3 IMMUNOLOGY

GLOSSARY

Adaptive immunotherapy	The transfer of immune cells for therapeutic benefit.
ADCC, antibody-dependent cellular cytotoxicity	A cytotoxic reaction in which the Fc receptor-bearing killer cells recognize target cells via specific antibodies.
Adhesion molecules	Cell surface molecules involved in cell–cell interaction or the binding of cells to extracellular matrix, where the principal function is adhesion rather than cell activation, e.g. integrins and selectins.
Adjuvant	Any foreign material introduced with an antigen to enhance its immunogenecity, e.g. killed bacteria, (mycobacteria), emulsions (Freund's adjuvant) or precipitates (alums).
Alloantibody	Antibody raised in one individual and directed against an antigen (primarily on cells) of another individual of the same species.
Allogeneic	See page 124.
Allotypes	The protein of an allele which may be detectable as an antigen by another member of the same species. Plasma proteins are an example of antigenically dissimilar variants.
Alternative pathway	The activation pathways of the complement system involving C3 and factors B, D, P, H and I, which interact in the vicinity of an activator surface to form an alternative pathway C3 convertase.
Anaphylatoxins	Complement peptides (C3a and C5a) which cause mast cell degranulation and smooth muscle contraction.
Anchor residues	Certain amino acid residues of antigenic peptides are required for interaction in the binding pocket of MHC molecules.
Antigenic peptides	Peptide fragments of proteins which bind to MHC molecules and induce T-cell activation.
APCs (antigen-presenting cells)	A variety of cell types which carry antigen in a form that can stimulate lymphocytes.
Apoptosis	Programmed cell death: a mode of cell death which occurs under physiological conditions and is controlled by the dying cell itself ('cell suicide').
Autologous	Originating from the same individual.
β_2-microglobulin	A polypeptide which constitutes part of some membrane proteins including the class I MHC molecules.
Bcl-2	A molecule expressed transiently on activated B cells which have been rescued from apoptosis.
CD markers (cluster of differentiation)	Used as a prefix (and number). Cell surface molecules of lymphocytes and platelets that are distinguishable with monoclonal antibodies, and may be used to distinguish different cell populations.

Cell adhesion molecules (CAMs)	A group of proteins of the immunoglobulin supergene family involved in intercellular adhesion, including ICAM-1, ICAM-2, ICAM-3, VCAM-1, MAd CAM-1 and PECAM.
Class I/II restriction	The observation that immunologically active cells will only operate effectively when they share MHC haplotypes of either the class I or class II loci.
Class switching	The process by which B cells can express a new heavy chain isotype without altering the specificity of the antibody produced. This occurs by gene rearrangement.
Clonal selection	The fundamental basis of lymphocyte activation in which antigen selectively causes activation, division and differentiation only in those cells which express receptors with which it can combine.
Collectins	A group of large polymeric proteins including conglutinin and mannose-binding lectin (MBL) that can opsonize microbial pathogens.
Colony-stimulating factors (CSFs)	A group of cytokines which control the differentiation of haemopoetic stem cells.
Constant regions	The relatively invariant parts of the immunoglobulin heavy and light chains, and the α, β, γ and δ chains of the T-cell receptor.
Co-stimulation	The signals required for the activation of lymphocytes in addition to the antigen-specific signal delivered via their antigen receptors. CD28 is an important costimulating molecule for T cells and CD40 for B cells.
Defensins	A group of small antibacterial proteins produced by neutrophils.
Dendritic cells	Derived from either the lymphoid or mononuclear phagocyte lineages. A set of cells present in tissues, which capture antigen and migrate to lymph nodes and spleen, where they are particularly active in presenting the processed antigen to T cells.
Domain	Segments or loops on heavy and light chains formed by intrachain disulphide bonds. Each immunoglobulin domain consists of about 110 amino acids.
Epitope	Part of an antigen that binds to an antibody-combining site or a specific T-cell surface receptor, and determines specificity. Usually about 9–20 amino acids in size.
Fas ligand	The ligand that binds to the cell surface molecule Fas (CD95) which is normally found on the surface of lymphocytes. When Fas ligand binds to its receptor, cell death (apoptosis) is triggered.
Genetic restriction	Describes the phenomenon where lymphocytes and antigen-presenting cells interact more effectively when they share particular MHC haplotypes.
Gut-associated lymphoid tissue (GALT)	Accumulations of lymphoid tissue associated with the gastrointestinal tract.

Haplotype	A set of genetic determinants coded by closely linked genes on a single chromosome.
Hapten	A substance of low molecular weight which is not itself immunogenic, but which can bind to an antibody molecule and produce a new antigenic determinant.
Helper (T_H cells)	A functional subclass of T cells which can help generate cytotoxic T cells and cooperate with B cells in the production of antibody responses. Helper cells recognize antigen in association with class II molecules.
Heterologous	Originating from a different individual or different inbred line.
Heterophile antigen	Antigen which occurs in tissues of many different species and is therefore highly crossreactive, e.g. Paul–Bunnell antigen which reacts with both sheep and beef erythrocytes.
HLA	See page 36.
Idiotype	Unique antigenic determinant on the antigen-binding region of an immunoglobulin molecule.
Hypervariable regions	Amino acid sequences within the variable regions of heavy and light immunoglobulin chains and of the T-cell receptor which show the most variability and contribute most to the antigen-binding site.
Immunoglobulin subclass	Immunoglobulin of the same class that is detectable in the constant heavy chain region, and differs in electrophoretic mobility and antigenic determinant, and function, e.g. IgG1, IgG2, IgG3 and IgG4.
Immunoglobulin supergene family (IgSF)	Molecules which have domains homologous to those seen in immunoglobulins, including MHC class I and II molecules, the T-cell receptor, CD2, CD3, CD4, CD8 ICAMs, VCAM and some of the Fc receptors.
Intercellular adhesion molecules	Cell surface molecules found on a variety of leucocytes and non-haematogenous cells which interact with leucocyte functional antigen (LFA-1); e.g. ICAM-1 (CD54), ICAM-2 (CD102) and ICAM-3 (CD50).
Integrins	One of the 'families' of adhesion molecules, some of which interact with cell adhesion molecules, and others with components of the extracellular matrix.
Isologous	Originating from the same individual or member of the same inbred strain.
Isotype	The class or subclass of an immunoglobulin common to all members of that species. Each isotype is encoded by a separate immunoglobulin constant region gene sequence that is carried by all members of a species.
Killer (K) cells	Type of cytotoxic lymphocyte that is able to mediate antibody-dependent cellular cytotoxicity (ADCC).
Langerhans' cells	Antigen-presenting cells of the skin which emigrate to local lymph nodes to become dendritic cells; they are very active in presenting antigen to T cells.

