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Clinical Immunology and Vaccination

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1. Autoimmune diseases

- Definition of autoimmune diseases and clinical burden
- Classification of autoimmune diseases
 - Immunological features of autoimmune disease
 - Antibody responses
 - T cell responses
- Etiology of autoimmune diseases
 - Genetic factors
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- Loss of tolerance

1. Definition and terminology of autoimmune disease:

Autoimmune diseases are diseases that involve an immune response against one or more self-antigens. These self-antigens are usually proteins that constitute part of the body; less often they are carbohydrates, lipids or DNA. The self-antigens that the immune system responds to in an autoimmune disease are called autoantigens and the immune response against an autoantigen is called an autoimmune response. The first disease to be identified as being autoimmune in origin was Hashimoto's thyroiditis in the mid-1950s. In this disease antibodies are produced against thyroglobulin and other thyroid-associated antigens. Antibodies against self-antigens are called autoantibodies and lymphocytes whose antigen receptors are specific for a self-antigen are called autoreactive cells. Nearly all autoimmune diseases involve the production of autoreactive CD4 T cells, which are also called autoreactive Th cells. Depending on the autoimmune disease there may also be the production of autoreactive B cells and/or autoreactive CD8 T cells. In Hashimoto's thyroiditis there is also an extensive infiltrate of lymphocytes

and monocytes into the thyroid (Figure.1). The result of anti-thyroid antibody production and the thyroid infiltrate is the destruction of a large proportion of the thyroid glandular tissue, leading to under functioning of the thyroid, or hypothyroidism. Hashimoto's thyroiditis demonstrates another feature of autoimmune diseases – the occurrence of primary pathology and secondary pathology. Primary pathology is the direct consequence of the autoimmune response; in the case of Hashimoto's thyroiditis this is destruction of thyroid tissue. The secondary pathology is a consequence of the altered tissue function caused by the primary pathology. Again, using Hashimoto's thyroiditis as an example, the secondary pathology due to hypothyroidism differs depending on the severity of the disease and the age at which it occurs. In infants it can cause cretinism, which is the retardation of physical and mental growth. In adults, some of the symptoms are fatigue, intolerance of the cold, dry skin and, in severe cases, mental impairment.

