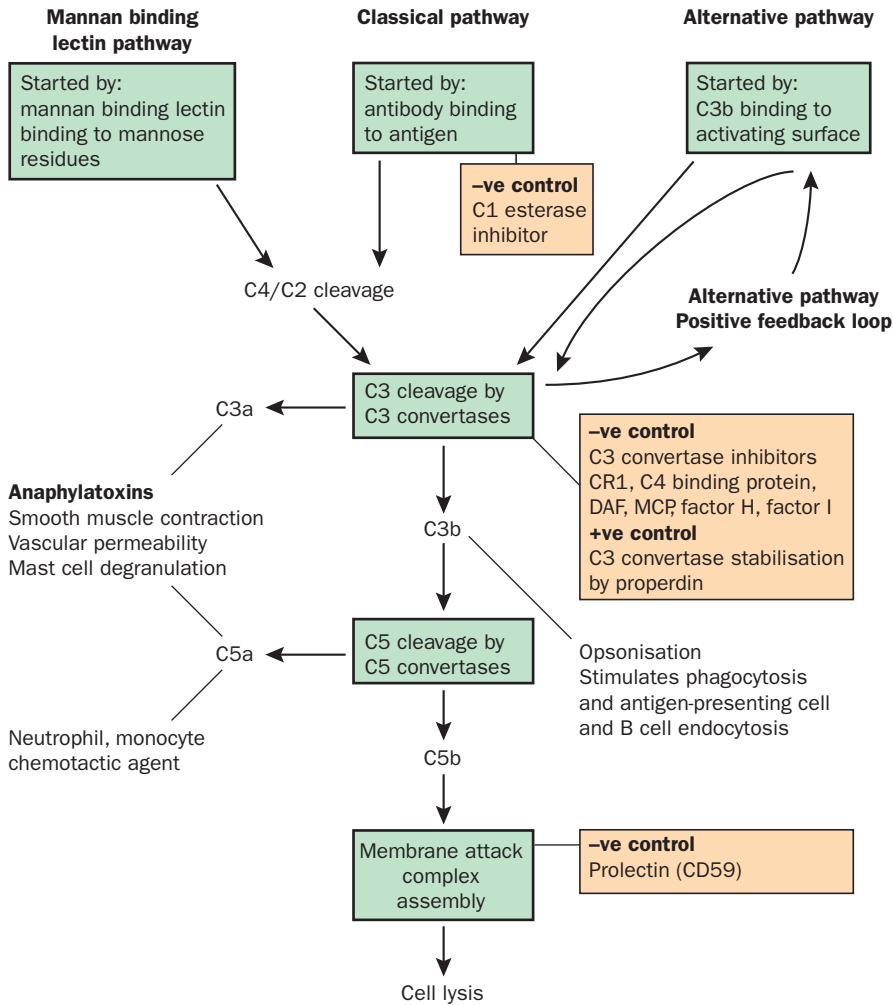


Figure 1: Complement pathways
The three pathways of complement activation. Regulatory proteins are shown in orange. Components of activation pathways are shown in green.

independently of the C1rs complex and is stimulated by mannose containing proteins and carbohydrates on microbes, including viruses and yeasts. Many of the components of the classical and alternative pathway are homologous, suggesting the pathways were initially derived from the same sequence. All three pathways converge with the activation of the central C3 component. This leads to a final common pathway, with assembly of C5–C9 forming a transmembrane pore (membrane attack complex) in the cell surface and death by osmotic lysis. The perforins, which are produced by cytotoxic T lymphocytes and natural killer cells, have a similar structure. Complement activation is focused on the surface of a cell or organism, which forms a protected site where the inhibitory proteins have limited access. Normal host cells bear the complement receptor type 1 and decay accelerating factor, which inhibit C3 convertase and prevent progression of complement activation. However, microbes lack these molecules and are susceptible to complement.

In addition to lysis of organisms, complement has other anti-infective functions. There is the opsonic action of C3b, the release of soluble C3a and C5a, which are anaphylatoxins and increase vascular permeability allowing proteins, such as antibody, to penetrate the tissue, and the chemotactic activity of C5a that induces an inflammatory infiltrate. Complement also has a role within the specific immune response; its activation

and deposition within immune complexes helps to target these to complement-receptor bearing antigen-presenting cells, such as B lymphocytes and follicular dendritic cells.

Eosinophils

The main physiological role of eosinophils is in protection of the host from parasitic (particularly nematode) infections. Such infections induce antigen-specific IgE production, the antibodies coating the organism. Eosinophils bind to the antibody using their low affinity receptors (Fc_εRII). Eosinophils are not phagocytic, but have large granules containing major basic protein, eosinophilic cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin, which are highly cytotoxic when released onto the surface of organisms. In more-developed countries the eosinophil is more often viewed as a pathological participant in allergic reactions.

Mast cells and basophils

Although basophils and mast cells are relatively few in number compared with the other white cells, they are involved in some of the most severe immunological reactions, such as angioedema and anaphylaxis. There are at least two populations of mast cells, based on the enzymes they contain and their tissue location. T mast cells (mucosal mast cells)

contain only trypsin, whereas connective tissue mast cells contain both trypsin and chymotrypsin. Basophils are morphologically similar cells found in the blood. Mast cells and basophils bear high-affinity receptors for IgE Fc_εRI (CD23) which rapidly absorb any local IgE. Crosslinking of these receptors by the binding of antigen to IgE leads to degranulation and release of preformed mediators, such as the vasoactive amines, histamine and serotonin. Membrane derived mediators such as leucotrienes B₄, C₄, D₄ and E₄, prostaglandins and platelet activating factor are also produced leading to increased vascular permeability, bronchoconstriction, and induction of an inflammatory response.

Natural killer cells

Natural killer cells have the morphology of lymphocytes but do not bear a specific antigen receptor. They recognise abnormal cells in two ways. First, they bear immunoglobulin receptors (FcR) and bind antibody-coated targets leading to antibody-dependent cellular cytotoxicity. Second, they have receptors on their surface for MHC class I. If on interaction with a cell, this receptor is not bound, the natural killer cell is programmed to lyse the target. This is achieved by secretion of perforins onto the surface of the cell to which the natural killer cell has adhered. Perforins make holes in the cell membrane and granzymes are injected through the pores. The granzymes

Adhesion molecule	Tissue distribution	Ligand
Immunoglobulin superfamily		
ICAM-1	Endothelial cells, monocytes, T and B cells, dendritic cells, keratinocytes, chondrocytes, epithelial cells	LFA-1
ICAM-2	Endothelial cells, monocytes, dendritic cells, subpopulations of lymphocytes	LFA-1
ICAM-3	Lymphocytes	LFA-1, Mac-1
VCAM-1	Endothelial cells, kidney epithelium, macrophages, dendritic cells, myoblasts, bone marrow fibroblasts	VLA-4
PECAM-1	Platelets, T cells, endothelial cells, monocytes, granulocytes	?
MAcCAM-1	Endothelial venules in mucosal lymph nodes	α 4 β 7 integrin and L-selectin
Selectin family		
E-selectin/ELAM-1	Endothelial cells	?
L-selectin	Lymphocytes, neutrophils, monocytes	CD34
P-selectin	Megakaryocytes, platelets and endothelial cells	?
Integrin family		
VLA subfamily		
VLA-1 to VLA-4	Endothelial cells, resting T cells, monocytes, platelets, and epithelial cells	Various molecules including laminin, fibronectin, collagen, and VCAM1
VLA-5 (fibronectin receptor)	Endothelial cells, monocytes, and platelets	Laminin
VLA-6 (laminin receptor)	Endothelial cells, monocytes, and platelets	Laminin
β 1 α 7	Endothelial cells, ?	Laminin
β 1 α 8	Endothelial cells, ?	?
β 1 α ,	Platelets and megakaryocytes	Fibronectin
β 2	Widely distributed	Collagen, laminin, vitronectin
Leucam subfamily		
LFA-1	Leucocytes	ICAMs-1 to 3
Mac-1	Endothelial cells, ?	ICAM-1, fibrinogen, C3bi
Cytoadhesin subfamily		
Vitronectin receptor	Platelets and megakaryocytes	Vitronectin, fibrinogen, laminin, fibronectin, von Willebrand factor, thrombospondin
B4 α 6	Endothelial cells, thymocytes, and platelets	Laminin
B5 α ,	Platelets and megakaryocytes, ?	Vitronectin, fibronectin
B6 α ,	Platelets and megakaryocytes, ?	Fibronectin
β 7 α 4/LPAM-1	Endothelial cells, thymocytes, monocytes	Fibronectin, Vcam-1
β 8 α ,	Platelets and megakaryocytes, ?	?

