Underwood: Chapter 7: Immunology and Immunopathology

Defence Against Infection

- Non-specific mechanisms include the skin barrier, lysozyme in some secretions, ciliary motion in respiratory tract, and colonisation by harmless bacteria

- Specific mechanisms are those of immunity

- Immunity is characterised by specificity and memory

Many *non-specific* defence mechanisms operate to prevent invasion of the body by micro-organisms. These include the epidermal covering of the body, the presence of lysozyme in tears and nasal secretions, gastric acidity, ciliary motion in the trachea and bronchi, and the colonisation of certain sites by non-pathogenic bacteria which prevent the multiplication of pathogens. *Immunity* is a term reserved for the *specific* response of the immune system.

Essential Features of the Immune System

The immune system has four essential features:

- specificity

- diversity

- memory

- recruitment of other defence systems.

Specificity

Immune responses in mammals have specificity for one particular antigen; usually there is no cross-reaction with other, closely related antigen, even when the chemical difference between the two antigens amounts to only a comparatively minor alterations in molecular structure.

Diversity

The immune system is likely to encounter many different antigens during the life of the individual. It therefore follows that it must have considerable diversity of response. This diversity is partly inherited and partly acquired during maturation of the immune system.

Memory

When an antigen reacts with a clone of immunologically competent cells with specificity for the antigen, there is expansion of the clone as well as adaptation of the cells to give the highest possible specificity for the antigen. During this process, memory cells are

generated so that, if the antigen is introduced a second time, the immune response will be more rapid and specific. This is the basis for all active immunisation procedures.

Recruitment of Other Defence Systems

Recognition of foreign material by the immune system does not, by itself, usually result in destruction of the material. The cells of the immune system release chemical messengers (such as lymphokines) which recruit and activate other cells (such as polymorphs, macrophages and mast cells) or chemical systems (such as complement, the amines, kinins, and lysosomal enzymes) to destroy the foreign material.

Antigens

An antigen is any substance capable of inducing an immune response (in which case it is also called an *immunogen*); this response may include the formation of specific antibodies or primed T-cells. To be more precise, an antigen is also a substance which reacts with antibodies or primed T-cells irrespective of its ability to generate them. Most antigens are large molecules (of MW over 1000). Smaller molecules do not usually provoke an immune response unless bound to a large *carrier molecule. The smallest topographical structure on the surface of a large molecule recognisable by the immune system is termed a hapten, epitope or antigenic determinant.*

The immune system can respond to an antigen in two ways:

- by a cell-mediated immunity (CMI)

- by humoral immunity (the production of antibodies).

Cellular Co-operation in the Immune Response

- Cell-mediated immunity is attributable to T-lymphocytes

- Humoral immunity is attributable to antibody (immunoglobulin) produced by plasma cells derived from B-lymphocytes

- T-lymphocytes can help and suppress B-lymphocyte activity

Both CMI and humoral immunity are dependent upon specifically responsive *lymphocytes* which recognise and react to the presented antigen. During development, lymphocytes become *committed,* that is, capable of recognising only one antigenic determinant. Thus, the presentation of one antigen stimulates only the relevant committed lymphocytes.

The lymphocytes responsible for CMI are called *T-lymphocytes* (thymus-dependent), because they or their precursors mature in the specialised environment of the thymus; from there they migrate out into the other lymphoid tissues. T-lymphocytes have antigen receptors on their surface and, on recognising an antigen, they proliferate to produce a clone of *specifically primed T-lymphocytes.*

The lymphocytes responsible for antibody production (humoral immunity) are called *B-lymphocytes.* This is because, in the chicken, they mature in a gut-associated organ called the *bursa of Fabricius.* There is no such structure in man, but the gut-associated lymphoid tissue is believed to perform a similar function. When stimulated by an antigen to which it is reactive, the B-lymphocyte proliferates to give a clone of cells all capable of reacting with the antigen. Some of these B-lymphocytes differentiate into *plasma cells* which are lymphocytes specially adapted for the manufacture and secretion of immunoglobulins.

Some of the specifically reactive T- or B-lymphocytes are long-lived and persist as *memory cells.* These cells are responsible for the increased rapidity and specificity of the *secondary immune response,* which occurs if the same antigen is encountered again.

Some T-cells co-operate with B-cells to assist them in the production of antibody: these are called *helper T-cells.* Others suppress the production of antibody and are called *suppressor T-cells;* these appear to exert a regulatory function over the immune system.

Humoral Immunity

- Immunoglobulins (Ig) are produced by plasma cells

- immunoglobulin molecules comprise light chains (either kappa or lambda) and heavy chains (gamma, mi, alpha, delta, epsilon)

- Molecules can be enzymatically separated into antigen-binding fragment (Fab) and crystallisable (Fc) fragment; some leucocytes have receptors for Fc

Antibodies are immunoglobulin (Ig) plasma proteins (sometimes called gamma globulins because of their mobility on a plasma electrophoresis strip). Their ultimate biological effect, by binding to antigen, includes:

- the lysis of bacteria

- neutralisation of toxins

- opsonisation of foreign material to promote engulfment by phagocytic cells

- antibody-dependent cell-mediated cytotoxicity (ADCC).

The word opsonisation is derived from a Greek word meaning 'to prepare for the table'; it is the surface coating of particles by complement to promote engulfment by phagocytic cells which have cell surface receptors for complement.

Antibody production

Antibodies are produced by plasma cells in the lymph nodes, bone marrow and spleen. The cells are ovoid in shape with an eccentrically placed nucleus with peripherally dispersed chromatin (clock-face nucleus). The cytoplasm is rather basophilic, and electron microscopy

shows it to contain abundant rough endoplasmic reticulum, correlating with its production of proteins for export. One plasma cell produces antibody reactive with only one antigen.

Any immunoglobulin consists of two pairs of identical polypeptide chains. The larger pair - the *heavy chains* - have about twice the molecular weight of the smaller pair - the *light chains.* If the molecule is digested by the enzyme papain, it cleaves into two Fab (fragment antigen-binding) fragments, which contain the antigen binding sites, and one Fc (fragment crystallisable) fragment. The Fc fragment of certain immunoglobulin clones has a role in complement activation.

There are five *classes* of immunoglobulin: IgG, IgM, IgA, IgD and IgE, characterised by the different structures of their heavy chains, which are called by the Greek letters gamma, mi, alpha, delta and epsilon respectively. There are only two types of light chains, called kappa or lambda and each Ig molecule has only one or the other type of light chain.

The antigen binding site of an Ig molecule is at the N-terminal end of the Fab polypeptide chains. This area has been shown, by amino acid sequencing, to contain hypervariable regions which account for the variations in tertiary structure of the antigen binding site, and the consequent range in specificities.

Immunoglobulin G

IgG is the most abundant immunoglobulin in the plasma and extracellular fluid. It can cross placenta, and is therefore important in the passive transfer of immunity to the fetus. It is capable of neutralising toxins and may be cytolytic through the activation of complement. Polymorphs and macrophages have surface receptors for the Fc fragment of IgG; thus binding of IgG to particulate antigen promotes adhesion of these cells and subsequent phagocytosis of the antigen.

Immunoglobulin A

IgA is secreted locally by plasma cells in the respiratory passages, salivary and lacrimal glands and the intestinal mucosa. It is an important constituent of breast milk. It is secreted as a dimer of two Ig molecules joined by a J (junction) chain. Coupled to a 'transport piece', it is secreted at these sites, where it has a local defensive function. It can activate complement by the alternative pathway.

Immunoglobulin M

IgM is formed by J chains into pentamers of Ig molecules and these attain the very high molecular weight of 900.000. The large molecular size prevents it from leaving the plasma, except when permitted by increased vascular permeability in inflammatory lesions. As it has ten antigen-combining sites, it has good agglutinating and complement-fixating properties. It is the first class of antibody to be formed in immune responses, and is characteristic of the primary immune response (first encounter with antigen); IgG is typically produced in large amounts in the secondary immune response.