Lectin pathway	A pathway of complement activation, initiated by mannose-binding lectin (MBL) which intersects the classical pathway.
Leucocyte functional antigens (LFAs)	A group of three molecules (LFA-1 (CD11a/CD18), LFA-2 (CD2) and LFA-3 (CD58)), which mediate intercellular adhesion between leucocytes and other cells in an antigen non-specific fashion.
Linkage disequilibrium	The association of two linked alleles more frequently than would be expected by chance.
Memory cells	Long-lived lymphocytes which have already been primed with antigen but have not yet undergone terminal differentiation into effector cells. They react more readily than naïve lymphocytes when restimulated with the same antigen.
Mixed lymphocyte reaction (MLR)	Proliferative response when lymphocytes from two genetically different (i.e. allogeneic) persons are mixed in cell culture. A vital test in matching donor and recipient prior to bone marrow transplantation.
Mucosa-associated lymphoid tissue (MALT)	Lymphoid tissue associated with the bronchial tree, gastrointestinal tract and other mucosa.
Natural killer (NK) cell	Type of cytotoxic lymphocyte that has the intrinsic ability to recognize and destroy virally infected cells and some tumour cells. Specializes in killing cells that express little or no MHC molecule.
NfkB	A transcription factor which is widely used by different leucocyte populations to signal activation.
Perforin	A granule-associated molecule of cytotoxic cells, homologous to complement C9. It can form pores on the membrane of a target cell.
Reactive oxygen/ nitrogen intermediates (ROIs/RNIs)	Bactericidal metabolites produced by phagocytic cells, including hydrogen peroxide, hypophalites and nitric acid.
Selectins	Three adhesion molecules, P-selectin (CD62P), E-selectin (CD62E), and L-selectin (CD62L) involved in slowing leucocytes during their transit through venules.
Superantigens	Antigens (often bacterial, e.g. staphylococcal enterotoxins) which bind to the MHC outside the peptide-binding groove and stimulate all or most of the T cells bearing particular T-cell receptor V regions. Antigens must normally be processed in order to trigger the T-cell receptor. Superantigens are not processed but bind directly to class II and Vβ.
Suppressor (TS) cell	Functionally defined populations of T cells which reduce the immune responses of other T cells or B cells, or switch the response into a different pathway to that under investigation.
Syngeneic	Genetically identical or closely related, so as to allow tissue transplant.

TAP transporters	A group of molecules which transport proteins and peptides between intracellular compartments.
T-cell receptor (TCR)	The T-cell antigen receptor consists of either an αβ dimer (TCR-2) or a γδ dimer (TCR-1) associated with the CD3 molecular complex.
T-dependent antigens	Require recognition by both T and B cells to produce an immune response.
T-independent antigens	Can directly stimulate B cells to produce specific antibody.
Titre	The highest dilution of a given substance, e.g. antibody, that will still produce a reaction with another substance, e.g. antigen.
Toll receptors	A group of evolutionarily ancient cell surface molecules, e.g. the IL-1 receptor, some of which are involved in transducing signals for inflammation.
Transforming growth factors (TGFs)	A group of cytokines, identified by their ability to promote fibroblast growth, that are also immunosuppressive.
Tumour necrosis factor (TNF)	See page 101.

THE IMMUNE RESPONSE SYSTEM

INNATE (NON-SPECIFIC) AND ADAPTIVE (ACQUIRED) IMMUNITY

(Fig 3.1 and Table 3.1)

The innate component functions as a first line of defence and involves antigen-independent mechanisms. The adaptive component results from antigen-dependent activation, proliferation and differentiation (clonal expansion) of lymphocytes. It takes longer to mobilize but confers specificity and exhibits memory. The two are functionally interrelated in several critical ways, e.g. through cytokines and complement components.

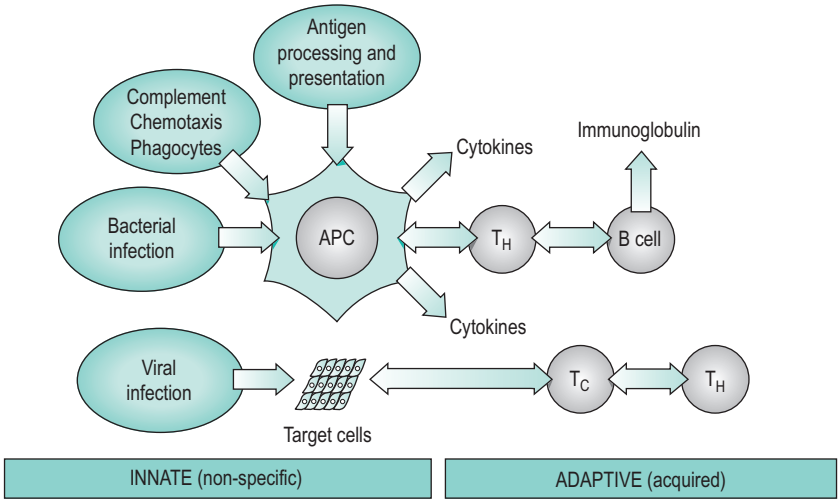


Fig. 3.1 Innate and acquired immunity.
APC = antigen presenting cells, T_H = helper T cells, T_C = cytotoxic T cells.

Table 3.1 Differences between the innate and adaptive immune response systems

Innate (non-specific system)	*Adaptive (acquired system)*
Components 1. Anatomical and physiological barriers 2. Inflammatory response with leakage of antibacterial serum proteins (acute-phase proteins) and phagocytic cells 3. Phagocytosis by neutrophils and macrophages 4. Complement system	**Components** 1. Cell-mediated response effected by T cells 2. Humoral immune response effected by B cells
Properties 1. Rapid: responds within minutes to infection 2. No antigenic specificity, i.e. the same molecules and cells respond to a range of pathogens 3. No memory, i.e. the response does not change after repeated exposure 4. Preformed or rapidly formed components	**Properties** 1. Slow: response over days to weeks 2. Antigenic specificity i.e. each cell is programmed genetically to respond to a single antigen 3. Immunological memory, i.e. on repeated exposure the response is faster, stronger and qualitatively different 4. Diversity: ability to recognize and respond to a vast number of different antigens 5. Self/non-self recognition: i.e. lack of response (tolerance) to self-antigens but response to foreign antigens

CELLS AND MOLECULES INVOLVED IN THE IMMUNE RESPONSE

1. **Antigen-recognition lymphoid cells (B and T lymphocytes)**
2. **Granulocytes**
3. **Macrophages**
4. **Dendritic cells**
5. **Natural killer cells**
6. **Cytokines**
7. **Accessory molecules**
8. **Other molecules**

1. ANTIGEN-RECOGNITION LYMPHOID CELLS (B AND T LYMPHOCYTES)

B lymphocytes (see also Immunoglobulins, p. 105).