ICAM=intercellular adhesion molecule, VCAM=vascular cell adhesion molecule, MAcCAM-1=mucosal addressin, E-selectin or ELAM=endothelial leukocyte adhesion molecule, LPAM=lymphocyte Peyer's patch adhesion molecule, PECAM=platelet/endothelial cell adhesion molecule, VLA=very late antigen. Reprinted from Kumar & Clark. Clinical Medicine, 4th edn, by permission of the publisher WB Saunders.

Table 1: Adhesion molecules

cause induction of apoptosis in the target. Normal host cells are MHC class I positive, the binding of this molecule to its receptor on the natural killer cell inhibits the death pathway. Tumour cells and viruses (especially those of the herpesvirus family) often cause downregulation of class I. Although this may offer some advantage to the pathogen impairing recognition by cytotoxic T cells, it does leave them open to natural killer cell attack.

Discrimination of pathogens by the innate system

Although not antigen-specific, the innate system is able to discriminate foreign molecules from self. Phagocytes bear pattern-recognition receptors, with lectin-like activity. These recognise structures termed pathogen-associated molecular patterns present on microbes, but not host cells.⁹ Examples are lipopolysaccharide, lipotechoic acid, and mannans on gram negative, gram positive, and yeast cell walls, respectively. The pattern-recognition receptor molecules fall into three groups depending on function; those inducing endocytosis and thus enhancing antigen-presentation; those initiating nuclear factor κ β transduction and cell activation (toll-like receptors)¹⁰ and those, for example mannan binding lectin, which are secreted acting as opsonins. The increasing knowledge of these recognition pathways, highlights the close relation between the innate and specific response—a pattern-recognition receptor recognises broad patterns on microbes and then presents the processed product to antigen-specific T cells.

The interactions allowing the innate response to eradicate infectious agents, such as phagocytosis, opsonisation, and complement-mediated lysis, require exposure to the surface of the microbe. The response is

therefore largely confined to eradicating extracellular organisms, mostly bacteria. This system is not able to detect intracellular organisms, notably viruses, mycobacteria, some fungi, protozoa, or other facultative intracellular pathogens. In addition, the response is fairly non-specific and often poorly targeted, leading to indiscriminate tissue damage.

Cellular communication

In order for cells to work effectively they need to be recruited to sites of inflammation and appropriately activated. This is achieved by the interaction of cellular receptors which signal internally to the nucleus, and external factors, such as cytokines, which are able to bind the receptors, and with other adhesion molecules.

Adhesion molecules

Adhesion molecules are surface-bound molecules involved in cell-to-cell interactions.¹¹ Their main function is in facilitating processes where close contact of cells is required—eg, in directing cell migration, phagocytosis, and cellular cytotoxicity. Adhesion molecules associate with cytoplasmic proteins and cytoskeletal components to cause cytoskeletal reorganisation, allowing cells to undergo directed movement. Signal transduction after ligation of the adhesion molecule, also leads to cell activation, alteration in receptor expression, cytokine production, and effects on cell survival. Cells can express adhesion molecules constitutively, or upregulate them on exposure to cytokines, chemokines, or other proinflammatory molecules, such as complement activation products and microbial metabolites. Some adhesion molecules are expressed mainly on leucocytes, others on endothelial cells enabling interaction between the two.

Chemokine	Class*	Sites of production	Biological activity
Macrophage chemoattractant protein-1	-CC-	Macrophages, fibroblasts, keratinocytes	Attracts monocytes and memory T cells to inflammatory sites
Macrophage inflammatory protein-1 α	-CC-	Macrophages	Attracts monocytes and T cells
MIP-1 β macrophage inflammatory protein-1 β	-CC-	Monocytes, macrophages, endothelial cells, T and B cells	Attracts monocytes and CD8+ T cells
RANTES	-CC-	Platelets and T cells	Attracts monocytes, T cells, and eosinophils
Interleukin 8	-CXC-	Macrophages	Attracts neutrophils, naive T cells

*Refers to a double cysteine amino acid structure (-CC-) within the cytokine; in some cases this is interspersed with another amino acid (-CXC-). RANTES=regulated on activation, normal T-cell expressed and secreted. Reprinted from Kumar & Clark. Clinical Medicine, 4th edn, by permission of the publisher WB Saunders.

Table 2: Chemokines and associated function

There are families of adhesion molecules based on structure and function (table 1). The main ones are the intercellular adhesion molecules, integrins, selectins, and cadherins (calcium-dependent adherins). In addition to the molecules on leucocytes, and vascular endothelium, there are also tissue-specific adhesion molecules called addressins. These are mainly involved in targeting lymphocytes to particular groups of lymphoid tissues such as in the gut, lung, skin, peripheral lymph nodes and possibly to brain, lung, and synovium.

Chemokines

Chemokines are particular members of the cytokine family that have a key role in leucocyte migration (table 2). They have substantial chemotactic function (inducing the directional movement of cells). Chemokines are named by the position of two cysteine (C) residues compared with the other amino acids (X). The two main subgroups are CXC (α -chemokines) and CC (β -chemokines). Chemokines are produced by most cells on stimulation with proinflammatory cytokines or bacterial products and chemokine receptors are found on all leucocytes. The effects of chemokines are more prolonged than other chemoattractants such as complement activation products, as they bind to glycosamino-glycans on cell surfaces and the extracellular matrix. Chemokines that cause recruitment of leucocytes are termed inflammatory. Additionally, there are lymphoid chemokines which regulate leucocyte positioning within the spleen and other lymphoid tissues.¹²

Neutrophil migration in response to infection or inflammation

A good example of how adhesion molecules and cytokines enable dynamic interactions between cells is given by neutrophil migration/recruitment. Chemokines and other chemoattractants use two mechanisms to recruit neutrophils. First, they induce local upregulation of adhesion molecule expression on vascular endothelium and neutrophils, making the cells stick to the vessel wall. Second, they activate neutrophil migration between the endothelial cells and into the tissue along the chemotactic gradient.

Release of tumour necrosis factor α , interleukin 1, and endotoxin from activated macrophages, mast cells, and organisms at the site of infection, causes local upregulation of E-selectin on endothelial cells. Neutrophils also bear their own type of selectin (L-selectin) and bind to the activated endothelium. Selectins cause the formation of tight bonds between the cells, however, these dissociate rapidly, releasing the neutrophil

to move downstream to attach to another selectin-bearing endothelial cell. This causes the intermittent tethering motion known as rolling.⁸ This slows the cell and allows the less strong, but stable bond to be formed between the integrin leucocyte function antigen on neutrophils and intercellular adhesion molecule type 1 on the vascular endothelial cell. At the same time there is the production of powerful neutrophil chemoattractants (effective at nanomolar concentrations) such as N-formyl-methionyl-leucylphenylalanine from bacterial cell walls, which causes the release of another chemotactic product, leucotriene B₄, from tissue mast cells; the chemokine interleukin 8 is secreted from stimulated macrophages and chemoattractant C5a from complement activation. Neutrophils move along the chemotactic gradient produced, and leave the circulation by diapedesis through spaces between endothelial cells. The same molecules also enhance intercellular adhesion molecule type 1 expression leading to further cell recruitment. Low concentrations of chemoattractants induce neutrophil migration. At high concentrations receptors for chemoattractants are downregulated and the cells remain at the inflammatory site. Activated neutrophils therefore accumulate. In large numbers this leads to pus formation, the characteristic green/yellow colour being due to the peroxidase enzymes within the cells. The importance of adhesion molecules in neutrophil migration is illustrated by individuals with a congenital deficiency of the common β chain of the β 2 integrins (LFA-1, Mac-1, and p150,95). The patients have severe infections due to paucity of neutrophils in the tissues. Paradoxically, there is a neutrophil leucocytosis in the blood due to the paralysed cells being unable to leave this compartment.¹³

The only endothelial cells that constitutively express adhesion molecules are the high endothelial venules of lymph nodes. These bind lymphocytes (but not neutrophils) and direct the trafficking of these cells from the blood into lymphoid tissue. Endothelial cells within other blood vessels express adhesion molecules only when activated by the presence of local tissue damage or microbes. Even then the adhesion molecules are only expressed on postcapillary venules, preventing the tissue anoxia that could result if large numbers of leucocytes accumulate in arteriolar or capillary vessels.