Immunoglobulin E

IgE binds selectively to mast cells and to basophils by its Fc fragment. The binding of antigen to its Fab fragment triggers release of histamine and other substances important in anaphylactic type hypersensitivity.

Immunoglobulin D

The function of IgD is largely unknown, but it may act as an antigen receptor on the lymphocyte surface.

Cell-Mediated Immunity

- T-lymphocytes are dependent upon the thymus gland for their development

- Helper T-lymphocytes recognise foreign antigens, secrete lymphokines, and help B-lymphocytes to produce a humoral response

- Suppressor/cytotoxic T-lymphocytes suppress B-lymphocytes and also destroy virus-infected cells

Cell-mediated immunity (CMI) is immunity dependent on the production of specifically primed immune cells and their actions.

T-lymphocytes

In the absence of T-lymphocytes, the host cannot mount cell-mediated immune responses; there may also be some impairment of humoral immunity.

Immature lymphocytes ('prothymocytes') reach the thymus via the blood, and mature in the specialised environment of the thymic epithelium into various types of T-lymphocytes. There is evidence that this differentiation is stimulated by various locally produced hormones, collectively termed 'thymic lymphopoietic hormone'. This process reaches its peak during fetal and early neonatal life; in later life the thymus atrophies, but still continues to export T-lymphocytes.

Originally, T-lymphocytes were identified by their ability, empirically, to form 'rosettes' in suspensions by binding to sheep red blood cells; B-lymphocytes were recognised by immunofluorescence techniques to detect their surface immunoglobulin. The availability of *monoclonal antibodies* to cell surface markers or antigens of lymphocytes has made it possible to distinguish not only T-lymphocytes from B-lymphocytes, but also between different subtypes of T-lymphocyte. Usually, the monoclonal antibodies are applied to frozen sections of fresh (unfixed) tissue, and their binding detected by an enzymatic reaction.

There are two main subsets of T-lymphocytes which can be distinguished by their surface antigens using monoclonal antibodies, or by their functional activity:

- helper T-lymphocytes

- suppressor/cytotoxic T-lymphocytes.

Helper T-lymphocytes

Helper T-lymphocytes recognise foreign antigens in conjunction with class II HLA antigens. When stimulated by antigens, they proliferate and transform into T-immunoblasts, which eventually differentiate into small T-lymphocytes responsive to the antigen.

On encountering antigen, helper T-lymphocytes release chemical messengers called *lymphokines* which regulate the proliferation of other lymphocytes, cause an acute inflammatory reaction and immobilise macrophages in the area. They 'help' B-lymphocytes to produce a humoral (antibody) immune response by 'presenting' antigens to B-lymphocytes in such a way as to stimulate them, and by the release of chemical messengers. Helper T-lymphocytes may be recognised by certain specific cell-surface antigens, detected by monoclonal antibodies.

Suppressor/Cytotoxic T-lymphocytes

The other major subset of T-lymphocytes, recognised similarly by their cell-surface antigens, are called suppressor/cytotoxic T-lymphocytes. These T-lymphocytes have a *suppressive* effect on B-lymphocytes and have a central role in the prevention of autoimmune disease. A further activity of this subset of lymphocytes is recognition and destruction of cells infected by viruses which express viral-coded antigen on their surfaces - hence the term *cytotoxic* T-lymphocytes.

T-Cell Receptors and Immune Recognition

T-cell receptors for antigens each consist of two cross-linked chains (alpha and beta) on the cell surface. The alpha- and beta-chains each have a constant region near the cell membrane and an outer variable region. The variable region offers considerable diversity necessary for the recognition of a wide range of possible antigens.

The mechanism responsible for the expression of a wide range of permutations in the variable region of the receptor is gene rearrangement. The same mechanism is responsible for antibody diversity in B-cells; indeed, there is a significant degree of homology between the structure of T-cell receptors and immunoglobulin molecules.

Gene rearrangement involves the excision, splicing and post-transcriptional modification of segments of 4-gene regions:

- V or variable region
- C or constant region
- D or diversity region
- J or joining region.

This results in the synthesis of numerous V-D-J-C RNA molecules each encoding for T-cell receptor chains with different antigen specificities.

Macrophages

They have a central role in CMI. Once they have phagocytosed particulate antigens, they sometimes re-secrete the antigen or present it on their cell surface, thereby presenting antigens to other cells of the immune system. They thus have a similar role to that of Langerhans cells, the antigen-presenting cells of the epidermis.

Macrophages also act as effector cells of the immune response by phagocytosing micro-organisms, especially if these have been coated with IgG, or with IgM and the C3b component of the complement.

Changes in the Lymph Node During the Immune Response

The cellular interaction in the immune response take place in the lymph nodes, tonsils, gut-associated lymphoid tissues (Peyer's patches) and in the peri-arteriolar lymphoid sheaths and lymphoid nodules (Malpighian bodies) of the spleen.

Lymph enters the node via the afferent lymphatics to reach the marginal sinus. Beneath the sinus is the cortex, which contains nodules of tightly packed lymphocytes called *follicles;* these contain proliferation foci called *germinal centres.* The follicles contain mainly B-lymphocytes and outside the follicle there is a rim, or *mantle zone* of pre-follicular B-lymphocytes. Towards the *hilum* of the node there are *medullary cords* packed with plasma cells and lymphocytes; the intervening *sinuses* are lined by histiocytes.

When the node is subjected to antigenic stimulation, the histological changes reflect the type of immune response being mounted. In B-lymphocyte-mediated (humoral) immune responses, for example to bacterial antigens, the follicles show hyperplasia with mitoses occurring in their germinal centres. In T-lymphocyte-mediated CMI, there is expansion of the paracortex, which contain many large T-cells. Drainage of particulate antigen into the node, and the presence of certain tumours in the zone of lymph drainage to the node, results in sinus histiocytosis, where the medullary sinuses become packed by histiocytes.

The Complement System

- Complement is a group of substances functioning as an enzymatic cascade

- Cascade may be activated by immunoglobulins (classical pathway) or by bacteria (alternative pathway)

- End products of the cascade are chemotactic for leucocytes, enhance phagocytosis, and lyse cell membranes.

The proteins of the complement system are mostly synthesised in the liver, and comprise about 10% of the total plasma protein. The protein components of the system are termed C1, 4, 2, 3, 5, 6, 7, 8, 9. C4 occupies second place in the sequence owing to the

anomalous order in which the components were discovered and named. C1 is a complex of three components; they function as an enzymatic cascade which can be activated at two main points along its sequence, but with common end results (the common pathway).

The *classical pathway* is activated when IgG or IgM antibodies combine with antigen. This causes activation of C1 to act-C1. One enzyme molecule can cleave many molecules of substrate, so there is amplification of the system. However, many of the activated components are destroyed spontaneously.

The *alternative pathway* is activated by lipopolysaccharide cell wall constituents (endotoxins) or by IgA immune complexes. Inhibitory factors such as C1-INH, and factors H and I are important in controlling activation of this pathway.

The biological activities of complement are mediated by the free products of the cascades and by the end product C5b6789.

Abnormalities of the Complement System

Deficiency of complement illustrates the importance of the system in defence against disease. For example, defects in the classical pathway result in frequent infections. Deficiency of the late components of the sequence, such as C5-8, results in disseminated infections with Neisseria organisms. Hereditary deficiency of C1-INH causes hereditary angio-oedema. Complement is also important in the clearance of immune complexes, and defects of components of the classical pathway result in immune complex glomerulonephritis.

Hypersensitivity Reactions

- Severe and harmful immunological reactions

- Type I: binding of antigen to IgE antibody on surface of mast cells causes release of histamine, etc.

- Type II: antigen on cell (i.e., bacteria) surface binds antibody, prompting lysis by either defensive cells or end products of complement cascade

- Type III: antigen combines with antibody to form immune complexes which activate complement

- Type IV: T-lymphocytes produce a delayed (> 12 hours) response to antigen

- Stimulatory hypersensitivity stimulates activity of target cell (i.e., effect of LATS on thyroid in Graves' disease).