Functions: Humoral immunity – antibody production; control of pyogenic bacteria; prevention of blood-borne infections; neutralization of toxins.

% of total lymphocytes: 12%; mainly fixed.

Site of production: Produced in germinal centre of lymph nodes and spleen.

Assessment of function: Serum specific immunoglobulin levels; specific antibodies; immunoglobulin response to pokeweed mitogen; endotoxin and EBV.

T lymphocytes

Functions: Cell-mediated immunity; protection against intracellular organisms, protozoa and fungi; graft rejection; control of neoplasms.

% of total lymphocytes: 70–80%; mainly circulating; long-lived memory cells.

Site of production: Produced in paracortical region of lymph nodes and spleen.

Assessment of function: Delayed hypersensitivity skin reactions using candida, mumps and purified protein derivative (PPD); active sensitization with dinitrochlorobenzene (DNCP); lymphocyte transformation: mitogenic response to phytohaemagglutinin (PHA) and concanavalin-A; mixed lymphocyte reaction (MLR); lymphokine release.

Identified by: T-cell surface phenotypes identified by reaction with monoclonal Abs (Table 3.2 and Fig. 3.2).

T cells express either γδ or αβ T-cell receptors. αβ T cells are divided into CD4 and CD8 subsets. T cells are further subdivided into T_H1 and T_H2 on the basis of their cytokine profiles (Fig. 3.3).

Table 3.2 T-cell surface antigens and CD markers (see also Fig. 3.3)

Surface antigen	*% of peripheral T cells*	*HLA restriction*	*Function*
T3 (CD3)	All		
T4 (CD4)	65	Class II MHC	T_H and T_{DH} cells
T8 (CD8)	35	Class I MHC	T_S and T_C cells

CD, cluster of differentiation; MHC, major histocompatibility complex; T_H helper T cells; T_{DH}, delayed hypersensitivity T cells; T_S suppressor T cells; T_C, cytotoxic T cells (see below).

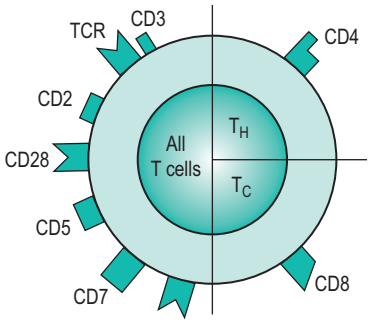


Fig. 3.2 T-cell CD markers.

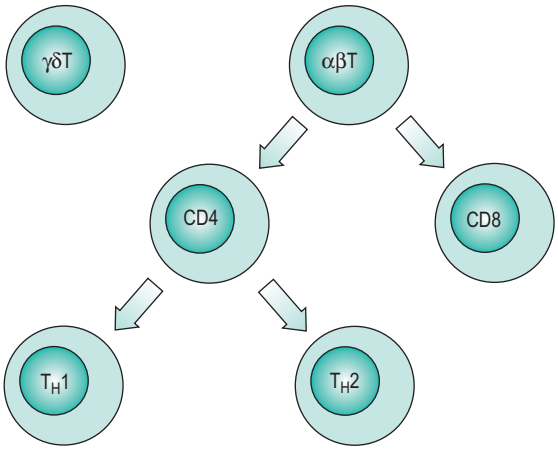


Fig. 3.3 T-cell subsets.

T-cell subpopulations

Regulatory and effector T cells
Regulatory cells:

1. **T_H helper T cells CD4$^+$:** recognize antigen by means of the T-cell receptors in association with macrophage receptors. Produces cytokines and helps generate cytotoxic T cells and cooperates with B cells in production of antibody responses. Recognizes antigen in association with class II MHC molecules on the surface of antigen-presenting cells.
2. **T_S suppressor T cells:** interfere with the development of an immune response of other T cells or B cells, either directly or via suppressor factors.

Effector cells:

3. **T_C cytotoxic T cells CD8$^+$:** regulate the immune response and can lyse target cells, e.g. viral or tumour antigens expressing antigen peptides presented by MHC class I molecules on the surface of all nucleated cells. Interleukin-2 (IL-2) is responsible for the generation of cytotoxic T cells.
4. **T_{DH} delayed hypersensitivity T cells:** release mediators that cause an inflammatory response attracting macrophages, neutrophils and other lymphocytes to the site.

Other selected important CD markers
CD28: Present in highest amounts in activated T cells. It is a T-cell costimulatory molecule which plays a major role in T cell activation.
CD45RA: An isoform of CD45 associated with active T cells that respond poorly to recall antigen.
CD45RO: An isoform associated with memory T cells. Responds well to recall antigen.
CD95: Also known as Fas, binds Fas ligand and mediates apoptosis of activated T cells.

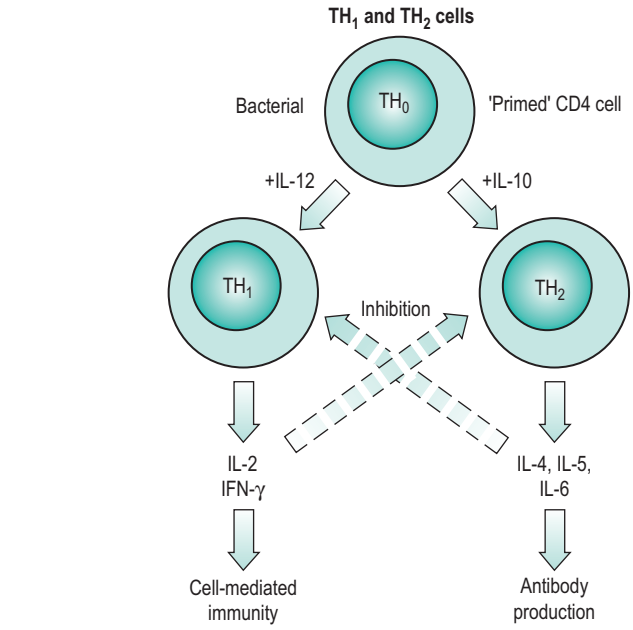
T_H1 and T_H2 populations (Fig. 3.4)

- CD4$^+$ MHC class II-restricted T cells can also be subdivided into T_H1 and T_H2 populations based on their profiles of cytokine production.
- The **T_H1 profile** is associated with production of IL-2, tumour necrosis factor (TNF)-β and interferon (IFN)-γ and is driven by IL-12.
- The **T_H2 profile** is associated with IL-4, IL-5, IL-6 and IL-13 and is driven by IL-10.
- T_H1 cytokines are involved in helping cell-mediated immunity and the T_H2 cytokines mediate humoral immunity.
- T_H1 cells can downregulate T_H2 cells and vice versa.