Cytokines

Cytokines are small molecular weight messengers secreted by one cell to alter the behaviour of itself or another cell (table 3). Cytokines send intracellular signals by binding to specific cell-surface receptors. Although most are soluble, some may be membrane-bound, making the differentiation between cytokine and receptor difficult. Cytokines are produced by virtually all cells and have a wide variety of functions. The biological effect depends on the cytokine and the cell involved, but typically these molecules will affect cell activation, division, apoptosis, or movement. They act as autocrine, paracrine, or endocrine messengers. Cytokines produced by leucocytes and having effects mainly on other white cells are termed interleukins. Cytokines that have chemoattractant activity are called chemokines. Those that cause differentiation and proliferation of stem cells are called colony-stimulating factors. Those that interfere with viral replication are called interferons.

Interferons

Interferons are a major class of cytokine that have a particular role in immunity. They are divided into type 1 (α and β interferons) and type 2 (γ or immune interferon).

Cytokine	Source	Mode of action
Interleukin 1	Macrophages	Immune activation; induces an inflammatory response
Interleukin 2	Mainly T cells	Activates T (and natural killer) cells and supports their growth. Formerly called T cell growth factor
Interleukin 3	T cells	Mainly promotes growth of haemopoietic cells
Interleukin 4	T helper cells	Lymphocyte growth factor; involved in IgE responses
Interleukin 5	T helper cells	Promotes growth of B cells and eosinophils
Interleukin 6	Fibroblasts	Promotes B cell growth and antibody production, induces acute phase response
Interleukin 7	Stromal cells	Lymphocyte growth factor; important in the development of immature cells
Interleukin 8	Mainly macrophages	Chemoattractant
Interleukin 10	CD4 cells, activated monocytes	Inhibits the production of interferon α , interleukin 1, interleukin 6, tumour necrosis factor α , and stops antigen presentation
Interleukin 12	Monocyte/macrophages	Augments T helper 1 responses and induces interferon γ
Interleukin 13	Activated T cells	Stimulates B cells
Granulocyte colony stimulating factor	Mainly monocytes	Promotes growth of myeloid cells
Monocyte colony stimulating factor	Mainly monocytes	Promotes growth of macrophages
Granulocyte-macrophage colony stimulating factor	Mainly T cells	Promotes growth of monomyelocytic cells
Interferon α	Leucocytes	Immune activation and modulation
Interferon β	Fibroblasts	Immune activation and modulation
Interferon γ	T cells and natural killer cells	Immune activation and modulation
Tumour necrosis factor α	Macrophages	Stimulated generalised immune activation as well as tumour necrosis. Also known as cachectin
Tumour necrosis factor β	T cells	Stimulates immune activation and generalised vascular effects. Also known as lymphotoxin
Transforming growth factor β	Platelets	Immunoinhibitory but stimulates connective tissue growth and collagen formation

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Table 3: Cytokines

Type 1 interferons have potent antiviral activity and are produced mainly by fibroblasts and monocytes as a reaction to infection. Both α and β interferon bind to the same cellular receptor and protect uninfected cells by inducing the intracellular production of molecules that inhibit or interfere with viral RNA and DNA production. They increase the expression of MHC class I molecules leading to enhanced recognition of virally infected cells by specific cytotoxic T lymphocytes. Type 1 interferons also have antiproliferative function. α interferon is used in the treatment of chronic hepatitis B and C infections in combination with antiviral drugs^{14,15} as well as in some forms of leukaemia.¹⁶ β interferon reduces the relapse rate in subgroups of patients with multiple sclerosis.¹⁷ Interferon γ has different functions, acting directly on the immune system to activate macrophage and neutrophil intracellular killing, stimulate natural killer cell function, and enhance antigen presentation by increasing MHC class II expression on antigen presenting cells. Interferon γ is

only produced by cells of the immune system and uses a separate receptor to that of the type 1 interferons. It is used in the treatment of a specific congenital neutrophil defect (chronic granulomatous disease)¹⁸ and in patients with defects in the production of interferon γ or its receptor, and in the adjunct therapy of some macrophage-based infections (leishmaniasis, atypical mycobacterial disease).¹⁹

Specific immunity

The characteristic of adaptive immunity is the use of antigen-specific receptors on T and B cells to drive targeted effector responses in two stages. First, the antigen is presented to and recognised by the antigen specific T or B cell leading to cell priming, activation, and differentiation (figure 2), which usually occurs within the specialised environment of lymphoid tissue. Second, the effector response takes place, either due to the activated T cells leaving the lymphoid tissue and homing to the disease site, or due to the release of antibody from

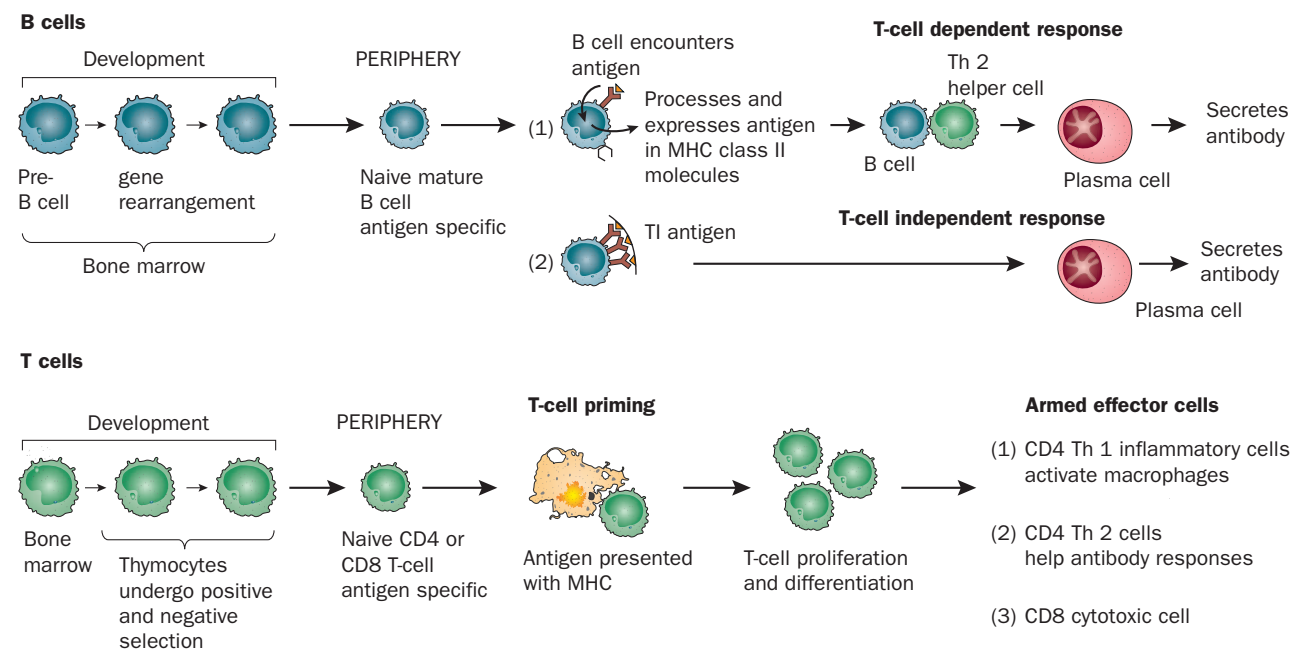


Figure 2: The role of T and B lymphocytes in specific immunity

activated B cells (plasma cells) into blood and tissue fluids, and thence to the infective focus.