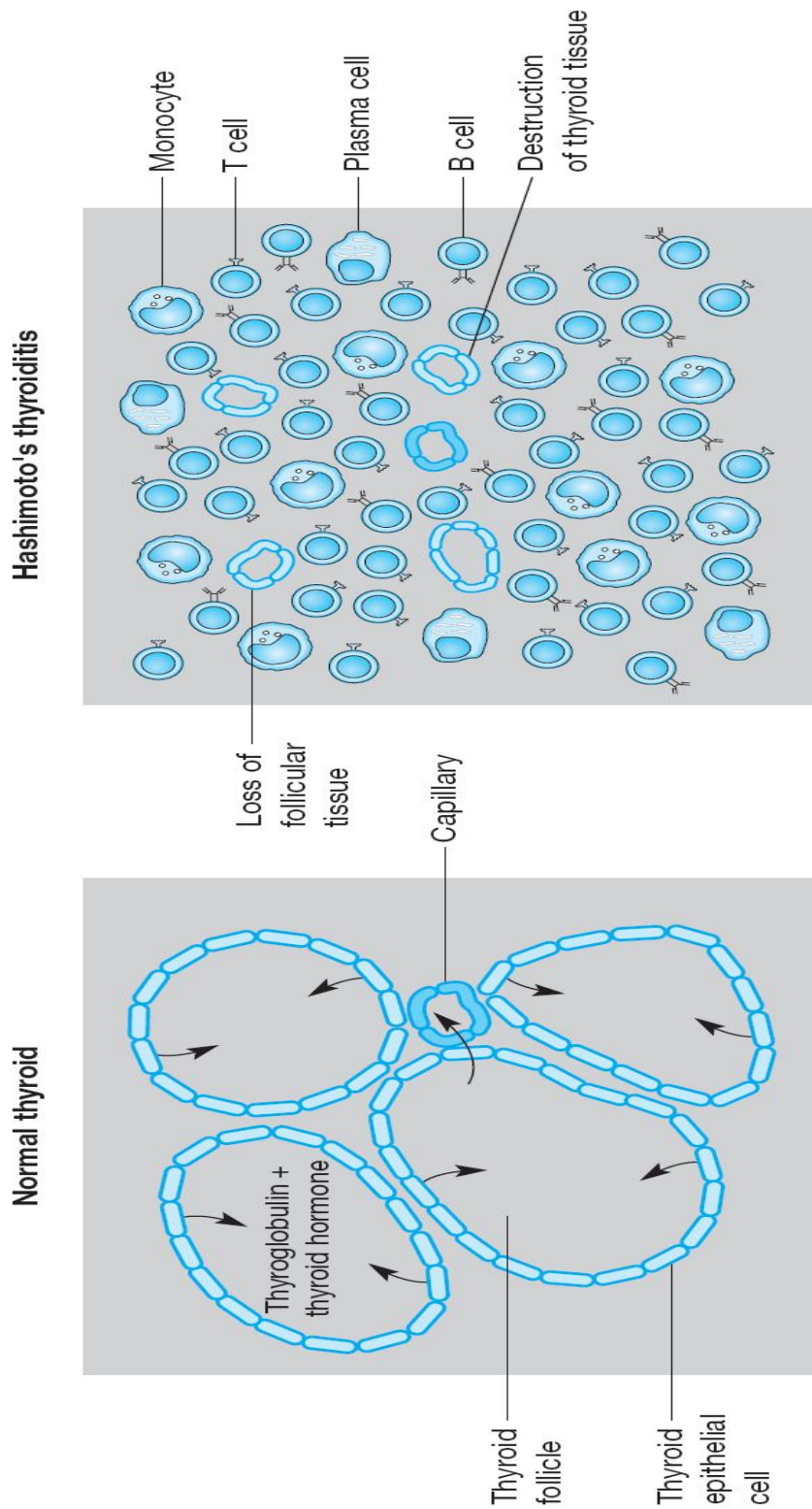


Figure .1 Thyroid infiltration and destruction in Hashimoto's thyroiditis. Normal thyroid consists of follicles containing thyroglobulin and thyroid hormone. The follicles are lined by thyroid epithelial cells. In Hashimoto's thyroiditis there is extensive infiltration of T cells, B cells, plasma cells and monocytes into the thyroid tissue, resulting in destruction of thyroid tissue and loss of thyroid hormone production.

2. Spectrum and clinical burden of autoimmune diseases:

The number of different autoimmune diseases is huge and essentially every tissue and organ can be affected by an autoimmune disease. (Table .1) is a partial list of autoimmune diseases. Some of these diseases, such as type I Insulin-dependent diabetes mellitus (IDDM), rheumatoid arthritis and multiple sclerosis are familiar to most people; however, most autoimmune diseases are not that well known. The full range of autoimmune diseases is probably not yet appreciated and there is speculation that other diseases whose cause is at present unknown, such as some forms of mental illness and alopecia (hair loss), may be due to autoimmunity. Autoimmune diseases are generally chronic in nature and can be quite debilitating; many of them are fatal if not treated. The number of people affected by all autoimmune diseases is about 5% of the population, so as a whole autoimmune diseases are quite common. The frequency of individual diseases varies considerably. Rheumatoid arthritis (RA) is the most common autoimmune disease, with an incidence of 1–2% of the population. Other autoimmune diseases are much rarer; pemphigus vulgaris, an autoimmune disease causing severe blistering of the skin, affects fewer than one in 100 000 people. It is not surprising that the more severe diseases with higher fatality rates usually have the lowest incidence. One issue of concern is that the incidence of autoimmune diseases has increased dramatically over the past 50 years or so, especially in industrialized countries.

Table .1 Spectrum of autoimmune diseases

<i>Biological system affected</i>	<i>Disease</i>	<i>Main effects</i>
Endocrine glands		
Thyroid	Hashimoto's thyroiditis	Thyroid destruction and underfunction
Thyroid	Grave's disease	Thyroid stimulation and overfunction
Islets of Langerhans (pancreas)	Insulin-dependent diabetes mellitus	Destruction of β cells (insulin-producing cells)
Adrenal gland	Addison's disease	Adrenal insufficiency
Haematopoietic system		
Red blood cells	Autoimmune haemolytic anaemia	Anaemia
Platelets	Autoimmune thrombocytopenia	Abnormal bleeding
Intrinsic factor (IF)	Pernicious anaemia	Autoantibody prevents absorption of vitamin B12
Nervous system		
Central nervous system	Multiple sclerosis	Progressive paralysis
Neuromuscular junction	Myasthenia gravis	Progressive muscle weakness
Skin		
Nuclear antigens	Scleroderma	Fibrosis of skin
Epidermal cell junctions	Pemphigus vulgaris	Severe blistering
Joints		
Synovium	Rheumatoid arthritis (RA)	Progressive destruction
Synovium	Systematic lupus erythromatosus (SLE)	Deformity
Kidney		
Basement membrane	Goodpasture's syndrome	Glomerulonephritis
Glomerulus	SLE	Glomerulonephritis