Hypersensitivity is a state of altered immunological responsiveness in which an excessively severe and harmful immune reaction occurs on exposure to an antigen. The reaction is brought about through either humoral immunity (antibodies) or CMI (sensitised T-lymphocytes); products of the reaction lead to lesions which can range from local inflammation to generalised shock, including possibly fatal circulatory collapse.

In many instances, hypersensitivity reactions are provoked by foreign antigens such as pollens, moulds, food substances and drugs. However, in some instances, the offending antigen is a bodily constituent; hypersensitivity to antigens of the host's own body is known as *autoimmune disease.* Transplant rejection is a special instance of hypersensitivity.

Type I (Anaphylactic or 'Immediate Type') Hypersensitivity

Type I is an immediate immune reaction, occurring within minutes of exposure to the causative antigen.

The commonest examples are hay fever, childhood eczema and extrinsic asthma. The tendency to develop this type of reaction is found in about one-tenth of the population and is termed *atopy.* For example, in hay fever, the commonest manifestation of atopy, exposure to minute quantities of allergens such as grass pollen leads to acute inflammation in the conjunctival and nasal mucosae. Besides these local reactions, in rare instances, entry of traces of an allergen into the body causes acute systemic anaphylaxis characterised by circulatory collapse, dyspnoea and convulsions, sometimes leading to death. The allergen may be a systemically administered drug, such as penicillin, or radiological contrast media, or the venom of a snake or insect.

Diagnosis

It is made by the demonstration of a relationship between exposure to a particular environmental antigen and onset of symptoms in a carefully taken clinical history. Provocation tests may be performed such as skin tests.

Further tests may include the measurement of serum IgE levels, which are elevated in atopy, and the radio-allergo-sorbent test (RAST). This is a type of radioimmunoassay, in which the levels of IgE class antibodies to suspected allergens can be measured. Such in vitro tests avoid the risks associated with the in vivo provocation tests.

Mechanism

IgE class antibodies with affinity specific for the provoking allergen, sometimes known as *reaginic antibodies* or *reagin,* are central to type I hypersensitivity. If blood from an atopic donor is transfused into a non-atopic recipient, the recipient may transiently develop the full range of hypersensitivities which the donor displayed.

The reaginic IgE binds by its Fc component to mast cells and basophil leucocytes, which have specific surface receptors for this class of immunoglobulin. Both cell types contain basophilic cytoplasmic granules which consist of stored histamine and other vasoactive compounds. These cells are commonly situated close to small blood vessels. Basophil leucocytes differ from mast cells by being motile.

The binding of relevant antigen of the IgE molecules attached to the cell surface causes cross-linking of the IgE molecules, which appears to be the stimulus to the cell to release its stored granules. These intracellular events are triggered by a rise in the intracellular messenger, cyclic-AMP (cAMP).

The mast cell degranulation reaction results in local release of the vasoactive compounds stored in the granules:

- histamine

- eosinophil chemotactic factors.

Other compounds, the 'slow reacting substances of anaphylaxis', are synthesised by the cell and also released:

- prostaglandins

- leukotrienes

- thromboxanes

- platelet activation factors.

The combined effect of these compounds is to produce the vasodilatation, increased vascular permeability, oedema and ingress of eosinophils typical of the local atopic response. In the case of asthma, they also cause hypersecretion by the bronchial mucous glands and bronchospasm.

While neutrophil polymorphs are the chief infiltrating cells of acute inflammation, eosinophil polymorphs are characteristic of atopic reactions. While tissue damage, and even necrosis, may be seen in acute inflammation, these are not a feature of the atopic response.

Acute Systemic Anaphylaxis

This is the most serious manifestation of atopy. Entry of the allergen into the circulating plasma causes degranulation of IgE-coated basophils with release into the circulation of chemical mediators. Arachidonic acid metabolites are the most important chemical mediators in this setting. Mast cells may also be activated by entry of antigen into the tissues. Generalised peripheral vasodilatation causes hypotension with shock, while contraction of bronchial smooth muscle result in dyspnoea. The skin may show widespread urticarial reaction. Death may result from circulatory collapse.

Modification of the Atopic Response

Mast cells may be stabilised and prevented from degranulating by glucocorticoids and drugs, such as disodium cromoglycate. Beta-adrenergic agonists increase mast cell cAMP and so inhibit degranulation. Anti-histamine drugs may antagonise the products of sensitised mast cells. Occasionally, hyposensitisation is attempted.

Individual Susceptibility to Atopic Responses

Genetic factors must predispose to the atopic tendency since there is evidence that it is familial. It appears that the IgE responses are genetically determined. Further evidence for genetic determination of atopy comes from its association with certain HLA types.

There is some evidence that atopic individuals may have defective secretion of IgA on to the mucous membranes. It is suggested that this may allow ingress of environmental antigen to cause hypersensitivity.

Regulation of the immune response by T-cells may be important in preventing atopy. For example, children with the rare inherited T-cell defect in Wiskott-Aldrich syndrome frequently become atopic.

Type II (Cytotoxic) Hypersensitivity

The characteristic feature is damage to cells by binding of specific antibodies to antigens on the cell surface.

Cytotoxic Antibodies to Blood Cells

The best known example of cytotoxic antibody development to blood cells is *autoimmune haemolytic anaemia,* in which auto-antibodies of the IgG or IgM class develop to antigens on the red cell surface. Such antibodies may be detected in the Coombs' (antiglobulin) test. Once coated by IgG, red cells become bound to macrophages because they have receptors for the Fc fragments of immunoglobulins. This result in phagocytic destruction of the red cells. IgM antibodies, which are powerful agglutinins, may agglutinate red cells in the red pulp of the spleen, resulting in cellular destruction. IgM may also activate complement, resulting in cellular lysis or promoting binding to macrophages.

In *idiopathic thrombocytopenic purpura,* antibodies develop to surface antigens on the platelets, resulting in their destruction, especially in the spleen.

In many instances, cytotoxic auto-antibodies to surface antigens on red cells are produced spontaneously. However, sometimes a drug or its metabolite may bind firmly to the cells surface to give a highly immunogenic epitope. For example, a metabolite of benzyl penicillin binds to the red cell membrane, and some individuals develop cytotoxic IgG class antibodies to this, resulting in the destruction of red cells, especially in the spleen. The antihypertensive drug alpha-methyldopa occasionally promoted the development of cytotoxic auto-antibodies to rhesus blood group antigens.

Naturally occurring antibodies to blood group antigens A and B are of IgM class.

Rhesus System

It is generally possible to prevent Rh-negative mothers from developing Rh antibodies by injecting them with Rh antibody within 48 hours of the birth of an Rh-incompatible child.

These probably serve to destroy any fetal red cells which enter her circulation before they can stimulate antibody production.

Auto-Antibodies to Other Tissues

In *Goodpasture's syndrome,* an auto-antibody develops to both glomerular capillary basement membrane, and that of alveolar capillaries in the lung. This results in local complement activation at these sites, causing pulmonary haemorrhages and glomerulonephritis.

In *myasthenia gravis,* antibodies develop to acetyl-choline receptors on skeletal muscle causing weakness.

An example of the development of auto-antibodies to an intracellular antigen is *systemic lupus erythematosus (SLE),* in which antibodies develop to a range of antigens found in the nucleus. This is one of the so-called non-organ-specific autoimmune diseases.

In some instances, the binding of auto-antibody of IgG class to cell surface antigen is believed to stimulate certain lymphocytes with cytotoxic properties, sometimes called K-cells, to destroy the sensitised cell. This mechanism, called antibody-dependent lymphocyte cytotoxicity, may be involved in Hashimoto's thyroiditis.

Type III (Immune Complex) Hypersensitivity

It is due to the formation of antigen-antibody complexes, which may activate complement and hence produce tissue injury.