Formation of antigen-specific receptors on T and B cells

B and T lymphocytes develop from progenitor cells within the bone marrow. B cells remain within the marrow for the duration of their development, but T cells migrate to the thymus at an early stage as thymocytes. The production of antigen-specific receptors in both cell types is the result of an unusual process of random rearrangement and splicing together of multiple DNA segments that code for the antigen-binding areas of the receptors (complementarity-determining regions). Gene rearrangement occurs early in the development of the cells, before exposure to antigen, which leads to the production of a repertoire of over 10^8 T-cell receptors and 10^{10} antibody specificities,¹⁹ adequate to cover the range of pathogens likely to be encountered in life.

The process for B-cell receptor rearrangement will be described, but the mechanism is similar for the T-cell receptor. There are four segments of gene involved in receptor formation called the variable (V), diversity (D), joining (J), and constant (C) regions. These are found on different chromosomes within the developing cell. The segments are cut out by nucleases and spliced together using ligases (a product of the recombination activation genes, RAG-1 and RAG-2). This forms the final gene sequence from which protein will be transcribed to form the receptor molecule. There are several ways in which clonal diversity occurs. First, there is a multiplicity of all these regions within the DNA (V=25–100 genes, D~25 genes, and J~50 genes), but only one of each is needed. There is combinational freedom in that any one of the genes can join with any one other to form the final VDJ region. Second, the splicing is inaccurate and frameshift in basepairs leads to the production of a different aminoacid (junctional diversity). Third, the enzyme deoxyribonucleotidyltransferase can insert nucleotides to further alter the sequence. A greater repertoire of B-cell receptors is produced as further immunoglobulin gene rearrangement occurs during B-cell division after antigen stimulation (somatic hypermutation).

In T lymphocytes the receptor has two forms. The most common consists of a heterodimer of an α and β chain, each with a constant and variable domain. The other form (<10% T cells) has γ and δ chains (the function of this type of T-cell receptor bearing cell remains uncertain). The T-cell receptor forms a complex with the CD3 molecule, with its associated signalling molecules (figure 5). In B cells the gene product is a membrane-bound form of IgM, initially expressed alone and later with IgD. Early in B-cell development this molecule acts as the antigen receptor, being able to induce signal transduction in a similar way to the T-cell receptor. The membrane bound molecule can also internalise antigen, inducing processing, and re-expression for antigen presentation to T cells. After B-cell activation the secreted form of antibody is produced by plasma cells. Despite the similarities in gene rearrangement processes, the T and B cell receptors recognise antigen differently. The T-cell receptor binds linear peptides usually of eight to nine aminoacids. This generally means antigen that has been broken down by intracellular processing. Antibody recognises the conformational structure (shape) of epitopes, and such antigens do not require processing.

The creation of new clones of T and B cells continues through life, although slows after the mid 20s, meaning that such individuals are slower to reconstitute their

immune system after bone marrow transplant or on introduction of effective antiretroviral therapy in HIV-related immunodeficiency.²⁰

Organisation of the immune system

The cells that emerge from the thymus and bone marrow having undergone gene rearrangement are naive—ie, they have not yet encountered their specific antigen within an immune response. These cells populate the secondary lymphoid tissues of the lymph nodes, spleen, tonsils, and mucosa associated lymphoid tissue. Because there are only a few naive T and B cells capable of reacting specifically with a foreign particle, in order for them to encounter their specific antigen, there has to be a system to bring them together. The lymphoid tissues provide the microenvironment for this process. In addition to T and B lymphocytes, they contain efficient antigen-presenting cells and are able to produce the cytokines necessary to maintain T and B lymphocytes. Lymphoid tissues express adhesion molecules in an ordered array, allowing cells to move through the tissue and increase the chance of lymphocytes being brought into contact with antigen. The lymphoid organs communicate with the tissues using lymphatics and blood vessels.

T lymphocytes

Development in thymus

Once receptor rearrangement has occurred, T and B cells are able to respond to their antigen and induce an immune response. However, cell activation is tightly regulated to ensure that only damaging antigens elicit a reaction. Regulation particularly involves the initiation of T lymphocyte activation. This requires that antigen is presented to the T cell within the peptide binding groove of a self MHC molecule. This is because the T-cell receptor does not just recognise the antigenic epitope, but recognises the complex of the peptide in association with the self-MHC molecule. The delicate process of positive selection of T cells that can react with self-MHC and peptide adequately to induce immune responses, but are not excessively MHC-reactive to the extent which would cause self-tissue destruction, occurs in the thymus (figure 3).

The meeting of naive T cell and antigen

Naive T cells bear receptors (peripheral node addressins) that bind to adhesion molecules on the high endothelial venules of lymph nodes, enter the nodes, and pass through binding transiently to the multiple antigen-presenting cells. Although about 95% of T lymphocytes are sequestered within the lymphoid tissue, they are not static but move continuously from one lymphoid tissue to another, via the blood or lymph, travelling around the whole body in 1–2 days. The traffic of lymphocytes is considerable, the output of cells in the efferent lymph being 3.0×10^7 cells/g of lymphoid tissue.²¹ Therefore, within a short while a T cell should meet its antigen. When the T cell meets an antigen-presenting cell bearing its antigen, activation occurs over the next 2–3 days.

The antigen is brought to the lymphoid tissue directly in the lymphatics, or within dendritic (or other antigen-presenting cells) cells that have endocytosed the antigen locally. Dendritic cells actively take up debris in their housekeeping role. However, if there is inflammation within a tissue, the dendritic cells are activated to leave the site, migrating to the downstream lymph node. Antigens in the blood are taken to the spleen, in the tissues to the lymph nodes, and from the mucosae to the mucosa associated lymphoid tissue. Dendritic cells express