T-cell antigen receptor (TCR) (Fig. 3.5)

TCR complex comprises a disulphide-linked heterodimeric glycoprotein that enables T cells to recognize a diverse array of antigens in association with MHC molecules. It consists of α and β subunits or occasionally γ and δ subunits. It is associated at the cell surface with a complex of polypeptides known collectively as CD3 which is required for activation of T cells.

- Consists of α, β subunits or, less commonly, γ or δ subunits.
- Differences in the variable regions of the TCR subunits account for the diversity of antigenic specificity among T cells.
- TCRs only recognize antigenic peptides bound to class I or class II MHC molecules.
- T cells can be divided into different subsets based on the expression of one or other T-cell receptor (TCR-1 or TCR-2).



TH_1 effects	TH_2 effects
• Reinforces early local responses • Promotes cell-mediated cytotoxic responses • Mediates type IV delayed type hypersensitivity	• Activates later systemic responses • Promotes humoral antibody responses • Promotes allergic type 1 hypersensitivity responses • Limits inflammatory responses

Fig. 3.4 Involvement of T_H1 and T_H2 cells in immunity.

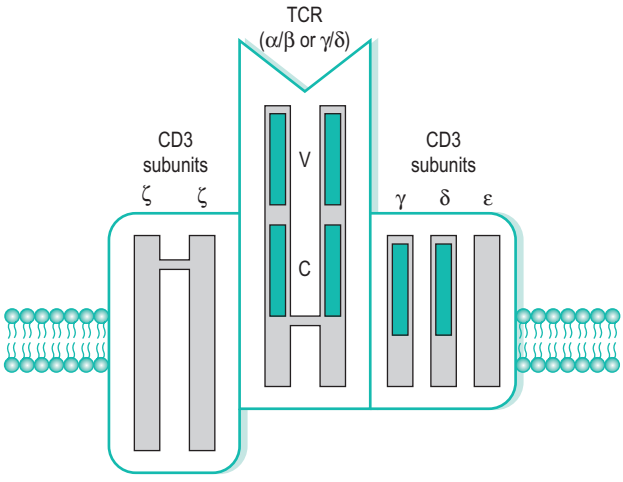


Fig. 3.5 T-cell receptor (TCR) complex.

- TCR-1 cells are thought to have a restricted repertoire and to be mainly non-MHC restricted.
- TCR-2 cells express either CD4 or CD8 which determines whether they see antigen in association with MHC class II or I molecules.

T-cell recognition of an antigen

- T cells recognize antigens that originate within other cells, such as viral peptides from infected cells.
- T cells bind specifically to antigenic peptides presented on the surface of infected cells by molecules encoded by the MHC.
- The T cells use their specific receptors (TCRs) to recognize the unique combinations of MHC molecule plus antigenic peptide (Fig. 3.6).

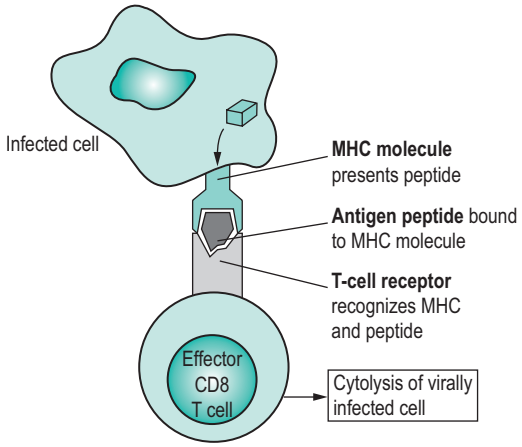


Fig. 3.6 T-cell recognition of antigen.

Summary

- **Class I MHC pathway:** presents antigenic peptides derived from intracellular viral, foreign graft and tumour cell proteins to **$CD8^+$ cells**.
- **Class II MHC pathway:** presents antigenic peptides derived from internalized microbes to **$CD4^+$ cells**.

The stages in the recognition and processing of a virally infected cell by a cytotoxic $CD8^+$ T cell are:

1. Entry of virus into the target cell.
2. Replication of the virus.
3. Processing of viral proteins to generate antigenic determinants which associate with MHC (HLA) class I molecules.
4. Presentation of the antigen–HLA complex for recognition by a specific CD8 cytotoxic cell, with killing of the infected cell.
5. The naïve T cells that emerge from the thymus are pre-cytotoxic T lymphocytes, and require further activation and differentiation to become the effector T cells that lyse virally infected target cells and tumour cells.

The γδ T-cell subset

- γδ TCR-expressing T cells are a minor population (> 5%) of all T cells and are a separate lineage from the αβ T cell that differentiates into $CD8^+$ and $CD4^+$ cells.

- The γδ TCR recognizes antigen differently without processing or presentation on a MHC class I or class II molecule, e.g. non-peptide antigen such as bacterial cell wall phospholipids.
- They act as part of the first line of defence, recognizing pathogens mainly in the skin and gut.
- They can secrete cytokines, help B cells, activate macrophages and lyse virally infected cells.

2. GRANULOCYTES

- Neutrophils (PMNs): strongly phagocytic cells important in controlling bacterial infections.
- Eosinophils: weakly phagocytic: main role is in allergic reactions and destruction of parasites.
- Basophils and mast cells: non-phagocytic granulocytes that possess cell-surface receptors for IgE. Mediate allergic and antiparasitic response due to release of histamine and other mediators.

3. MACROPHAGES

Monocytes are released from the bone marrow, circulate in the blood and enter tissues, where they mature into macrophages.

Functions

1. Phagocytose microbes.
2. Secrete inflammatory mediators and complement components.
3. Present antigen associated with class II MHC and $CD4^+$ cells.
4. Secrete numerous cytokines that promote immune responses (IL-1, TNF-α, IL-6 and IL-12).

Phagocytosis of microbes by neutrophils and macrophages

1. Bacteria are opsonized by IgM, IgG, C3b and C4b, promoting their adherence and uptake by phagocytes.
2. The killing activity of neutrophils and macrophages is enhanced by highly reactive compounds: oxygen-dependent (hydrogen peroxide H_2O_2, superoxide anion, hydroxyl radicals, hypochlorous acid and nitric oxide (NO)) and oxygen-independent (acids, lysozyme–degrades, bacterial peptidoglycan, defensins (damage membranes), lysosomal proteases, lactoferrin (chelates iron). Their formation by NADPH oxidase, NADH oxidase or myeloperoxidase is stimulated by a powerful oxidative burst following bacterial phagocytosis.