Immune complexes result from the reaction of antibody, usually IgG or IgM class, with antigen, with subsequent activation of complement.

Persistent infection. In infective endocarditis due to alpha-haemolytic streptococci, malaria, or viral hepatitis, immune complexes are formed which contain antigens from the infecting organism. These immune complexes may become deposited in the tissues.

Autoimmune disease. Sometimes, the offending antigen is a self-antigen, as in SLE, where immune complexes of various nuclear antigens with IgG may be formed in large amounts, exceeding the capacity of the mononuclear phagocytic system to dispose of them.

Extrinsic disease. Immune complexes may be formed locally at the point of entry of an environmental antigen into the body, most commonly in the lung as in extrinsic allergic alveolitis. Farmers sometimes develop IgG antibodies to inhaled spores from moulds growing in hay. Subsequent inhalation of the spores results in immune complex formation in the alveolar walls, resulting in an acute inflammatory response called 'farmer's lung'. This response is different from extrinsic asthma, which involves IgE bound to mast cells.

Experimental Immune Complex Disease

Nicholas Maurice Arthus developed an experimental model for local immune complex disease in 1902. Animals were repeatedly injected with doses of foreign antigen until they developed high levels of IgG class antibodies. Further subcutaneous injection of the antigen resulted in severe oedema and haemorrhage at the injection site.

Histologically, there is margination and emigration of neutrophil polymorphs, platelet aggregation and vascular thrombosis. Immune complex deposition in the walls of venules results in activation of complement, yielding reaction products such as C3a and C5a which are known as *anaphylatoxins.* These cause acute inflammation, are chemotactic for neutrophils, and release histamine from mast cells. The neutrophil polymorphs, in phagocytosing the immune complexes, release their lysosomal enzymes, resulting in tissue damage.

A similar response, the *Arthus reaction,* was seen in man when antisera raised in animals were injected to neutralise the toxins of bacteria such as tetanus and diphtheria. The animal proteins were highly immunogenic, resulting in an IgG antibody response. This reaction is now rarely seen because human antisera have largely replaced those raised in animals.

Disease due to circulating immune complexes may be produced in animals by the single injection of a large amount of antigen. At first, antigen is present in excess over the concentration of antibody. This results in the formation of small, soluble immune complexes which are not easily phagocytosed and which persist in the circulation. As the concentration of free antigen continues to fall and the concentration of antibody rises, the stage is reached where immune complexes are formed 'at equivalence' (at the same molar concentration of both antigen and antibody). These complexes are large and readily phagocytosed, being cleared away by the RES. However, soluble immune complexes, still present in circulation, result in complement activation and an increase in vascular permeability, and may be deposited in the walls of blood vessels, especially in the glomerular basement membrane, resulting in glomerulonephritis.

Clinical Immune Complex Disease

The commonest antigenic causes of immune complex disease are microbial antigens, and self-antigens in autoimmune diseases. A sore throat due to beta-haemolytic streptococci of certain types may be followed by deposition in the glomerular basement membrane of immune complexes containing streptococci antigens, causing post-streptococcal acute diffuse proliferative glomerulonephritis. A similar effect may be seen in chronic infections, such as malaria, syphilis and leprosy. Drugs may also be immunogenic, causing similar renal damage through immune complex deposition.

Polyarteritis nodosa is believed to be due to immune complex deposition in the arterial wall; in a small proportion of cases the offending antigen is hepatitis B surface antigen.

In rheumatoid arthritis IgM antibodies develop to the Fc fragment of IgG, especially when it is already bound to antigen. The resulting immune complexes may cause local tissue

injury by activating complement and, rarely, vasculitis. SLE in immune complexes between antibodies and various antigens from the cell nucleus may cause damage to various tissues, notably the kidney and skin.

Type IV (Delayed Type) Hypersensitivity

The common factor shared by delayed type hypersensitivity (DTH) responses is involvement of specifically primed T-lymphocytes, and that humoral immunity (the antibody response) is not involved.

DTH is involved in the normal cell-mediated immune response to viruses, fungi and certain bacteria, notably mycobacteria. Local tissue damage is the unwanted side-effect of this otherwise protective immune response.

DTH responses may also develop to transplanted organs, to self-antigens which have become altered by foreign haptens and to various environmental antigens. All of these are unwanted effects of the immune response. However, there is evidence that T-lymphocyte-mediated immune responses may also occur to antigens on the surface of tumour cells. The extent to which such responses may be useful in combating tumour growth is the subject of intensive investigation.

Histological Features

The best-known example of DTH is the tuberculin reaction. If a small amount of purified protein derivative (PPD) of tubercle bacilli is injected intradermally (Mantoux or Heaf test) in non-immune individuals, there is no effect. However, in individuals with cell-mediated immunity to tubercle bacilli, either as a result of previous tuberculous infection or to immunisation with BCG (bacille Calmette-Guerin, a live, but non-virulent, strain of *Mycobacterium bovis*), an area of reddening and induration develops after 12-24 hours.

Histologically, the dermis of the reaction site shows accumulation of lymphocytes and macrophages around small blood vessels, and oedema and vascular dilatation. In contrast to the situation in the acute inflammatory response, polymorphs are rarely seen. In some naturally occurring DTH responses, notably those to mycobacteria and some fungi, macrophages may undergo terminal differentiation into epithelioid cells (characteristic of granulomatous inflammation) or to multinucleate giant cells.

Granulomatous Hypersensitivity

It is the form of T-cell-mediated immunity most likely to produce disease. The usual cause is the ingestion by macrophages of antigenic materials which they are unable to destroy. Examples include mycobacteria such as *M. tuberculosis and M. leprae,* inorganic antigens such as zirconium, inert minerals such as silica, and locally produced immune complexes in extrinsic alveolitis. In the systemic granulomatous disease, sarcoidosis, the stimulus to granuloma formation is unknown.

A *granuloma* is defined as a collection of epithelioid histiocytes. These epithelioid cells are larger than their parent macrophages and have eosinophilic cytoplasm. Electron

microscopy shows them to have increased endoplasmic reticulum but few phagolysosomes, unlike activated macrophages. The reason for this adaptation is not understood. In addition to the formation of epithelioid cells, macrophages may differentiate into multinucleate giant cells. This is especially seen in the reaction to *M. tuberculosis,* where cells with a peripheral crescent of nuclei (Langhans' giant cells) form.

Mechanism

The interaction of specifically primed T-lymphocytes with antigen is central to the DTH response. It is not known how such primed T-cells leave the circulation to reach the site of antigen accumulation in the tissues. It may be that only a very small number of primed T-cells need to reach the antigen, perhaps through random circulation, to trigger the response. Generally, helper T-lymphocytes do not react with free antigen, recognising antigens only when presented in conjunction with class II HLA molecules. Such HLA molecules are present on the surface of the so-called *antigen-presenting cells* which include macrophages and a specialised cell with dendritic processes in the epidermis, called the *Langerhans' cell* (not to be confused with the Langhans' giant cell). This reaction stimulates the helper T-cells to secrete a range of compounds, collectively termed *lymphokines,* which activate cytotoxic and suppressor T-cells and recruit macrophages into the area.

There are other important chemical mediators of the DTH response in addition to the lymphokines; these include the many factors secreted by macrophages (*monokines*). One important monokine is interleukin 1, which promotes the release of the acute phase reactants by the liver, increases the proliferation of T-cells and acts on the hypothalamic thermoregulatory centre to induce fever. It is thus responsible for some of the systemic symptoms of DTH.

Contact Dermatitis

It is a specific example of DTH due to haptens.

Relatively simple chemicals may be absorbed by the skin and, acting as a hapten-protein complex, stimulate a cell-mediated immune response via the Langerhans' cells. Chemicals known to do this include nickel (present in jewellery and clothing-fasteners), chromium salts, formaldehyde, cyanoacrylate adhesives, photographic developers, and substances from the primula plant. Subsequent exposure to the antigen induces lymphocytic infiltration around dermal blood vessels, together with dermal and epidermal oedema leading to vesicle formation.