3. Classification of autoimmune diseases:

The wide spectrum of known autoimmune diseases has led to attempts to classify them. One distinction that became apparent between different autoimmune diseases was that some affect only one organ while others are more widespread. Diseases such as IDDM and thyroiditis affect the β cells of the islets of Langerhans of the pancreas and the thyroid specifically; no

other organs or tissues are affected directly by the autoimmune response, although other tissues may be affected by the loss of insulin production or thyroid function caused by the autoimmune destruction of the tissue. Other autoimmune diseases affect many tissues, for example systemic lupus erythematosus (SLE) is an autoimmune disease affecting the skin, kidneys and joints. Autoimmune diseases such as IDDM or thyroiditis, in which one organ is affected, are called **organ-specific**. Diseases such as SLE, where many tissues are affected, are called **systemic or non-organ-specific**.

4. Immunological features of autoimmune diseases:

During an autoimmune response the immune system produces effector cells and molecules that attack particular parts of the body. These effectors may include antibody, cytotoxic CD8 T cells and effector CD4 T cells. Therefore, depending on the autoimmune disease, one or more of the serological and histological features associated with an attack by the immune system on a particular tissue or tissues will be seen.

a. Serum autoantibodies: The serum of many patients with autoimmune disease contains autoantibodies. In the past these could sometimes be identified by **immunofluorescence** or **immunocytochemical** techniques using sections of relevant tissue (Figure.2). As more autoantigens are identified it is becoming easier to measure autoantibodies using enzyme-linked immunosorbent assay (**ELISA**) (see Figure.3).