4. DENDRITIC CELLS

Found in various tissues, e.g. Langerhans cells of the skin, peripheral blood and lymph glands.

Functions

1. Antigen-presenting cells: efficient at presenting antigen to both $CD4^+$ and $CD8^+$ cells.
2. Have phagocytic activity and release cytokines.

Antigen-presenting cells
- Include macrophages, monocytes or their derivatives (microglial cells, Kupffer cells and skin Langerhans cells).
- Characterized by their ability to phagocytose, internalize and process antigen.
- Possess Ia antigen, Fc receptors and C3b receptors and produce interleukin 1.

5. NATURAL KILLER (NK) CELLS

Functions
1. Similar function to lymphocytes – kill virus-infected cells and some tumour cells, and produce cytokines.
2. Recognition of target differs from lymphocytes – they do not bind MHC, and a carbohydrate receptor selects target. NK cells express two major classes of inhibitory receptors for MHC molecules: lectin-like receptors of the CD94 family and immunoglobulin superfamily molecules (KIRs).
3. Act rapidly, and constitute an early antiviral defence.
4. Identified by: Fc receptor for IgG.
5. Previously referred to as large granular lymphocytes (IGL) because of their appearance.

Mechanisms of NK cell killing
- Direct cytotoxicity involving contact with target cell and lysis by perforin-mediated mechanism similar to that used by T_C cells, except it is antigen independent and non-MHC restricted.
- Antibody-dependent cellular (ADCC) cytotoxicity. Binding of Fc receptors on NK cells to antibody-coated target cells initiates killing. (Neutrophils, eosinophils and macrophages also exhibit ADCC).

6. CYTOKINES (Fig. 3.7)

- Small protein signalling molecules (usually glycoproteins) of relatively low molecular weight.
- They regulate important biological processes: proliferation and differentiation, growth inhibition, apoptosis, chemotaxis and chemokinesis, resistance to viral infection, induction of cytotoxic effector cells, induction of phagocytes, promotion of intercellular adhesions and regulation of adhesion to extracellular matrix.
- Many cytokines act by causing aggregation of receptors at the cell surface, which leads to activation of second messenger system.
- The main cytokines are interferons, interleukins, tumour necrosis factor, growth factors, colony stimulating factors and chemokines.

1. Interferons (IFNs) (Table 3.3)
These glycoproteins are produced by virus-infected cells.
- Three species of interferon:
  1. Alpha-interferon (IFN-α) produced by human leucocytes
  2. Beta-interferon (IFN-β) produced by human fibroblasts
  3. Gamma-interferon (IFN-γ) produced by human T lymphocytes in response to antigenic stimulation.
- Properties:
  1. Prevent viral replication
  2. Antitumour activity
  3. Activate macrophages and natural killer (NK) cells.

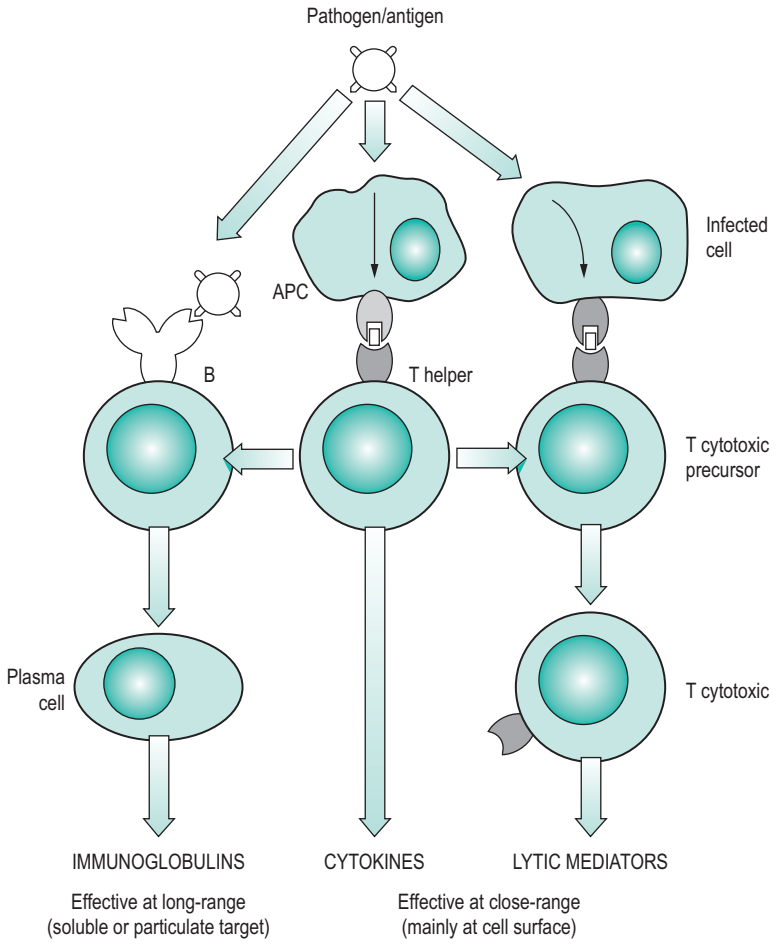


Fig. 3.7 Interrelationship of immune cell populations.
APC = antigen-presenting cell.

Table 3.3 Interferons (IFNs)

Cytokine	*Immune cells*	*Induced by*	*Immunological effects*
IFN-α,-β	T and B cells, monocytes or macrophages	Mainly viruses; also some bacteria, protozoa and cytokines	Antiviral activity Stimulation of macrophages and large granular lymphocytes (LGL) Enhanced HLA (MHC) class I expression
IFN-γ	T cells and NK cells	Recognition of antigen by T-cell receptor	Antiviral activity Stimulation of macrophages and endothelium Enhanced HLA (MHC) class I and class II expression Suppression of T_H2 cells

2. Interleukins (ILs) (Table 3.4)

These cytokines stimulate proliferation of T helper and cytotoxic cells and B cells.

Interleukin-1 (IL-1) is a central regulator of the inflammatory response.

- Synthesized by activated mononuclear phagocytes.
- IL-1β is secreted into the circulation and cleaved by interleukin-1β converting enzyme (ICE).
- IL-1β levels in the circulation are only detectable in the following situations: after strenuous exercise, in ovulating women, sepsis, acute organ rejection, acute exacerbation of rheumatoid arthritis.
- Acts in septic shock by increasing the number of small mediator molecules such as PAF (platelet-activating factor), prostaglandins and nitric oxide which are potent vasodilators.
- The uptake of oxidized low density lipoproteins (LDL) by vascular endothelial cells results in IL-1 expression which stimulates the production of platelet-derived growth factor. IL-1 is thus likely to play a role in the formation of the atherosclerotic plaque.
- IL-1 has some host defence properties, inducing T and B lymphocytes, and reduces mortality from bacterial and fungal infection in animal models.