Stimulatory Hypersensitivity

There is only one example in clinical medicine. In the autoimmune disease Graves' thyroiditis an IgG class auto-antibody is formed to thyroid epithelial cells. The binding of this antibody, 'long-acting thyroid stimulator' (LATS), has a similar effect on the thyroid epithelial cells to that of the binding of thyroid stimulating hormone to its receptor: the cells is activated into secreting thyroxine. Patients develop thyrotoxicosis. The binding of an auto-antibody to cell-surface antigens is the same mechanism as that of type II hypersensitivity, but in the case of Graves' thyroiditis this has, paradoxically, a stimulatory rather than cytotoxic effect.

Hypersensitivity and the Normal Immune Response

Autoimmune Disease

- Tissue damage due to immune reactions with self-antigens

- May be humoral or cell-mediated

- Examples include Hashimoto's disease of the thyroid, rheumatoid disease, and some haemolytic anaemias

- Female preponderance

In the normal individual, however, although recognition of self-antigens by clones of lymphocytes does, occur, a harmful autoimmune response is kept at bay by active control mechanisms within the immune system.

Auto-Antibodies

Auto-immunisation appears to occur quite commonly, since many individuals have low concentrations of circulating antibodies to the DNA of nuclei, to gastric parietal cells and to thyroglobulin. However, high concentrations of such auto-antibodies are strongly associated with clinical disease. For example, patients with high concentrations of antibodies to thyroglobulin and to thyroid epithelial cells commonly have hypothyroidism due to Hashimoto's thyroiditis.

In some cases, microbial infection tricks the immune system into producing antibodies which cross-react with self-antigens. For example, *Streptococcus pyogenes* contains certain antigens which are similar to antigens in the normal myocardium. Thus defensive antibodies raised to infecting streptococci may cross react with the myocardium causing rheumatic fever.

Cell-Mediated Immunity

It appears that cell-mediated immunity may be involved in the tissue damage in diseases such as *juvenile-onset diabetes mellitus,* in which lymphocytes accumulate in the islets of Langerhans at the onset of the disease.

The Range of Autoimmune Disease

They may be *organ-specific, non-organ specific and connective tissue disease.*

Organ-Specific Autoimmune Disease

In organ-specific autoimmune conditions, the target tissue is destroyed by type II (cytotoxic) hypersensitivity, or antibody-dependent lymphocyte toxicity. The affected tissue shows eventual loss of the target cells, fibrosis, and infiltration by lymphocytes and plasma cells. Some of the plasma cells are producing the auto-antibody locally. The best-known example of such destruction is *Hashimoto's thyroiditis.* The thyroid is the site of two related

autoimmune diseases: *Graves' thyroiditis*, in which stimulatory type hypersensitivity leads to thyrotoxicosis, may occasionally evolve into *Hashimoto's thyroiditis.*

Addison described two autoimmune diseases before the aetiology of either was known. In *Addisonian pernicious anaemia*, antibodies develop to an antigen associated with the microvilli of the canalicular system of gastric parietal cells and to intrinsic factor itself. The result is achlorhydria caused by chronic gastritis due to parietal cell destruction, with failure to absorb vitamin B_{12} in the absence of intrinsic factor.

Two notable features of the organ-specific autoimmune diseases are:

- many of them affect endocrine tissues

- there is a tendency of the diseases to be associated with each other; thus, patients with pernicious anaemia very commonly also have autoimmune thyroid disease.

It has been suggested the *Type I (juvenile onset) diabetes mellitus may follow Coxsackie or mumps viral infection.*

Non-Organ-Specific Autoimmune Diseases: 'Connective Tissue' Diseases

Rheumatoid Disease

Rheumatoid disease is the commonest 'connective tissue' disease and an important cause of disability. Although its main feature is a destructive polyarthritis characterised by infiltration of the synovium by lymphocytes, plasma cells and macrophages, the disease has multisystem effects.

The serum of most patients contains rheumatoid factors, immunoglobulins (normally IgM, but sometimes IgG) which react with the Fc fragment of the host's own IgG once it has bound to antigen. These are assayed in the *'latex test'*, which measures the agglutinating titre of patient's serum for latex beads coated with IgG. This replaces the Rose-Waaler test, in which sheep red cells coated with IgG were agglutinated.

The rheumatoid factors thus bind to IgG, forming immune complexes which are formed locally in, or deposited in, the synovium. This gives rise to a type III hypersensitivity response with complement activation, resulting in tissue damage. Deposition of the immune complexes at other sites (for example, arterial walls) accounts for some of the systemic effects of the disease. There is also evidence that cell-mediated immunity may be involved in rheumatoid disease.

An important systemic complication of the continuing inflammation occurring in this disease is the development of secondary amyloidosis, which commonly leads to renal failure.

Systemic Lupus Erythematosus (SLE)

The multisystem disease, SLE, causes lesion in the skin, joints, renal glomeruli, blood vessel walls and other sites. It is characterised by the development of auto-antibodies known

as *antinuclear antibodies.* These react with various constituents of the nucleus, including DNA. The antibodies are not cytotoxic, and probably do not damage normal cells. However, when cells break down, nuclear antigens are released and these form circulating immune complexes (type III hypersensitivity) with the autoantibodies. The deposition of these immune complexes in small blood vessel walls, especially near the renal glomerular basement membrane, accounts for the diverse effects of the disease. Auto-antibodies to DNA and other nuclear antigens are usually detected in the indirect immunofluorescent test.

Other Autoimmune Diseases

There is evidence that the bile duct destruction characteristic of primary biliary cirrhosis is caused by autoimmunity, while antibodies to constituents of hepatocytes are found in the lupoid type of chronic active hepatitis and in progressing alcoholic cirrhosis.

In some diseases, auto-antibodies develop to extracellular antigen: for example, in bullous pemphigoid, antibody develops to the epidermal basement membrane.

Aetiopathogenesis of Autoimmune Disease

Autoimmune disease may arise for a number of different reasons:

- defective suppression mechanisms

- increased or abnormal antigenic stimulation

- enhanced immunogenicity of self-antigens

- antigen presentation by class II HLA molecules

- HLA antigen type.

Defective Suppression Mechanisms

Tolerance to self-antigens was originally thought to be brought about by destruction of clones of T- and B-cells reactive to 'self-antigens in the fetus - the 'forbidden clone' hypothesis. However, there is now evidence from in vitro experiments that such clones do exist, but are controlled by *active suppression mechanisms.* Central to these mechanisms are the *suppressor T-lymphocytes,* which appear capable both of recognising self-antigens, and of specifically suppressing helper T-lymphocytes and B-lymphocyte reactivity to self-antigens.

For example, in SLE there is evidence of thymic dysfunction, and suppressor T-cell activity has been shown to be defective. The association of myasthenia gravis with thymic hyperplasia and thymic tumours suggests some modifications in T-cell control mechanisms in this disease. In certain types of liver disease, such as primary biliary cirrhosis, anti-mitochondrial antibodies are commonly present in the serum, but the role of these in the pathogenesis of the disease is not known.

Abnormal Antigenic Stimulation

In other instances, the immune system appears to be normal, but subject to increased or abnormal antigenic stimulation. For example, antigens in certain sites (the 'immunologically privileged sites') appear to be hidden from the immune system. Such sites include the eye, parts of the CNS and the testis. Not only are there no clones of helper T-lymphocytes or B-lymphocytes reactive to antigens at these sites, but there are also no primed suppressor T-lymphocytes to identify antigens in these sites as self-antigens. Thus, for example, following a penetrating testicular injury or the rupture of an epididymal cyst, agglutinating auto-antibodies may develop to spermatozoa, resulting in sterility. Particularly serious are perforating injuries to the eye; not only is the injured eye under threat, but there may also be autoimmune destruction of the non-injured eye (sympathetic ophthalmitis).