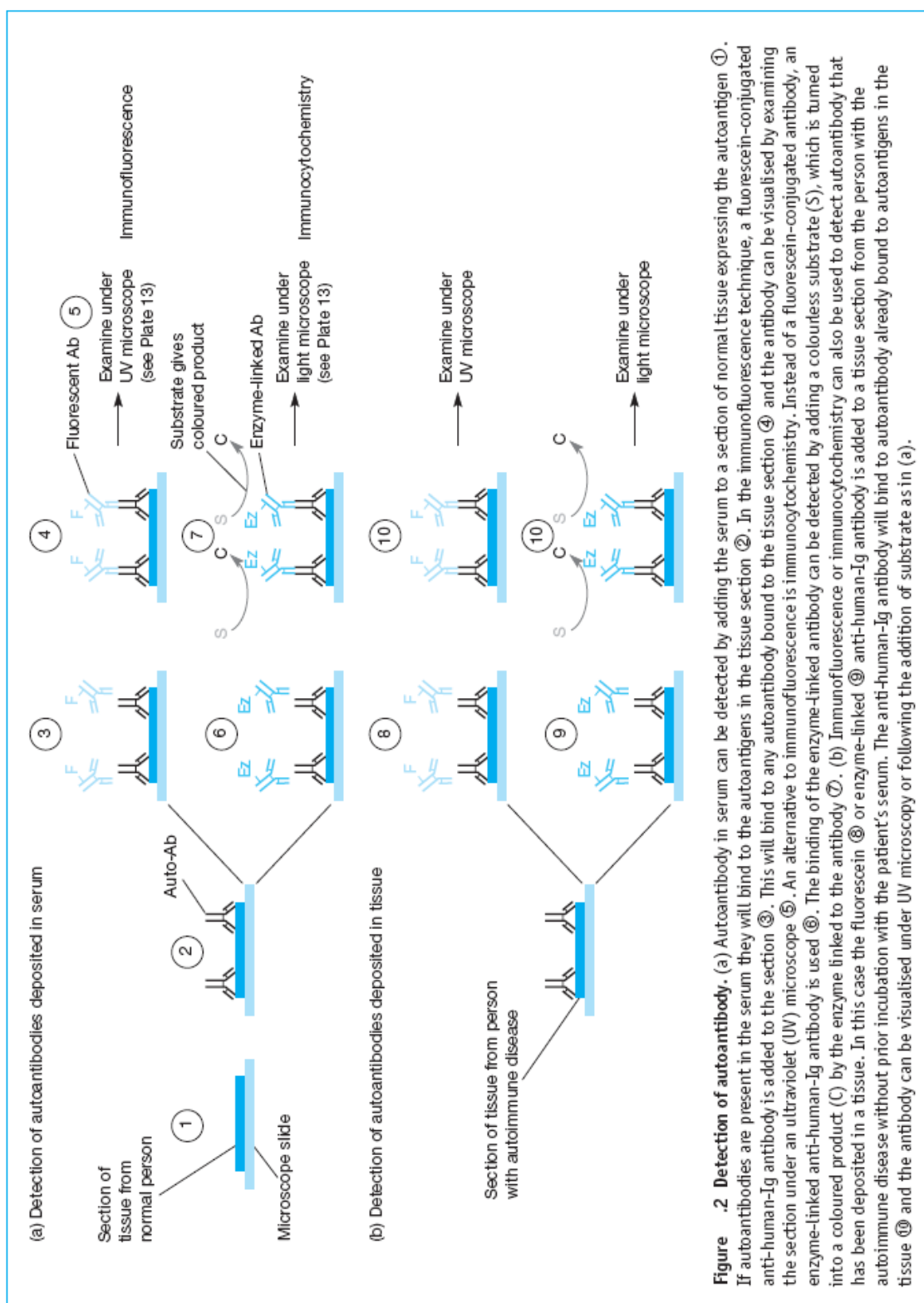


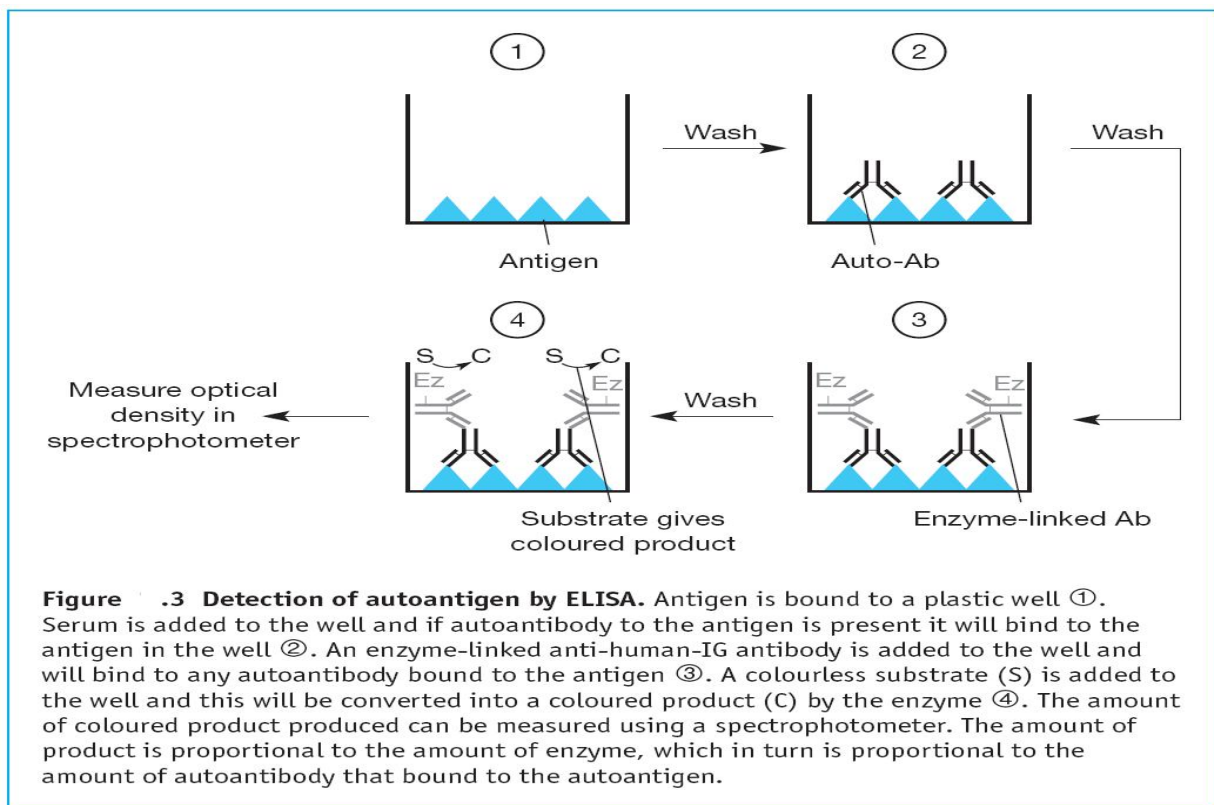
Figure 2 Detection of autoantibody. (a) Autoantibody in serum can be detected by adding the serum to a section of normal tissue expressing the autoantigen ①. If autoantibodies are present in the serum they will bind to the autoantigens in the tissue section ②. In the immunofluorescence technique, a fluorescein-conjugated anti-human-Ig antibody is added to the section ③. This will bind to any autoantibody bound to the tissue section ④ and the antibody can be visualised by examining the section under an ultraviolet (UV) microscope ⑤. An alternative to immunofluorescence is immunocytochemistry. Instead of a fluorescein-conjugated antibody, an enzyme-linked anti-human-Ig antibody is used ⑥. The binding of the enzyme-linked antibody can be detected by adding a colourless substrate (S), which is turned into a coloured product (C) by the enzyme linked to the antibody ⑦. (b) Immunofluorescence or immunocytochemistry can also be used to detect autoantibody that has been deposited in a tissue. In this case the fluorescein ⑧ or enzyme-linked ⑨ anti-human-Ig antibody is added to a tissue section from the person with the autoimmune disease without prior incubation with the patient's serum. The anti-human-Ig antibody will bind to autoantibody already bound to autoantigens in the tissue ⑩ and the antibody can be visualised under UV microscopy or following the addition of substrate as in (a).