Interleukin-2 (IL-2) is also known as T-cell growth factor.

- Induces proliferation of other T lymphocytes; generates new cytotoxic cells, and enhances natural killer cells.

Table 3.4 Interleukins (ILs)

Cytokine	*Immune cells*	*Immunological effects*
IL-1α, β	Monocytes/macrophages, dendritic cells	Activation of T and B cells, macrophages, and endothelium Stimulation of acute phase response
IL-2	T_H1 cells	Proliferation and/or activation of T, B and LGL
IL-4	T_H2 cells, macrophages, mast cells and basophils, bone marrow stroma	Activation of B cells Differentiation of T_H2 cells and suppression of T_H1 cells
IL-5	T_H2 cells, mast cells	Development, activation and chemoattraction of eosinophils
IL-6	T_H2 cells, monocytes or macrophages	Activation of haemopoietic stem cells Differentiation of B and T cells Production of acute phase proteins
IL-8	T cells, monocytes, neutrophils	Chemoattraction of neutrophils, T cells, basophils Activation of neutrophils
IL-10	T_H2 and B cells, macrophages	Suppression of macrophage functions and T_H1 cells Activation of B cells
IL-12	Macrophages, dendritic cells, B cells	Suppression of macrophage functions and T_H1 cells Activation of B cells

3. Tumour necrosis factor (TNF) (Table 3.5)

- The principal mediator of the host response to Gram-negative bacteria. May also play a role in the response to other infectious organisms, and is a key cytokine in the pathogenesis of multiorgan failure.
- Activates inflammatory leucocytes to kill microbes; stimulates mononuclear phagocytes to produce cytokines; acts as a costimulator for T-cell activation and antibody production by B cells; and exerts an interferon-like effect against viruses.

Table 3.5 Tumour necrosis factor (TNF)		
Cytokine	*Immune cells*	*Immunological effects*
TNF-α	Macrophages, lymphocytes, neutrophils, eosinophils,	Activation of macrophages, granulocytes, cytotoxic cells and endothelium
	NK cells	Enhanced HLA class I expression
		Stimulation of acute phase response
		Anti-tumour effects
TNF-β	T_H1 and T_C cells	Similar to TNF-α

4. Growth factors

Transforming growth factor-beta (TGF-β)

- Generally limits inflammatory response.
- Enhances IgA synthesis.
- Initiates and terminates tissue repair.
- Undergoes autoinduction.
- Released by platelets at the site of tissue injury and promotes the formation of extracellular matrix.
- Implicated in diseases of tissue fibrosis such as cirrhosis and glomerulosclerosis.

5. Colony stimulating factors (CSFs) (Table 3.6)

- These are involved in directing the division and differentiation of bone-marrow stem cells, and the precursors of blood leucocytes.

Table 3.6 Colony stimulating factors (CSFs)		
Cytokine	*Immune cell source*	*Immunological effects*
GM-GSF	Many cells	Myeloid growth
G-CSF	T cells, macrophages, neutrophils	Development and activation of neutrophils
M-CSF	T cells, macrophages, neutrophils	Development and activation of monocytes/ macrophages
GM-CSF	T cells, macrophages, mast cells, neutrophils, eosinophils	Differentiation of pluripotent stem cells Development of neutrophils, eosinophils and macrophages
Transforming growth factor (TGF)-β	T cells, monocytes	Inhibition of T and B cell proliferation and LGL activity
Erythropoietin*	Kidney	Erythropoiesis
*Not a typical cytokine as it has a single origin.		

6. Chemokines

- Large family of cytokines that have chemoattractant properties.
- Responsible for recruiting leucocytes to inflammatory lesions, inducing release of granules from granulocytes, regulating integrin avidity and in general exhibiting proinflammatory properties.
- Chemokines are secreted by many cell types.
- The receptors for the chemokines are also family-specific (Table 3.7).

Table 3.7 Cytokine subgroups		
Cytokines	*Immune cell source*	*Immunological effects*
α Subgroup CXC-type (e.g. IL-8)	Macrophage, neutrophil, endothelium, fibroblast	Attracts neutrophils and promotes their migration into tissues
β Subgroup CC-type (e.g. MIP, RANTES)	Macrophage, neutrophil, endothelium, T cell	Attracts macrophages, eosinophils, basophils and lymphocytes

Cytokine disorders

Both cytokine overexpression and underexpression or their receptors can be pathogenic:

1. Septic shock: production of IL-1, IL-6 and TNF due to endotoxin stimulation of macrophages following Gram-negative infection.
2. Toxic shock syndrome: massive release of cytokines due to superantigen stimulation of T-cells by TSST-1, a bacterial exotoxin.
3. Chagas' disease (*T. cruzi* infection): causes reduced expression of IL-2 receptor, leading to marked immune suppression.

7. ACCESSORY MOLECULES

Promote adhesion of T cells and/or signal transduction leading to T-cell activation.

Adhesion molecules (Table 3.8)

- Involved in cell–cell communication and recognition (i.e. help bind T cells to antigen-presenting cells and target cells), and control leucocyte migration (i.e. help direct T cells to sites of inflammation and lymph nodes).
- They fall into families that are structurally related:
  - **The cell adhesion molecules (CAMs)** of the immunoglobulin superfamily (antigen presentation)
  - **the cadherin superfamily** (neuromuscular interaction)
  - **integrins** (interaction between cells and the extracellular matrix)
  - **selectins** (leucocyte adhesion to endothelium during inflammation).

Table 3.8 Adhesion molecules involved in lymphocyte interactions		
	Receptor on lymphocyte	*Ligand on interacting cell*
T cells	CD4	HLA class II
	CD8	HLA class I
	CD28	CD80
	CD2	LFA-3
	VLA-4	VCAM-1
B cells	LFA-1	ICAM-1, -2 or -3
	CD40	CD40-ligand
LFA, lymphocyte function-associated antigen; VLA, very late antigen; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule.		

Coreceptor activating molecules (e.g. CD28, CTLA-4)

- Transduce signals important in regulating functional responses of T cells.

8. OTHER MOLECULES

Heat shock proteins

The *heat shock response* is a highly conserved and phylogenetically ancient response to tissue stress that is mediated by activation of specific genes. This leads to the production of specific heat shock proteins that alter the phenotype of the cell and enhance its resistance to stress. Their principal function appears to be to act as molecular chaperones for damaged protein to direct it into degradation pathways such as ubiquitination.