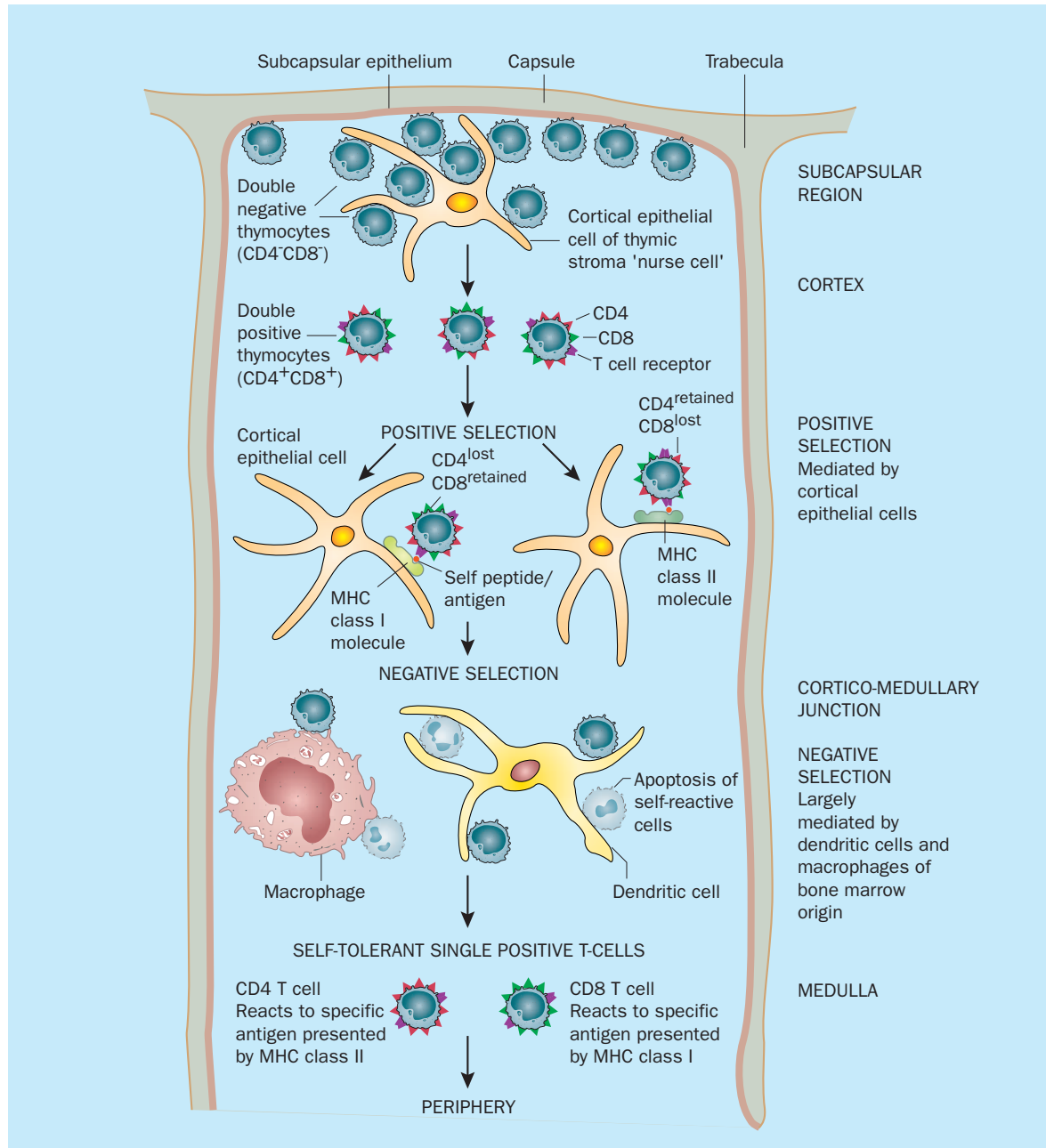


Figure 3: **T cell development in the thymus**

Thymocytes enter the thymus in the subcapsular region. Cells bearing a T-cell receptor that recognises self MHC are positively selected in the cortex and pass into the corticomedullary junction. Here, T cells that react with self-antigens are deleted by apoptosis in a process known as negative selection. The cells that exit are self-tolerant but able to recognise foreign antigen when presented with self MHC.

receptors for lymphoid chemokines and migrate into the lymphoid tissue which expresses these constitutively. Dendritic cells are particularly important in activating primary naive T cells. The antigens may be further processed by antigen-presenting cells (macrophages, interdigitating dendritic cells, and B cells) ready to attract antigen-specific T cells and induce an immune response.

Antigen presentation and the MHC molecules

There are two ways in which antigen loading onto MHC can occur. The antigen may have been produced endogenously within the cell (such as viral or tumour proteins) and is complexed with MHC class I through intracellular processing pathways (figure 4a). Alternatively, specialised professional antigen-presenting cells might have

taken up exogenous antigen by endocytosis. Antigen-presenting cells include dendritic cells (the interdigitating dendritic cells of lymph nodes, veiled cells in the blood, and Langerhan's cells in the skin), B cells, and macrophages. Exogenous antigen is processed via a different pathway to endogenous, and re-expressed with MHC class II molecules (figure 4b). MHC class II has restricted expression, in normal circumstances being expressed only on these specialised cells.

Antigen recognition by T cells

This recognition of antigen by the T-cell receptor is different for $CD4^+$ and $CD8^+$ cells. $CD4^+$ lymphocytes only recognise antigen presented with MHC class II and $CD8^+$ cells with MHC class I. Since $CD4^+$ and $CD8^+$

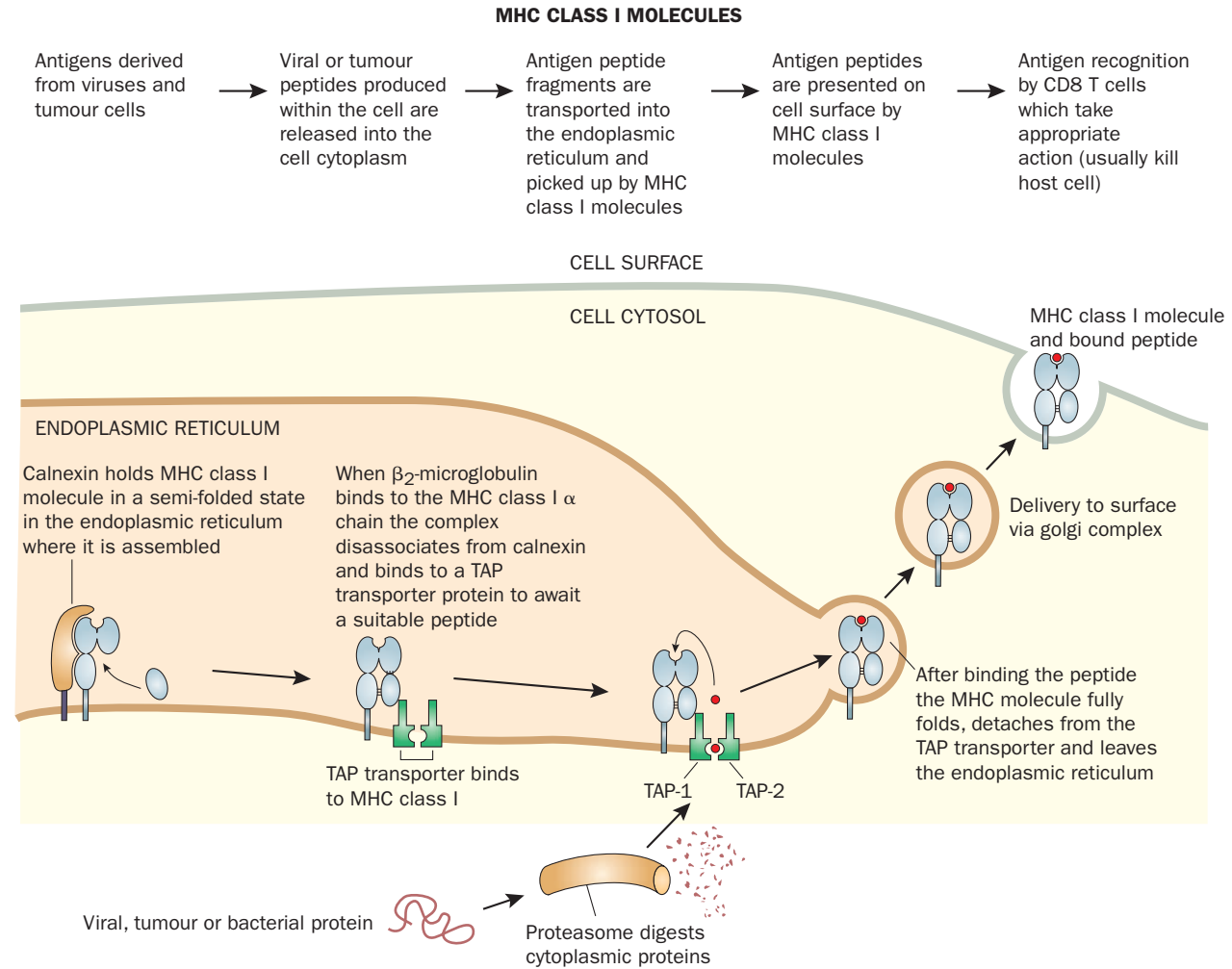


Figure 4A: **The pathway of endogenous antigen delivery to class I MHC molecules**

cells have very different functions, the MHC molecule that is used to present an antigen will determine the type of effector response generated. Endogenous antigens complexed with MHC class I molecules activate CD8+ cytotoxic T cells. Because all nucleated cells express MHC class I, this means that any such cell that is infected with a virus or other intracellular pathogen, or is producing abnormal tumour antigens can present these antigens with class I and be removed by cytotoxic attack. Whereas these CD8 responses are highly targeted to the cell that they recognise, CD4 activation leads to production of cytokines which in turn activate a wide range of cells around them. The reaction therefore needs to be kept in check, which is achieved by only a small number of class II antigen-presenting cells being able to drive the response.