b. Deposition of antibody and complement in affected tissue:

Another common feature of autoimmune diseases is the deposition of autoantibody in tissue. Autoantibody can bind directly to autoantigens in the tissue or it can be deposited in the form of immune complexes. Both of these situations can lead to complement fixation and activation, resulting in the generation of inflammatory responses. Deposition of antibody and complement can be detected by immunofluorescence or immunocytochemistry (Figure.2).

c. Infiltration of cells in the affected tissue:

In many autoimmune diseases a cellular infiltrate is seen in the affected organ or tissue. Typically the infiltrate will consist of lymphocytes and monocytes but other immune related and inflammatory cells can sometimes be seen.



5. Antibody-mediated autoimmune diseases:

In some autoimmune diseases autoantibody is the only or main autoimmune feature of the disease and the pathology can be explained by the actions of the autoantibody. Therefore it is easy to classify these as antibody-mediated autoimmune diseases. Different classes of autoantibody, with different functions, can be produced in autoimmune diseases and cause pathology in a number of ways:

- a. **Complement-mediated lysis:** Autoantibody binding to red blood cells can result in complement fixation and lysis of the red cells, leading to **autoimmune haemolytic anaemia** (Figure.4).
- b. **Opsonisation:** In **autoimmune thrombocytopenia** autoantibody binds to platelets and promotes their opsonisation by phagocytes in the liver and spleen (Figure.4). This leads to platelet deficiency (thrombocytopenia) and **poor** clotting.