Free radicals

- A *free radical* is literally any atom or molecule which contains one or more unpaired electrons, making it more reactive than the native species.
- Free radical species produced in the human body are:
  - $OOH^{\bullet}$ (peroxide radical)
  - $O_2^{\bullet}$ (superoxide radical)
  - $OH^{\bullet}$ (hydroxyl radical)
  - $NO^{\bullet}$ (nitric oxide).
- The hydroxyl radical is by far the most reactive species, but the others can generate more reactive species as breakdown products.
- When a free radical reacts with a non-radical, a chain reaction ensues which results in the formation of further free radicals and direct tissue damage by lipid peroxidation of membranes (particularly implicated in atherosclerosis and ischaemic reperfusion injury within tissues).
- Free radical scavengers bind reactive oxygen species.
- Principal dietary antioxidants:
  - Vitamin E
  - β-Carotene
  - Vitamin C
  - Flavonoids.
- Patients with dominant familial forms of amyotrophic lateral sclerosis (motor neuron disease) have mutations in the gene for Cu–Zn SOD-1, suggesting a link between failure of free radical scavenging and neurodegeneration. Protection against heart disease and cancer may be conferred by dietary antioxidants.

Nitric oxide (NO)

NO is an important transcellular messenger molecule which is involved in a diverse range of processes.

- NO is synthesized from the oxidation of nitrogen atoms in the amino acid L-arginine by the action of *NO synthase* (NOS; Fig. 3.8).
- NO acts on target cells close to its site of synthesis, where it activates guanylate cyclase, leading to a rise in intracellular *cGMP* which acts as a second messenger to modulate a variety of cellular processes. It has a very short half-life.
- There are at least three distinct *isoforms* of NO synthase:
  1. Neuronal (constitutive) NO synthase (CNS neurotransmission, memory formation)
  2. Endothelial (constitutive) NO synthase (vasodilator tone modulation, organ-specific microcirculatory control, e.g. kidney)
  3. Macrophage (inducible) NO synthase.

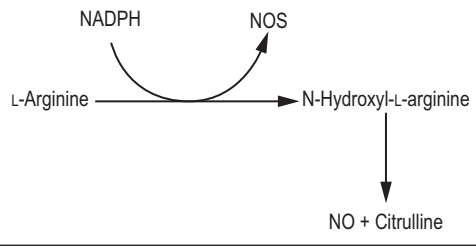


Fig. 3.8 Synthesis of nitric oxide.
NOS = nitric oxide synthase.

Clinical relevance of NO

1. Septic shock (NO is released in massive amounts and results in decreased vascular tone, cardiac output with low BP). This is because endotoxin release triggers the innate immune response when macrophages are directly activated through Toll-like receptors. Macrophage activation results in the secretion of TNF, prostaglandins and NO. There have been three main approaches to preventing septic shock:
   (i) Blocking nitric oxide production by macrophages, endothelium and smooth muscle.
   (ii) Blocking TNF with monoclonal antibodies.
   (iii) Recombinant bactericidal protein to bind to endotoxin and prevent macrophage activation. All have failed in clinical trials, probably because the innate immune response has already mediated its damage by the time symptoms develop.
2. Atherosclerosis (where NO synthesis may be impaired, leading to tonic vasoconstriction and vasospasm).
3. 1° and 2° pulmonary hypertension (inhaled NO reverses pulmonary hypertension).
4. Hepatorenal syndrome and the hypertension of chronic renal failure.
5. Glutamate-mediated excitotoxic cell death in the CNS, such as in Alzheimer's disease, and also in acute brain injury, such as stroke.
6. Tissue damage in acute and chronic inflammation (probably by interacting with oxygen-derived free radicals).
7. ARDS (adult respiratory distress syndrome).

APOPTOSIS (see also p. 35)

- Is the process of programmed cell death, and is a mechanism for the elimination of excess or damaged cells.
- Several genes have been identified that either promote *(bax, bak, bcl-Xs)* or inhibit *(bcl-2, bcl-XL, bcl-w)* apoptosis. Antiapoptotic genes could confer characteristics such as longer survival.
- It is mainly triggered through the Fas–Fas ligand interaction. Binding of Fas ligand (expressed on a killer T cell) to Fas expressed on a target cell triggers a cascade of intracellular biochemical changes in the target cell. Fas interacts with several proteins in the 'death pathway' to activate a proteolytic enzyme, caspase. The caspase proteolytic cascade then activates a cytoplasmic enzyme (caspase-activatable DNAase (CAD)) which can then migrate to the nucleus and cleave DNA into small fragments, which are the end-point of apoptosis.
- It has several important roles in shaping the adaptive immune response, e.g. after an immune response to a pathogen, redundant lymphocytes are cleared by apoptosis.
- It is also involved in some pathological processes, e.g. destruction of $CD4^+$ cells in HIV infection; can lead to the production of autoantibodies against DNA and result in autoimmune disease; clones of B cells that have increased levels of *bcl-2* through mutations may be protected from apoptosis and develop into a B-cell malignancy.

IMMUNOGLOBULINS

PROPERTIES, FUNCTIONS AND REACTIONS

The properties and functions of the major classes of immunoglobulins are shown in Table 3.9, and the immunological reactions of IgG, IgA and IgM are summarized in Table 3.10.

Table 3.9 Properties and functions of the major classes of immunoglobulin

Ig class	*Heavy chains*	*Molecular weight*	*% total Ig level*	*Normal plasma level*	*Function*
IgG	γ	150 000 (monomer)	80	8–16 g/l	1. Distributed in blood and interstitial fluids 2. The major immunoglobulin of the secondary immune response* 3. The only immunoglobulin that crosses the placenta, and therefore the major protective immunoglobulin in the neonate. Most maternally transmitted IgG has disappeared by 6 months† 4. Opsonization, toxin neutralization and agglutination. Coats cells prior to killing by killer cells. Activates complement via classical pathway
IgA	α	160 000 370 000 (dimer and secretory form)	13	1.4–4 g/l	1. Principal immunoglobulin in secretions of respiratory and gastrointestinal tract and in sweat, saliva, tears and colostrum. Key defence role for mucosal surfaces 2. Polymerizes to a dimer intracellularly by binding through a cysteine-rich polypeptide (J-chain), synthesized locally by submucosal cells 3. Secreted through epithelia as the dimer bound to a secretory transport piece, synthesized locally by epithelial cells 4. When aggregated binds polymorphs and activates complement by the alternative pathway
IgM	μ	900 000 (pentamer)	6	0.5–2 g/l	1. Macroglobulin made up of five monomeric immunoglobulin subunits linked by a J-chain 2. Mainly intravascular 3. Principal immunoglobulin of the primary immune response 4. Does not cross the placenta. Fetal production of high levels of specific IgM in intrauterine infection may be of diagnostic significance, e.g. rubella 5. Agglutinates and opsonizes particulate antigens. Activates complement via the classical pathway. Blood group antibodies: IgM

* **Secondary antibody response characterized by:** 1. lowering of the threshold of immunogen; 2. shortening of the lag phase; 3. a higher rate of antibody production; 4. longer persistence of antibody production.