Enhanced Immunogenicity of Self-Antigens

In some autoimmune diseases, self-antigens appear to have been modified or rendered more immunogenic by chemicals, such as drugs. An example is the antihypertensive drug, methyldopa, which stimulates antibody production to rhesus blood group antigens on red cells.

Antigen Presentation

The context in which antigens are presented to the immune system may be important in determining whether reactivity to the antigen develops. Helper T-cells can recognise antigens only in association with class II HLA molecules, and the majority of cells do not express these. There is now increasing evidence that cells of some target tissues in autoimmune diseases express class II HLA molecules (HLA-DR antigens) on their surface, thus enabling helper T-cells to respond to antigens in their vicinity.

HLA antigens

Certain HLA antigen types are associated with an increased risk of developing autoimmune disease. This could be because certain HLA antigens are themselves similar to various environmental antigens, exposure to which then results in autoimmunity to an HLA antigen. Alternatively, certain HLA haplotypes could be linked to genes regulating the immune response, so that the defects resulting in autoimmunity would be genetically determined.

Immunology of Organ Transplantation

- Rejection can be minimised by matching ABO blood groups and HLA antigens
- Class I HLA antigens (loci A, B and C) are expressed on all nucleated cells
- Class II HLA antigens (locus D) are expressed only on B-lymphocytes and antigen-presenting cells
- Graft rejection may be humoral or cell-mediated
- Grafted bone marrow may cause graft-versus-host disease if not matched

The basic immunology of organ transplant rejection was established in 1943 by Gibson and Medawar. Skin grafts between allogeneic (unrelated) mice were rejected 10-20 days after

grafting, while those transplanted between syngeneic animals (inbred strains) were permanently accepted. However, if mice were injected at birth with cells from an allogeneic animal, they were found to accept permanently skin grafts from that donor animal; in other words, immunological tolerance had been induced. If the tolerant animal was then injected with lymphocytes from a mouse which had previously rejected a graft from the allogeneic strain, the tolerance was overcome and the graft promptly rejected. Thus, the immune system displays memory and specificity in transplant rejection, and these attributes lie in the lymphocytes.

Tissue Typing and the HLA System

In humans, the *major histocompatibility complex (MHC)* is a series of genes on chromosome six which code for various antigens most readily detected on the surface of leukocytes, and hence called *human leukocyte antigens (HLA).* Closely linked to these genes is a family of genes controlling immune responses. All nucleated cells express HLA-A, -B and -C antigens, but HLA-D and -DR antigens (sometimes called class II HLA antigens) are normally expressed only on B-cells and antigen-presenting cells.

HLA antisera are obtained from recipients of previous blood transfusions or tissue transplants, or are produced artificially as monoclonal antibodies. The typing antibody and complement are mixed with cells of the individual to be typed. If these cells express the corresponding iso-antigen, they are lysed.

The rarity of heart, lung and liver donors means that cross-matching in these cases is restricted to the ABO blood group system. Failure to cross-match for blood group substances results in hyperacute graft rejection.

Functions of the HLA System

The existence of the MHC was discovered by observations on the fate of experimental organ grafts in laboratory animals. The human analogue, the HLA system, has been characterised by using antibodies in sera from transplantation and transfusion recipients. Although HLA-matching between donor and recipient is important to minimise the risk of graft rejection, the *natural function of the HLA system is to enable T-cells to interact more specifically with other host cells by a process known as dual recognition.* Class I and class II substances have different roles in this process.

Cytotoxic T-cells recognise and eliminate host cells bearing foreign antigens (e.g. viral products) and class I HLA substances (HLA-A, -B and -C) on their surfaces. Cytotoxic T-cells would be ineffective against free virus particles, but the dual recognition of adjacent viral antigen and class I HLA substances on the surface of virus-infected cells restricts the cytotoxic effects of T-cells.

Class II HLA substances (HLA-DR, -DP and -DQ) on antigen-presenting cells enable their recognition by helper T-cells which then interact with B-cells and plasma cells to induce specific antibody synthesis; class II HLA substances are present on antigen-presenting cells and B-cells. Class II HLA substances on antigen-presenting cells bearing foreign antigens protect them selectively from recognition and elimination by cytotoxic T-cells. This

requirement for the juxtaposition of foreign antigen and class II substances is another example of the importance of dual recognition in immune responses.

Graft Rejection

Different tissues vary in their ability to provoke an immune response from the recipient. For example, the bone marrow and skin appear to be highly immunogenic, while failure of liver transplantation is more often through technical problems than immunological reactions.

Hyperacute rejection, characterised by vascular thromboses, necrosis and polymorphonuclear infiltration, is rarely seen in clinical practice due to the practice of ABO cross-matching, but the other types of rejection are a major clinical problem. Accelerated rejection may be seen in sensitised patients who have received previous grafts, while acute and chronic rejection are primary immune responses to the donor antigens.

In the context of the kidney, rejection via cell-mediated immunity has two main histological components. There is interstitial infiltration by lymphocytes (many are T-cells) and macrophages, together with vascular damage including arterial intimal swelling and disruption of the internal elastic lamina.

The action of antibodies in transplant rejection is variable. In some instances (for example, hyperacute rejection) they are cytotoxic, while in others they may bind to HLA antigens, thus masking them from recognition by T-lymphocytes (graft enhancement). The factors which favour prolonged graft survival (taking renal transplants) are:

- ABO compatibility

- good class II HLA cross-match (especially identical sibling)

- previous blood transfusion (induces tolerance).

Factors impairing graft survival include:

- drug side-effects

- infection

- recurrence of the disease which originally necessitated transplantation.

Immunosuppression

Some form of immunosuppressive therapy is usually needed in all grafts other than autografts. Such therapeutic methods include: immunosuppressive drugs, including steroids and azathioprine; cytotoxic drugs, such as cyclophosphamide; lymphoid irradiation; and the relatively new drug, cyclosporin A. This drug is a major advance in immunosuppressive therapy because it shows selective cytotoxicity for antigen-primed T-cells, thus disrupting cell-mediated immunity against the graft.

Graft-Versus-Host Disease

The typical recipient of a bone marrow transplant has severe immunodeficiency, either as a consequence of the disease necessitating transplant (i.e., leukaemia), or as a result of cytotoxic drugs or radiotherapy. Transplanted bone marrow is immunocompetent, containing viable T-lymphocytes which may stimulate a severe, and sometimes fatal, immune response against the host's antigens - *'graft-versus-host' (GVH) disease.* The clinical features of GVH disease include diarrhoea due to malabsorption, a characteristic dermatitis, destruction of blood cells, and cholestatic liver disease.

The risk of GVH disease can be reduced by careful HLA cross-matching, and by selective destruction of T-lymphocytes in the graft using monoclonal antibodies.

Immune Response to Tumours

- Clinical importance of anti-tumour immune responses is controversial

- Lymphocytic infiltration of tumours is associated with better prognosis

- Tumour immunotherapy regimes include monoclonal antibodies, lymphokine activated killer cells, and interferon.

There is plentiful histological evidence to suggest that the host mounts some form of immune response to tumours. At the invasive edge of tumours, the surrounding stroma often contains an infiltrate of lymphocytes and macrophages. For example, medullary carcinoma of the breast is typically surrounded by masses of lymphocytes, seminoma of testis often contains may lymphocytes, and malignant melanomas of the skin are commonly surrounded by a cellular infiltrate. Ly-nodes draining tumours sites show a variety of tissue reactions suggestive of antigenic stimulation, including follicular hyperplasia and sinus histiocytosis.

Lymphocytic infiltration in human tumours has prognostic significance and may correlate with improved survival in carcinoma of the breast, stomach, colon and rectum. Some studies have shown a correlation between the presence of sinus histiocytosis in ly-nodes draining the breast, and a better prognosis. Less clear evidence for the role of the immune system in defending the host against tumours comes from well-documented reports of total or partial tumours regression. For example, in patients with disseminated malignant melanoma it is sometimes impossible to find the primary tumour, implying that it has regressed.