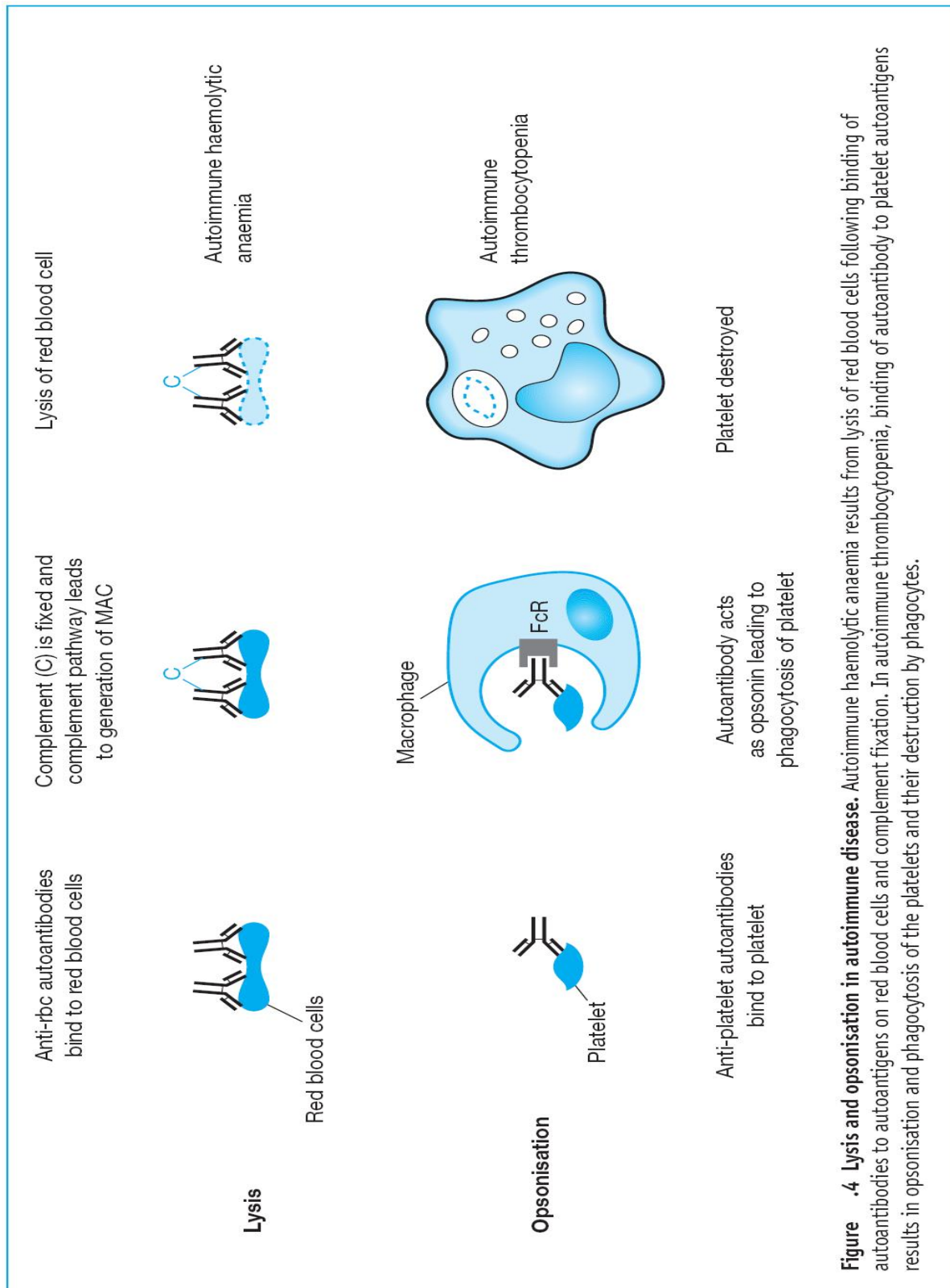
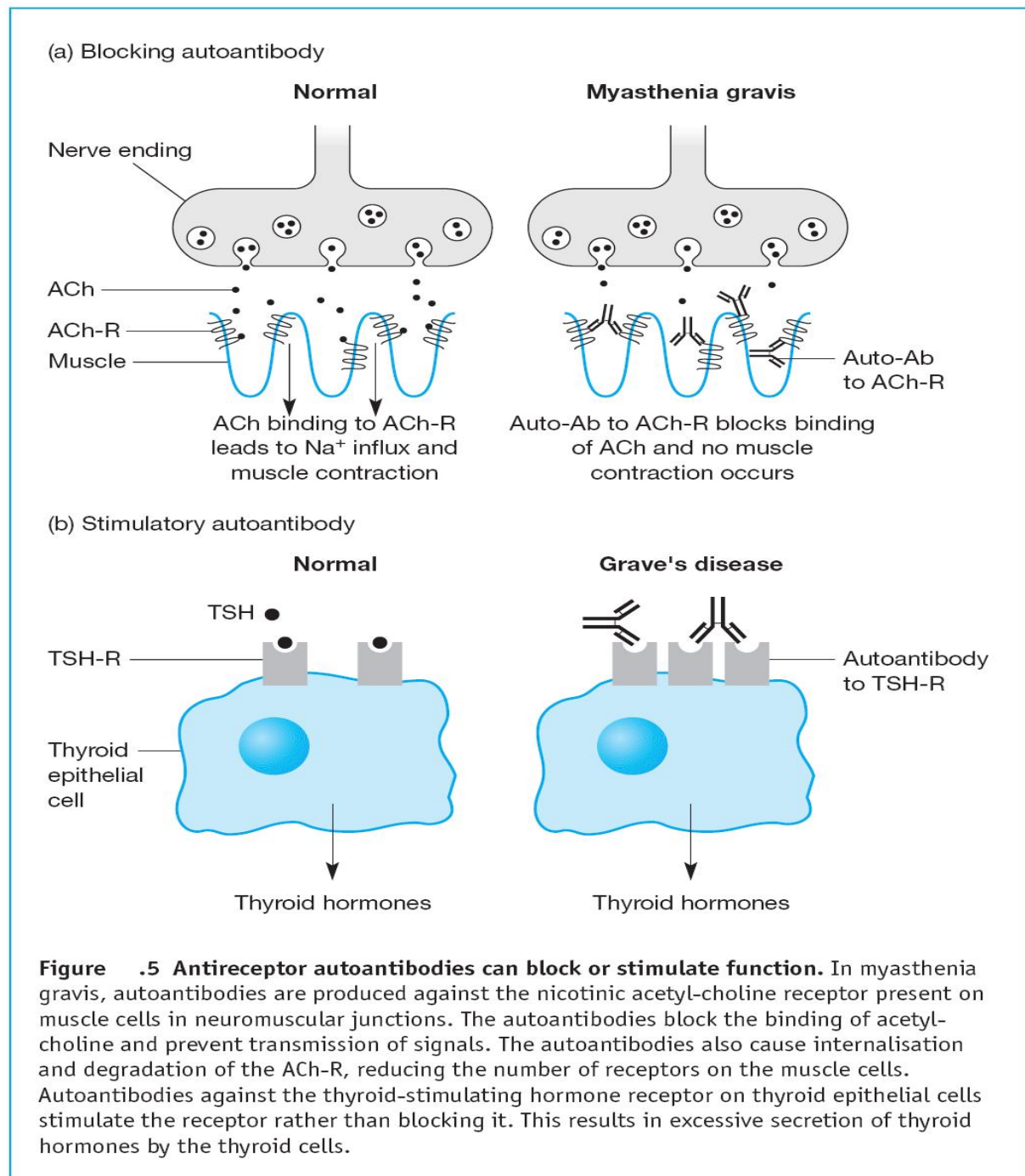