The need for intracellular processing and expression with MHC ensures that only antigens derived from foreign molecules that have either invaded the interior of a host cell, or have induced an inflammatory response to activate endocytosis by antigen-presenting cells, are recognised as foreign. Innocuous antigens are largely ignored. Another safety net to avoid inappropriate antigen-presentation or effector cell attack is in place because binding of the T-cell receptor to the antigen-MHC complex alone, is not adequate to induce activation of the cell: coreceptor stimulation is also required.

T-cell receptor signalling

T-cell receptors on the surface of cells are associated with the CD3 complex of molecules that transmit signals into the cell when antigen is bound to the T-cell receptor. Aggregation of the receptor causes phosphorylation of tyrosines within the cytoplasmic tail of the CD3 complex and the transduction of signals downstream to the nucleus leading to activation of gene sequences leading to T-cell proliferation (figure 5). Recruitment of the receptor and associated molecules into lipid rafts enhances the interaction.²² Coreceptors are molecules on the surface of the T cell that send signals to the cell to cause activation if the T-cell receptor is also engaged. Without these cosignals the cell will either become anergic (unreactive) or die by programmed cell death. The main coreceptors for T-cell activation (apoptosis) are CD80 (B7-1), CD86 (B7-2), and CD40, that bind CD28, CTLA-4, and CD40 ligand on the T cell, respectively. Activated dendritic cells are the most potent stimulators of naive T cells, bearing large amounts of B7 and CD40. Inflammatory mediators induce the upregulation of costimulatory molecules, therefore a T cell is much more likely to be activated if it meets its specific antigen via an antigen-presenting cell, which has been exposed to an inflammatory environment.

Division and clonal expansion of each T cell produces up to 1000 progeny. Most are armed effector cells, which upregulate receptors enabling them to leave the lymphoid tissue and be guided to the site of inflammation. Organ-specific adhesion molecules attract both the effector and long-lived effector memory cells to the disease site.²³ There

MHC CLASS II MOLECULES

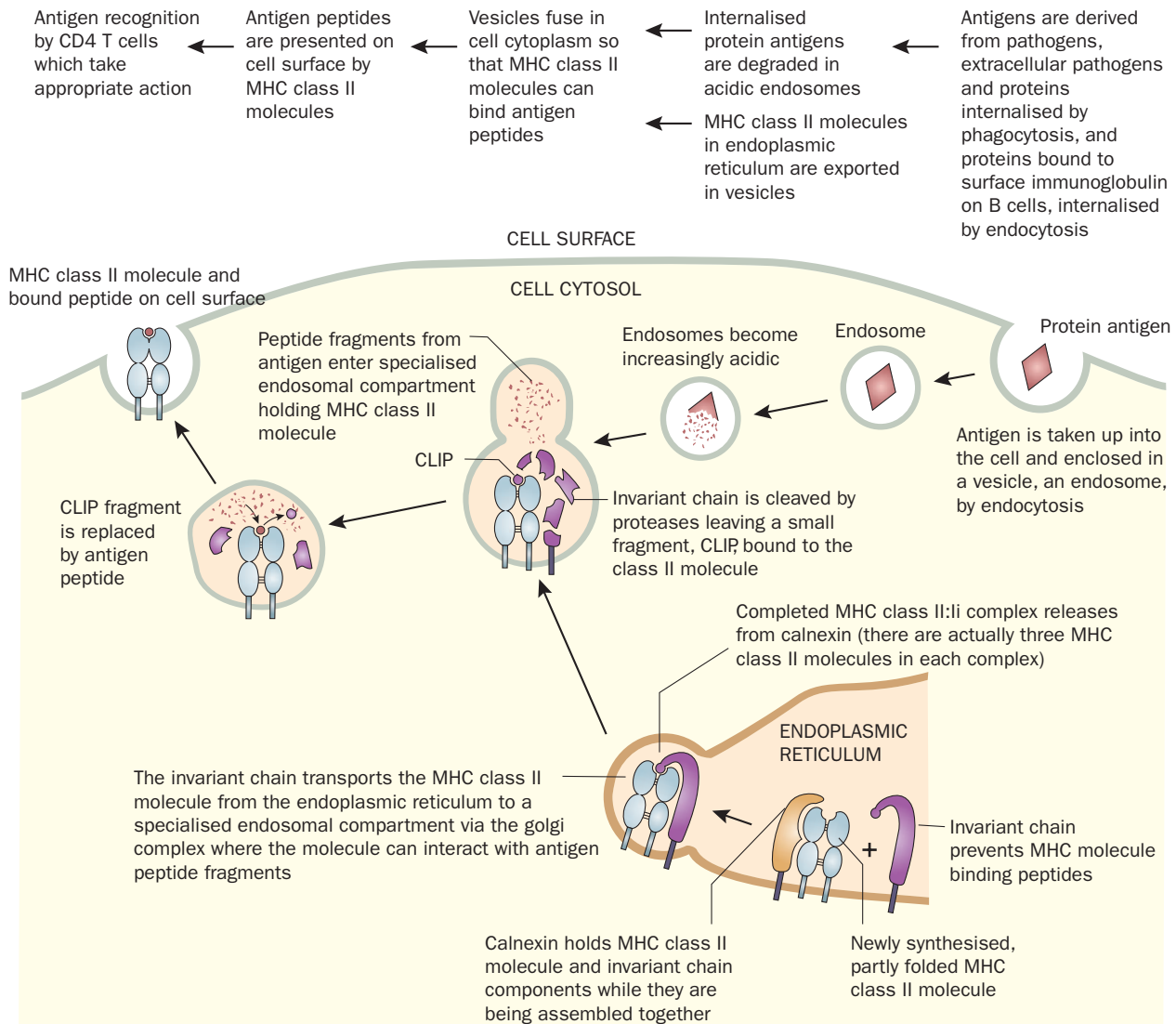


Figure 4B: **The pathway of exogenous antigen delivery to class II MHC molecules**

the T cells will recognise target cells expressing the specific foreign antigen with MHC and initiate either a cytotoxic attack, or stimulate an inflammatory response. Some of the activated T cells remain in the lymph nodes as central memory cells. Naive and memory T cells are partly differentiated by the presence of CD45RA (naive) and CD45RO (memory) surface molecules. Memory cells may live for 10 years or more. They react more quickly on subsequent exposure because the log phase for their cell division is short (12 h compared with 24 h) and they have a longer lifespan due to decreased apoptosis.

Effector T cells

Two major types of effector T cells have been identified, T helper (Th) and T cytotoxic (Tc), bearing either CD4 or CD8 molecules on their surface, respectively. CD4⁺ Th cells are the orchestrating cells of the immune response, recognising foreign antigen, and activating other parts of the cell-mediated immune response to eradicate the pathogen. They also play a major part in activation of B cells. CD8⁺ cytotoxic cells are involved in antiviral and

possibly antitumour activity. Both types have a major role in the control of intracellular pathogens.

T helper CD4⁺ cells

Th cells are subdivided functionally by the pattern of cytokines they produce.²⁴ On stimulation, precursor Th 0 lymphocytes become either Th 1 or Th 2 cells. The difference between these cells is only in the cytokines secreted; they are morphologically indistinguishable. However, the response they generate is very different. Th 1 cells produce interleukin 2, which induces T cell proliferation (including that of CD4⁺ cells in an autocrine response). Interleukin 2 stimulates CD8⁺ T cell division and cytotoxicity, by decreasing activation thresholds. The other major cytokine produced by Th 1 cells, interferon γ activates macrophages to kill intracellular pathogens such as mycobacteria, fungi, and protozoa and induces natural killer cells to cytotoxicity. Its importance has been shown in patients lacking the interferon γ receptor who suffer severe mycobacterial infections.²⁵ The Th 1 cytokines therefore induce mainly a cell-mediated inflammatory response—eg, the granulomatous lesions of tuberculosis. There is a positive feedback loop as interferon γ stimulates other Th 0 cells to become Th 1 and inhibits Th 2 differentiation.