The advent of monoclonal antibodies, which enable different classes of lymphocytes to be demonstrated immunohistochemically in frozen tissue sections, has revolutionised the study of the host's cellular response to tumours. Cellular infiltrates around tumours often contain a predominance of T-lymphocytes, including the helper and suppressor/cytotoxic subsets. The natural killer (NK) cell, a class of lymphocyte which possesses the ability, at least in vitro, to kill tumour cells without prior antigenic stimulation, has been detected in these infiltrates. The in vivo function of NK cells is not known.

The presence of Langerhans' cells, the dendritic antigen-presenting cells of the normal epidermis, has been demonstrated in certain epithelial tumours. It is postulated that these cells

may be able to pick up tumour antigens and carry them to regional ly-nodes, presenting them, together with class II HLA antigens, to T-lymphocytes.

Are Tumour Cells Antigenic?

There is experimental evidence that *tumour cells express the HLČA and blood group antigens of the host,* although sometimes at reduced concentrations. However, a tumour cell can be recognised by the host as distinct from the normal tissues only if it expresses new antigens.

Several human tumours express the co called *oncofetal antigens.* These are immunologically detectable substances which are expressed by normal populations of cells in the developing fetus, but are not usually expressed by cells in the adult. The reappearance of these 'antigens' is an example of the 'cellular anarchy' of tumour cells. Examples include *alpha-fetoprotein,* which may be produced by hepatocellular carcinoma, and *carcino-embryonic antigen* which may be expressed by various adenocarcinomas, but there is no evidence that these induce a useful immune response in the host.

In experimental tumours in animals, a class of cell surface antigens called *tumour associated transplantation antigens (TATAs)* has been discovered. These cause rejection of transplanted tumour cells in a pre-immunised syngeneic host, and appear to be unique for the individual experimentally produced tumour. Antigens of this class may be the most important immunogens responsible for the host's immune response to tumour.

Immunological Surveillance Against Tumours

Macfarlane Burnet suggested that T-lymphocytes monitor the host's cells and react against any which have developed novel surface antigens. In this way, clones of potentially malignant cells would be destroyed. There is evidence favouring immunological surveillance in tumours. *Patients with congenital and acquired immunodeficiency states have a high risk of malignant tumour development, and the range of tumours is different from that seen in otherwise normal individuals. For example, renal transplant recipients, commonly treated by long-term immuno-suppression, have a greatly increased incidence of non-Hodgkin's lymphoma; this may arise in sites such as the brain, which is a most unusual site for such lesions in normal individuals. Hepatocellular carcinoma and various skin tumours also have an increased incidence.*

KS is an exceedingly rare tumour in the general population, yet commonly develops in patients with AIDS.

These findings could suggest that some normally operating surveillance mechanism, which destroys mutant clones of cells before spread can occur, has broken down. Some immunosuppressive agents, such as radiotherapy and the alkylating agents, are oncogenic in their own right.

Immunosuppressed patients are known to be prone to chronic viral infection, and some viruses (HBV, EBV, human papilloma and herpes) have proven oncogenic properties.

Tumour Evasion of the Host's Immune Response

Despite the many immune mechanisms known to be active against tumour cells, the natural history of most cancers, if not surgically ablated, is one of relentless progression culminating in death.

The immunogenicity of some tumours appears to be reduced by shedding or endocytosis of antigens, or masking them with mucin substances. Tumour antigens free in the circulation may block antigen recognition sites. Alternatively, antibodies to tumour antigens may bind to these antigens on the cell surface, masking them from T-cell receptors. Antigen-antibody complex bind to T-cells, blocking their tumour-recognition mechanisms. Alternatively, a growing tumour, by initially presenting only a very small antigen load, may induce tolerance to itself.

Immunotherapy

Early attempts at non-specific immunotherapy, such as the administration of BCG at a skin site draining to the axillary ly-nodes in patients with mammary carcinoma, have been disappointing.

Monoclonal Antibodies

The development of monoclonal antibodies offers exciting potential for specific immunotherapy. It is possible to generate large quantities of monoclonal antibodies to tumour antigens. Cytotoxic agents may be chemically bound to the antibodies, thus ensuring their delivery to the target cell.

Lymphokine-Activated Killer (LAK) Cell Therapy

In lymphokine-activated killer (LAK) cell therapy, an experimental treatment, patients are first subjected to leukapheresis (the white cells extracted from their blood using a special centrifuge), and the harvested lymphocytes are then cultured for several days with interleukin-2 (IL-2) to induce LAK cells. These autologous LAK cells are then returned to the patient intravenously together with high doses of IL-2. Early results suggest that reduction in apparent tumour bulk occurs with some renal cell carcinomas (hypernephromas), melanomas and colorectal tumours; sarcomas appear unresponsive.

Interferon Therapy

Most studies of interferon therapy to date have used interferon-alpha. The most encouraging responses have been with the rare, hairy cell leukaemia and with mycosis fungoides, a T-cell lymphoma of the skin. Responses have also been reported with renal cell carcinomas, melanomas, colorectal tumours, lymphomas and Kaposi's sarcoma. The mechanism of action is unknown but could include:

- direct antiproliferative action on tumour cells
- activation of natural killer cells and/or macrophages
- increased expression of class I HLA antigens on tumours cells.

Immunodeficiency

- Present with abnormal susceptibility to infections

- Primary deficiencies are due to congenital defects of B- or T-lymphocyte function or both

- Secondary deficiencies are due to causes such as malnutrition, immunosuppressive drugs, AIDS, etc.

Immunodeficiency presents clinically as an abnormal tendency to develop infections. Immunodeficiency should be suspected in any patient who develops infections by organisms which are not pathogenic in the normal individual ('opportunistic pathogens'). Such infections include *Pneumocystic carinii* pneumonia, cytomegalovirus infection, or infection by atypical mycobacteria. Organisms which normally cause only superficial infections may produce systemic infection in the immunocompromised, for example fungi such as *Candida, Aspergillus* and *Mucor*. Hence any unusually extensive infection by a common infective agent should be viewed with suspicion.

Immunodeficiency may be classified into two major types:

- deficiencies of non-specific resistance

- deficiencies in specific immune responsiveness.

Deficiencies of non-specific resistance include defects of neutrophil function, abnormalities of the complement system, and systemic diseases such as diabetes mellitus. In addition, there may be local impairment of resistance to infection in an otherwise normal patient, for example in a gangrenous limb.

Deficiencies in specific immune responsiveness are usually classified as:

- *primary immunodeficiencies,* which usually present in infancy and are often genetically mediated

- *secondary immunodeficiencies,* which are usually acquired later in life and are secondary to some other disease.

Primary Immunodeficiencies

Those are congenital immunodeficiencies not resulting from any other disease states.

Defects Mainly of B-Lymphocyte Function

The *Bruton type of agammaglobulinaemia* is the leading example of immunodeficiency due to defective B-lymphocyte function. It is transmitted as an *X-linked recessive* genetic defect and is first noticed in infancy in males. *Pre-B-cells fail to differentiate into B-*

lymphocytes. Germinal centres are absent from the ly-nodes, the tissues do not contain plasma cells, and B-lymphocytes are virtually absent from blood. Circulating levels of IgG, IgM and IgA are negligible. Immunisation procedures have no effect, and there are repeated severe infections with pyogenic bacteria once initial protection by placentally transferred maternal IgG has been lost. Pneumococcal and meningococcal septicaemias, alimentary infections by *Giardia lamblia* and various opportunistic infections occur commonly. While viral infections are less of a problem in defects of B-cell function, persistent hepatitis B virus infection can occur. Diagnosis is based upon demonstration of very low levels of serum IgG, IgM and IgA after making allowance for the IgG transferred across the placenta. Treatment is by lifelong injections of human IgG. Immunisation with live organisms (e.g. Sabin polio vaccine) is dangerous, as this may produce virulent disease.