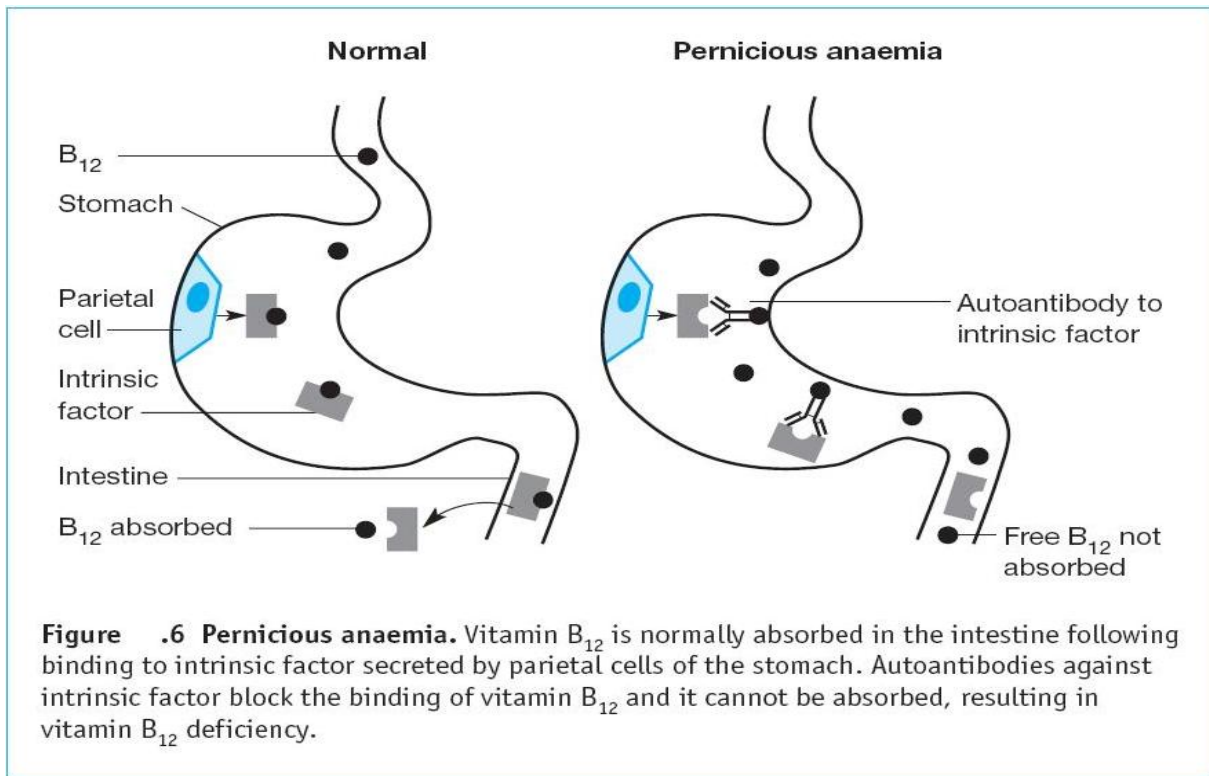