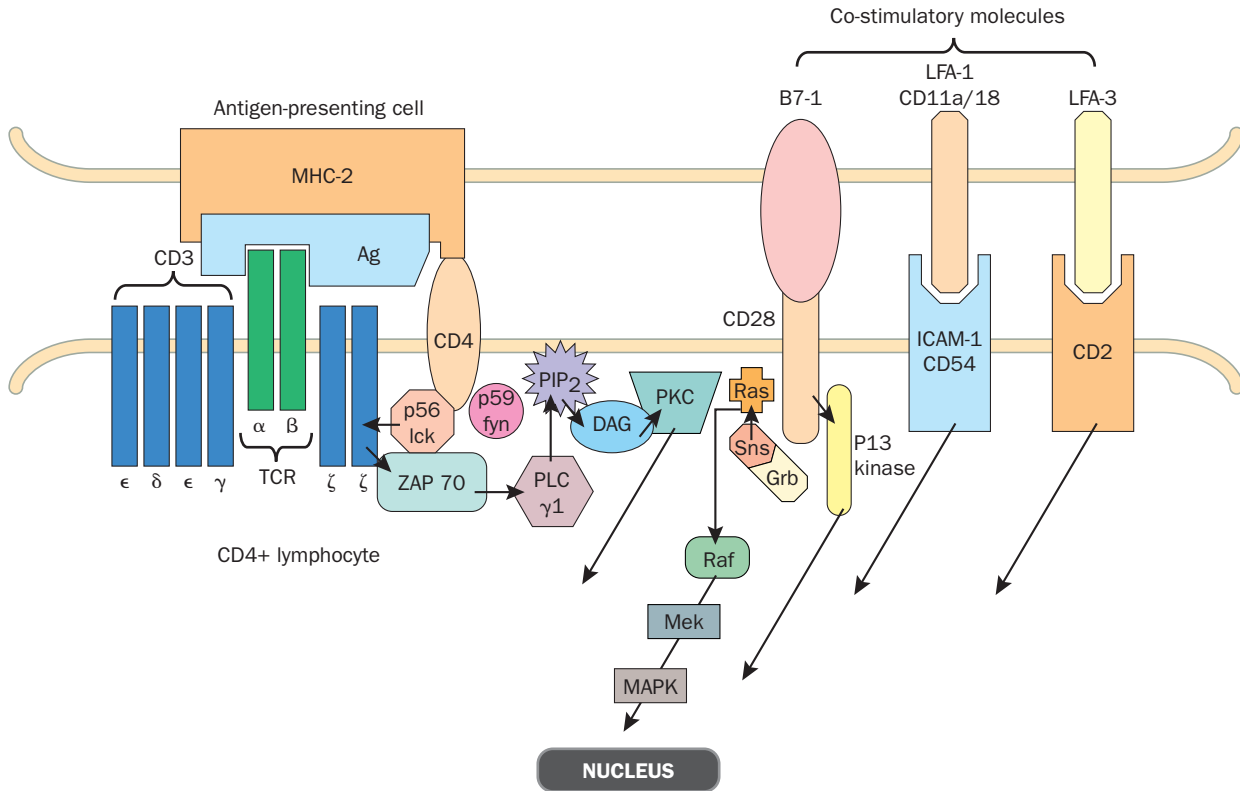


Figure 5: **Activation of T cells**

Lck=lymphocyte cytoplasmic kinase, ZAP=zeta-associated protein, DAG=diacyl glycerol, Ras=rous adenocarcinoma, Sos=son of sevenless, Raf=ras associated factor, Mek=mitogenic extracellular kinase, MAPK=mitogenic associated proliferation kinase, PKC=protein kinase C, PLC=phospholipase C, EPK=extracellular receptor associated kinase. Crosslinking of the T-cell receptor causes aggregation with the CD3 complex containing ϵ , δ , γ chains together with the three dimers and activation of phosphorylation and differentiation. If the costimulatory molecules are not activated at the same time a different sequence of signals is activated leading to cell death and apoptosis.

Interleukin 12 secreted by the interferon- γ -stimulated macrophages, further increases interferon γ production by T cells. A Th 1 response is essential to the host to control the replication of intracellular pathogens, but possibly contributes to the pathogenesis of autoimmune disease such as rheumatoid arthritis and multiple sclerosis. Conversely, Th 2 cells produce interleukin 4, interleukin 5, interleukin 6, and interleukin 10, that favour antibody production. Interleukin 4 induces class-switching in B cells to IgE production and interleukin 5 promotes the growth of eosinophils. Interleukin 4 provides positive feedback to induce further Th 2 responses and suppress Th 1 differentiation. Thus the Th 2 response is associated with allergic disease.

T cytotoxic (CD8+) cells

These are directly cytotoxic to cells bearing their specific antigen. After binding to the target cell, Tc insert perforins into the cell membrane, in the same way as natural killer cells. Cytoplasmic granules containing granzymes pass through the pores from the T cell into the target cytoplasm. These activate caspase enzymes that induce DNA fragmentation and cell apoptosis. Tc also bind target cell surface Fas (death inducing) molecules by their Fas ligand (FasL), which also activates apoptosis. In the same way as Th differentiate to Th 1 and Th 2, Tc 0 cells have been shown to differentiate to Tc 1 and Tc 2 based on cytokine secretion is documented. These subtypes have a limited cytokine repertoire and their role is not yet clear. It is also postulated that some CD8+ T cells have a suppressor function in downregulating lymphocyte responses.

The activation of macrophages via CD4+ cell cytokines to kill facultative intracellular pathogens, and the role of CD8+ T cells in killing of virally infected cells, provide the control of intracellular infections that cannot be achieved by the innate system.

B lymphocytes

B cells produce antibody. This serves to neutralise toxins, prevents organisms adhering to mucosal surfaces, activates complement, opsonises bacteria for phagocytosis, and sensitises tumour and infected cells for antibody-dependent cytotoxic attack by killer cells. Thus antibody acts to enhance elements of the innate system. Although ultimately antibody is the secreted product of activated B cells with the functions listed, early in B-cell development it is a membrane bound molecule that acts as the B-cell receptor. In this role it internalises antigen and processes it to act as an antigen-presenting cell for T-cell responses (figure 6).

Different classes of antibody predominate at different compartments of the body (IgM being intravascular, IgG the main antibody of the blood and tissues, IgA in secretions). Mucosa associated lymphoid tissue consists of lymphoid tissue at several mucosal sites (bronchus, gut, urogenital tract). However, these are all linked functionally as subpopulations of B cells home to these tissues specifically. A response generated at one site will induce immune responses to the same antigen at other sites. This effect can be used therapeutically because vaccination at one mucosal site can potentially induce generalised mucosal immunity.²⁶ For example, an oral vaccine could induce vaginal and rectal immunity