A less serious form of B-cell deficiency is *transient hypogammaglobulinaemia;* this appears to be a delay in maturation of the B-cell system, as it is relatively common in premature infants of both sexes. immunoglobulin deficiency is usually limited to IgG, and the defect usually recovers by the age of 3 years.

One of the commonest selective immunodeficiencies is IgA deficiency. The lack of IgA in exocrine secretions exposes the patient to repetitive and persistent gastrointestinal infections. Paradoxically in an immune deficiency state, there is an association with allergies and autoimmune disorders.

Some patients with immunodeficiency have a reduced concentration of all classes of immunoglobulin. This is *common variable immunodeficiency*, a combined defect of T-helper cells and B-lymphocytes.

Defects Mainly of T-Lymphocyte Function

The leading example of defective T-lymphocyte function is the *Di George syndrome*, a rare defect in development of the third and fourth branchial arches resulting in an almost complete absence of the thymus and the parathyroid glands. The condition appears to arise sporadically, and there is no clear mode of inheritance. Apart from the hypocalcemic problems of hypoparathyroidism, affected infants have decreased circulating levels of lymphocytes, especially of T-lymphocytes. Ly-nodes show a selective lack of paracortical (T-cell) areas. Immunoglobulin levels are usually normal, but specific immunoglobulin production to some antigens is reduced, probably because of a lack of helper T-cell activity. Infection with pyogenic bacteria is not a feature of this condition. However, patients develop severe infections with fungi, viruses and opportunistic pathogens such as *Pneumocystis carinii;* cell-mediated immunity is known to be essential in defence against these agents. In its severest form, the condition is fatal, but transplantation of thymic tissue from a well cross-matched donor offers some hope of a cure.

Combined Defects of T- and B-Lymphocyte Function

The most serious example of a combined defect of T- and B-lymphocyte function is *severe combined immunodeficiency (SCID).* The thymus in SCID is hypoplastic, while the lymph nodes show defective germinal centres. Very few circulating lymphocytes of any type

can be found, circulating immunoglobulin levels may be nearly undetectable and cell-mediated immunity is greatly reduced. The condition may be inherited as an X-linked or an autosomal recessive disorder. Immunisation by live agents is likely to produce virulent disease. Death usually occurs in infancy from multiple infections, although bone marrow transplantation has effected cure in some cases.

In the rare condition, *ataxia telangiectasia* (inherited as an autosomal recessive gene), apart from the cerebellar ataxia and telangiectatic lesions of blood vessels which characterise the syndrome, there are combined defects of cell-mediated and humoral immunity.

Combined immunodeficiency is also seen in the *Wiskott-Aldrich syndrome,* an X-linked genetic defect in which there are platelet function defects, T-lymphocyte defects, and immunoglobulin deficiency, especially of IgA. Affected children show atopic eczema and recurrent infections, particularly otitis media.

Secondary Immunodeficiencies

Immunodeficiencies may be acquired secondary to various disease processes or drug effects. Examples include:

- protein deficiency

- haematological malignancy

- acute infection

- chronic renal failure

- immunosuppressive drug therapy, cancer therapy

- splenectomy

The commonest cause of secondary immunodeficiency worldwide is *protein deficiency* due to malnutrition, which causes defects in cell-mediated immunity. Similar effects may be seen in the cachexic state of disseminated cancer, where defects of both T- and B-cell function may be observed.

The *haematological malignancies* such as leukaemias and lymphomas cause severe acquired immunodeficiency states, because the normal cell populations of the marrow and ly-nodes are replaced by neoplastic cells which do not function normally.

Acute viral infections may depress immunological responsiveness: for example patients with infectious mononucleosis due to Epstein-Barr virus are prone to develop other infections. Similarly, overwhelming *bacterial infections* may disturb immune functions. An example is the reactivation of cold sores (due to Herpes simplex type I virus) during pneumococcal pneumonia.

Patients with *chronic renal failure,* even when treated by regular dialysis, develop combined acquired immunodeficiencies, probably due to toxic effects of accumulated metabolites.

In Western countries, many cases of acquired immunodeficiency are *iatrogenic,* for example due to steroid or other immunosuppressive drug therapy following organ transplantation, or following cytotoxic or radiotherapy for treatment of malignant disease.

Splenectomy, which is sometimes carried out as a staging procedure for Hodgkin's lymphoma or following traumatic splenic rupture, leads to a characteristic immunodeficiency state in which patients are susceptible to infection by pyogenic bacteria, especially pneumococcal septicaemia.

Acquired Immune Deficiency Syndrome (AIDS)

Since 1980, an increasing number of cases of the newly defined AIDS have occurred, initially in the USA. The syndrome was first described in homosexuals, IV drug abusers, Haitians and haemophiliacs. While the prevalence in Western countries continues to increase, disturbing evidence is becoming available about its endemic nature in some 'Third-world' countries including those in Africa.

AIDS is characterised by a profound defect in cell-mediated immunity, with lymphopenia and diminished T-lymphocyte responses.

The circumstances in which AIDS develops point to some parenterally transmitted infective agent, present in various body fluids, as the cause. In 1984, Gallo and colleagues isolated human T-lymphocytotrophic virus III (HTLV-III) from patients with AIDS. The virus has since been renamed HIV. Antibodies to HIV have been detected in virtually all patients with AIDS, and in a large proportion of population groups known to be at risk of AIDS.

HIV infection may be clinically silent, or may present in a variety of ways. Some patients, on developing antibodies to HIV, develop the *acute seroconversive illness* characterised by symptoms resembling glandular fever, possibly associated with acute encephalopathy and acute myelopathy. Other patients chronically infected with HIV develop haematological cytopenias, minor opportunistic skin infections and lymphadenopathy. The ly-pathy seen in chronic HIV infection is termed *persistent generalised ly-pathy (PGL).* This is defined as enlarged nodes at least 10 mm in diameter in two or more (non-contiguous) extra-inguinal sites persisting for at least 3 months in the absence of any current illness or medication known to cause enlarged nodes.

A proportion of patients go on to develop *AIDS-related complex* which is characterised by constitutional symptoms and abnormal laboratory tests of immunological competence falling short of AIDS. The onset of full-blown AIDS is usually signalled by the development of multiple opportunistic infections, which may include *Pneumocystis carinii* pneumonia, cytomegalovirus infection, cerebral toxoplasmosis, atypical mycobacterial infections, systemic fungal infections and parasitic infestations of the gastrointestinal tract. About one-third of patients develop an otherwise rare sarcoma, possibly of vascular endothelial cells, called

Kaposi's sarcoma (KS). Before the recognition of AIDS, KS was virtually confined to the southern European countries, being most common in Italians and Jews, and to central Africa. The prognosis for AIDS patients is very poor; about 90% die within two years of diagnosis.

The development of HIV infection does not necessarily lead to clinical AIDS, but why the syndrome develops in only some infected individuals is not known. HIV binds to the CD4 molecules on the surface of the helper/inducer subpopulation of T-lymphocytes, causing them to be lethally damaged. The consequent depletion of T-helper/inducer cells is responsible for the immune suppression and susceptibility to opportunistic infections.

Since the initial description of the syndrome, the natural history of AIDS has been changing: it is becoming less confined to homosexuals and more prominent in the heterosexual population, having the same epidemiology as other sexually transmitted diseases.

It is clear that HIV may be transmitted by blood and blood products, hence its prevalence in haemophiliacs who received pooled clotting factor VIII concentrates. The development of screening tests for antibodies to HIV in potential blood donors is a major advance in the prevention of transmission through blood products.

HIV has been demonstrated in other body fluids including semen, tears and saliva. This has major implications for the handling of patients who have AIDS or are carrying HIV, and for the conduct of autopsy examination on victims of the disease.

It has recently become apparent that AIDS may be caused by more than one virus, and a second virus, designated HIV2, has been isolated in certain parts of Africa. The presence of more than one serotype of the virus may be a serious hindrance to the development of a reliable vaccine for prevention of the disease.