†**Transient disease in the newborn caused by maternal IgG:** rhesus incompatibility, autoimmune thrombocytopenia, thyrotoxicosis, myasthenia gravis, lupus erythematosus.

Table 3.9 (*Cont'd*)

Ig class	*Heavy chains*	*Molecular weight*	*% total Ig level*	*Normal plasma level*	*Function*
IgD	δ	170 000 (monomer)	0.1	4–40 mg/l	1. Precise functions are unknown 2. Nearly all immunoglobulin is present as cell surface receptor on human B cells and may be involved in B-cell activation
IgE	ε	185 000	0.002	0.1–1.3 mg/l	1. Immediate hypersensitivity reactions: binds to mast cells and basophils via its Fc fragment, which degranulates and releases biologically active mediators, e.g. histamine, when exposed to the appropriate antigen. Possibly of benefit in controlling certain parasitic infections 2. Serum levels correlate with severity of asthma 3. Activates various cells involved in allergic and inflammatory disease, which are activated by 2 main types of cell surface receptor: the FESR1 receptor on mast cells, basophils and eosinophils, and the FqSR2 receptor on lymphocytes. Receptor blocking monoclonal antibodies have been developed as possible asthma therapies

Table 3.10 Summary of reactions of various immunoglobulins

Reaction	*IgG*	*IgA*	*IgM*
Agglutination	+	+	++
Precipitation	+	+	+
Virus neutralization	+	+	+
Complement fixation	+++	–	+
Complement-dependent lysis	+	–	+++
Immune complex	+	–	+

STRUCTURE OF IMMUNOGLOBULIN MOLECULE (Fig. 3.9)

- An immunoglobulin molecule is a 4-polypeptide chain structure with two heavy and two light chains linked covalently by disulphide bonds.
- Treatment of the antibody unit with papain produces:
  - Two identical univalent *antigen-binding fragments (Fab),* each containing one antigen-binding site, and
  - One *crystallizable fragment (Fc),* which contains sites for complement fixation, reactivity with rheumatoid factors, skin and macrophage fixation and regulation of catabolism.
- *Light chains*
  - Molecular weight of approximately 23 000.
  - Two types: kappa (κ) and lambda (λ). Each immunoglobulin molecule has either two κ or two λ chains.
- *Heavy chains*
  - Molecular weight of about twice that of light chains (i.e. 50 000–75 000) and twice the number of amino acids.
  - Five classes of immunoglobulin are recognized on the basis of the Fc fragment of the heavy chain, i.e. heavy chain isotypes IgG, IgA, IgM, IgD and IgE. Heavy chain classes are also divided into subclasses of molecules, e.g. IgG1, IgG2, etc.

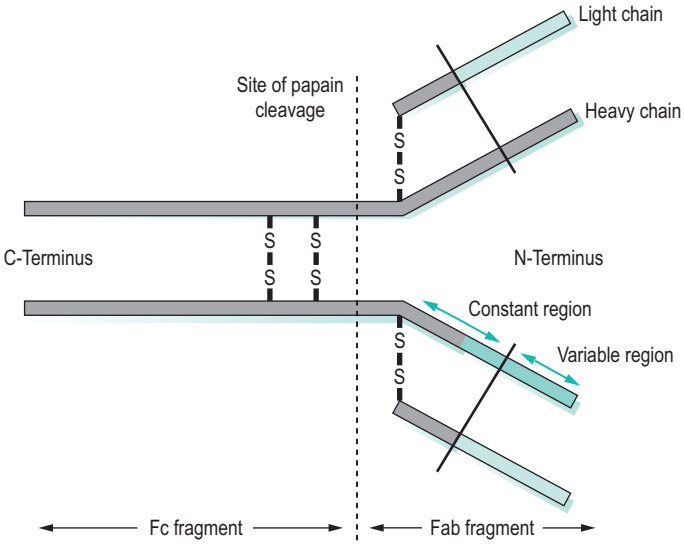


Fig. 3.9 Structure of the immunoglobulin molecule.
Fc = crystallizable fragment, Fab = antigen-binding fragment.

- Both heavy and light chains consist of two regions (Table 3.11):
  1. A *constant region* (C_H and C_L), in which the amino acid sequence of immunoglobulins of the same class is more or less identical.
  2. A *variable region* (V_H and V_L) where the amino acid sequence varies considerably from molecule to molecule and contributes to the antigen-binding site.

Table 3.11 Antigenic determinants on antibodies

Epitope class	*Location*	*Comment on epitope*
Isotype	Constant region	5 human isotopes are IgA, IgD, IgE, IgG, IgM. Each class of Ig heavy chains are identical in all members of a species
Allotype	Constant region	Vary among individuals of the same species. IgG exhibits the most allotypic difference
Idiotype	Variable region	Differ among antibodies with different antigen-binding specificities. Monoclonal antibodies have the same idiotype. Anti-idiotypic antibodies will resemble the original antigenic determinant group

Development and activation of B cells

- Direct B cell/T_H cell interaction and cytokines secreted by T_H cells are required for B cells to respond to most antigens.
- Stimulation of B cells by protein antigens induces generation of memory B cells and antibody-secreting plasma cells.
- During this clonal expansion and differentiation, the antibody affinity for antigen may change (affinity maturation), and the biological activities of the antibody can change (isotype class switching).

CLINICAL CONSIDERATIONS (see Table 3.12)

Table 3.12 Immunoglobulin products for replacement therapy

Product	*Indications*
Immunoglobulin replacement therapy (pooled from normal humans)	Primary immunodeficiency
High-dose immunoglobulin (pooled from normal humans)	Immunosuppressive effects used in autoimmunity
Anti-D (pooled from women with high levels of anti-D)	Prevention of haemolytic disease of the newborn
Hyperimmune immunoglobulin (pooled from humans with high titres of antibodies)	Prevention of tetanus, rabies, varicella zoster and hepatitis B
Antivenom	Treatment of snake bite
Monoclonal antibodies (raised against specific human cells in mouse hybridomas)	Used as immunosuppressants and cancer treatment

PARAPROTEIN

- A homogeneous band of one immunoglobulin, usually IgG, IgM or IgA. Its presence implies proliferation of a single clone of cells.