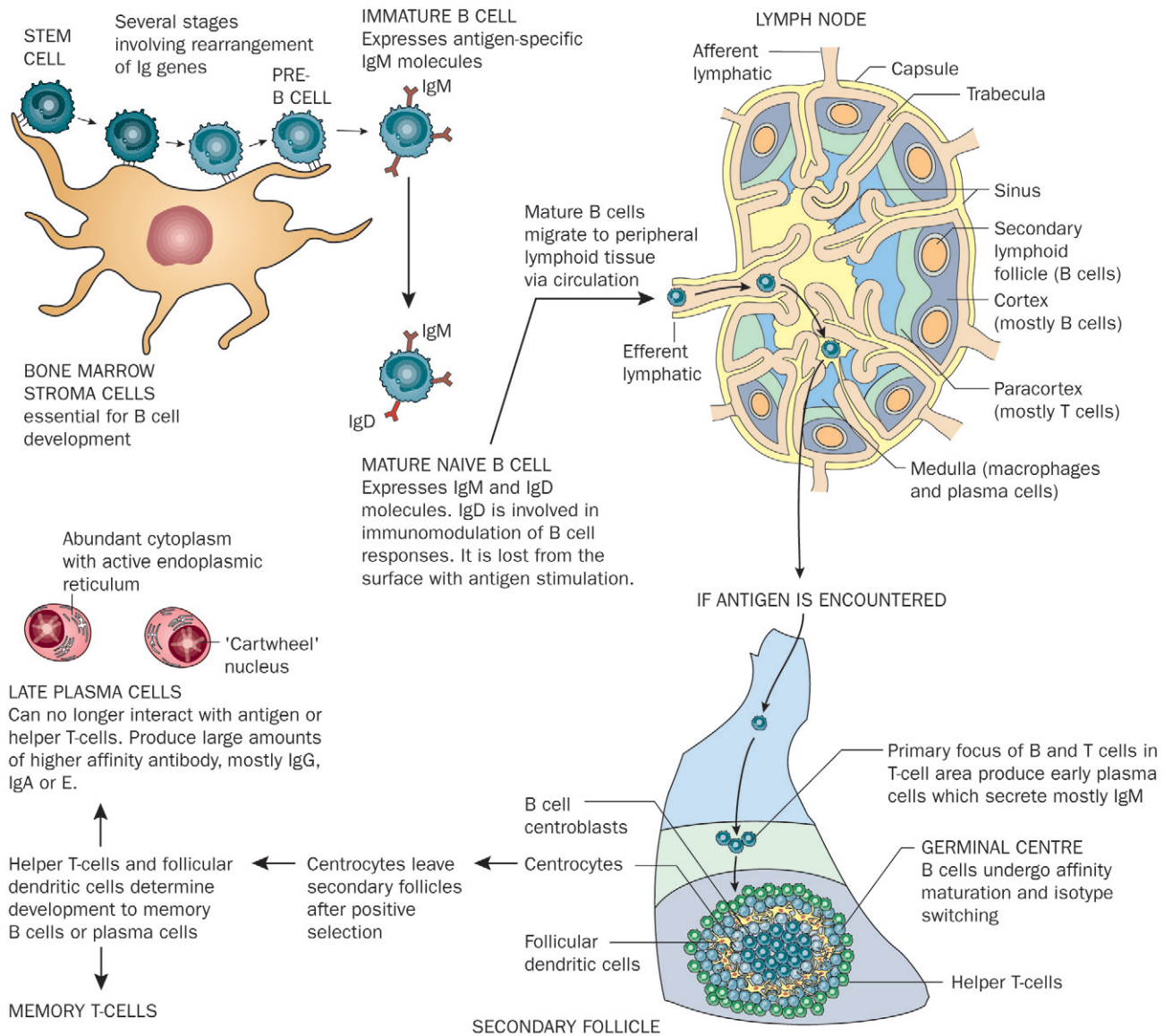


Figure 6: **The pathway of B lymphocyte development from stem cell to plasma cell**
Reproduced with permission by Keith Nye.

which could be particularly relevant in infections such as HIV.

B cell activation

Most B cells remain in the lymphoid tissue, the recirculating pool being small. B cells usually recognise free antigen brought to lymphoid tissues by the routes described previously. However, during subsequent infections by the same pathogen B cells can be activated by follicular dendritic cells which bear Fc and complement receptors, bind immune complexes containing antigen, and trap this to activate the B-cell response (follicular dendritic cells are a different family to dendritic cells and do not endocytose and present antigen).

T-cell dependent responses

Antigen recognised by the surface IgM of the B cell, is internalised, processed, and re-expressed on the MHC class II molecule of the B cell. This can then present the antigen to a primed specific T cell (which recognises a different part of the same antigen). The T cell in turn

produces cytokines (B-cell growth factors) leading to B-cell division and maturation to antibody secreting cells. Further T-cell interactions, in particular the binding of CD40 on B cells with the CD40 ligand on T cells induces isotype switching from the initial IgM response. However, as the *VDJ* gene is not further altered the same antigen-binding site is used throughout. Thus, a mature but naive B cell, that has rearranged its *VDJ* gene, will initially make an IgM response on primary antigen stimulation because this is the first constant chain to be translocated. IgG and other isotype responses develop later and require additional T cell help. The process of B-cell activation occurs mainly within the germinal centres of lymph nodes. At this site somatic hypermutation occurs, leading to a greater diversity of antibody. Those cells whose surface antibody binds the antigen most avidly proliferate most efficiently and therefore the antibody response matures with increased affinity. Once the switch from IgM to another isotype has occurred, some of the activated cells become long-lived memory cells. These react rapidly to rechallenge and the characteristic IgG production of the

secondary response occurs. The activated B cells leave the lymphoid tissue as plasma cells. The spleen has a particular role in antibacterial polysaccharide (capsule) responses, especially in the production of the IgG₂ subclass of antibody, which is important in protection from pneumococcus, haemophilus, and meningococcus. Marginal zone B cells in the spleen are important in this process. The low number of these cells in infancy or their removal as a result of splenectomy, correlates with poor antibody responses to this type of organism.

T-cell independent responses

B cells can also respond to some antigens in a T-cell independent reaction.²⁷ The antigens that can induce this have numerous repeating epitopes (mainly polysaccharides) that bind multiple B-cell receptors and activate the B cell directly to secrete IgM antibody. However, as there is no germinal centre formation, no affinity maturation takes place, and there is no class switching or generation of memory. Therefore T-cell independent responses are IgM limited, of poor specificity, and shortlived.

Regulation of autoimmune responses

This process of random antigen receptor production inevitably leads to development of autoreactive receptors that bind self antigens.²⁸ However, there are systems in place to induce tolerance (a state in which the immune system fails to respond to an antigen) and reduce the risk of autoimmune disease. First, the binding of specific antigen to the T or B cell receptor in immature lymphocytes (within the thymus or the bone marrow), leads to programmed cell death (apoptosis) and clonal deletion.²⁹ This is due to the lack of costimulatory molecule activation, either because these are not expressed or because of low production of cytokines. Over 90% thymocytes die by apoptosis (either due to failing to be positively selected, or due to self-reactivity, and negative selection) highlighting the degree of regulation that occurs during thymic processing.³⁰ However, there will be some autoantigens that are not expressed in the primary lymphoid tissues, but will be met in the periphery. Exposure in this circumstance induces the autoreactive cell to anergy (unresponsiveness). This is again the result of the lack of costimulatory molecules being activated as there is no tissue damage. T cell tolerance would be expected to reduce the chances of a B cell reacting to autoantigens, in addition to the clonal deletion of self-reactive B cells in the marrow. However, there are additional mechanisms postulated to prevent autoimmunity in B cells. Those cells that inadvertently produce self-reactive antibody might be able to undergo receptor editing to change antibody specificity. Anti-idiotypic antibodies that bind to the idiotype marker (antigen-binding site) on B cells, may also suppress autoantibody production. Immature B1 cells, which express the CD5 molecule, produce low affinity natural antibodies which often recognise autoantigens. These cells could play a part in autoimmune disease. More mature B cells lack this molecule (B2 cells).

Interactions within and outside of the immune system

The immune system is a major target for development of treatment strategies, in particular to improve the management of infections, tumours, and autoimmune disease resistant to conventional therapies. Approaches include immunomodulation with cytokines or their antagonists,³¹ therapeutic vaccination with designer adjuvants to drive specified types of immune response,³² and regulation of cell function and survival by

manipulation of coreceptor signalling molecules.³³ The immune system is easily accessible through stem cells in the bone marrow. The possibilities of manipulation through gene therapy has been raised with the successful integration of the adenosine deaminase gene into the cells of children with severe combined immunodeficiency.³⁴ However, immune reactions are complex, changes in one component could affect several others; this is illustrated in the cytokine network theory, where alteration of the concentration of one cytokine will lead to a cascade of effects on others. High concentrations of cytokines will commonly cause shedding of the receptors for the cytokines from cell surfaces reducing further responses. Such soluble receptors could absorb cytokine from tissue fluids, either reducing its function, increasing its clearance, or possibly extending its half-life by preventing breakdown. An understanding of these interactions are crucial to the use of cytokines or their inhibitors in clinical practice.³⁵ It is becoming clear that the immune system does not work in isolation, but has close communications with other tissues. The interaction of immune cells and lymphokines with the neurological and endocrine systems is now documented.³⁶ Lymphoid cells bear steroid and insulin-like growth factor receptors on their surface and can respond to changes in concentrations of hormones. Conversely, lymphokines such as interleukin 1 can affect the central hypothalamic-pituitary axis. A better knowledge of these interactions may have far-reaching effects on our understanding of the effects of social, psychological, and environmental factors on the development and evolution of illness.

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