Figure 4. Lysis and opsonisation in autoimmune disease. Autoimmune haemolytic anaemia results from lysis of red blood cells following binding of autoantibodies to autoantigens on red blood cells and complement fixation. In autoimmune thrombocytopenia, binding of autoantibody to platelet autoantigens results in opsonisation and phagocytosis of the platelets and their destruction by phagocytes.

- c. **Inhibition of receptor function:** The disease **myasthenia gravis** involves the production of antibodies against the acetyl choline receptor, which is present on muscle fibers of the **neuromuscular junction**. The autoantibodies bind to the **acetyl choline receptors** and prevent the binding of acetyl choline released at the nerve endings. This blocks transmission of signals across the neuromuscular junction, leading to **muscle weakness** (Figure.5).
- d. **Stimulation of receptors:** For reasons that are not understood, in some cases antibodies against receptors can stimulate the receptor rather than block it. This happens in **Grave's disease** where autoantibodies are produced against the **thyroid-stimulating hormone (TSH) receptor** present on thyroid epithelial cells (Figure.5). Stimulation of these cells results in thyroid overactivity with the symptoms of **hyper-thyroidism** – nervousness, tiredness, weight loss despite a good appetite and **proptosis** (bulging of the eyes).
- f. **Blockage of biological function:** Some autoantibodies block the function of molecules other than receptors. **Pernicious anemia** results from **autoantibodies** against **intrinsic factor**. Intrinsic factor is produced in the **stomach** and binds to **vitamin B12**, enabling **B12** to be absorbed from the **intestine** (Figure.6). The autoantibodies against intrinsic factor prevent the binding of B12 and hence its absorption, resulting in vitamin B12 deficiency. The vitamin deficiency leads to a lack of platelets and leukocytes and neurological changes.
- g. **Deposition of immune complexes:** In SLE, immune complexes fail to be cleared from the blood and are deposited in various sites such

as the kidney, skin and joints. The immune complexes then fix complement, which leads to an inflammatory response and damage to the affected tissues. In SLE this results in kidney and joint damage and characteristic skin rashes.





6. Autoimmune diseases with unknown causes of damage:

In many autoimmune diseases it is not possible to blame the pathology simply on the action of antibody. In these diseases, which include IDDM, Hashimoto's thyroiditis and rheumatoid arthritis, there is extensive infiltration of immune-associated cells into the affected tissue and the production of autoantibodies (. The primary pathology in these diseases is damage to, or destruction of, part of the tissue, but the exact cause of the damage is hard to determine. Often the cellular infiltrate consists of CD4 and CD8 T lymphocytes, B cells, monocytes/macrophages and other inflammatory cells. Therefore the potential exists for any of the immune effector mechanisms to be responsible for the actual tissue damage. Autoantibody bound to cells in the tissue can fix complement, resulting in direct complement-mediated damage. Complement activation can also

stimulate an inflammatory response with infiltration into the tissue and activation of neutrophils and monocytes, causing further tissue damage. Antibody can also act as an opsonin, promoting phagocytosis, or contribute to ADCC. CD8 T cells can kill target cells presenting autoantigens on their class I MHC molecules. Finally CD4 T cells can act as effector cells in promoting a delayed-type hypersensitivity response leading to the recruitment and activation of monocytes that cause tissue damage not involving antibody. The difficult task in these autoimmune diseases is sorting out the actual effector mechanism from the potential mechanisms. Unfortunately the presence of a particular cell type in an infiltrate, or the presence of autoantibody, does not necessarily mean it is contributing to the pathology. In experimental IDDM, autoantibodies against β cell antigens are produced but do not appear to contribute to the destruction of the β cells. This means that for many autoimmune diseases, including the examples above, the nature of the damaging autoimmune effector mechanism is not known. It is also quite possible that for many autoimmune diseases more than one effector mechanism contributes to the tissue damage.

7. Etiology of autoimmune disease:

The factors that contribute to the development of autoimmune disease are very complex. It has become clear in recent years that both genetic and environmental factors contribute to the development of autoimmune disease. Autoimmune diseases tend to run in families, so if one family member has an autoimmune disease there is an increased likelihood of another member having the disease. However, because families tend to live together, it is not always clear whether it is the sharing of genes or the environment that increases the incidence of an autoimmune disease in families. Some of the

best evidence for both genetic and environmental contributions to the development of autoimmune disease comes from the study of concordance between identical and non-identical twins. 'Concordance' is a term used to describe the incidence of a particular disease or trait in twins. This is easiest to understand if you consider a situation where a disease is caused solely by inherited genetic factors. In this situation, if one identical twin gets the disease the other twin will always get the disease because they are genetically identical. The concordance in this situation is 100%. Nonidentical twins share on average 50% of their genes and therefore the concordance for a purely genetically determined disease would be 50% if one gene caused the disease and less if more than one gene was involved. Because twins are usually brought up in the same household and environment it is safe to assume that the difference in concordance in identical and non-identical twins is due to genetic factors. If a disease is due to a combination of genetic and environmental factors the concordance for identical twins will be less than 100% but it will still be higher than for non identical twins. This is the case with autoimmune diseases where concordance between identical twins is about 25% and that for non-identical twins is nearer 5%. The exact percentages vary from autoimmune disease to autoimmune disease but identical twins always show higher concordance than non-identical twins. These studies show that susceptibility to autoimmune disease can be inherited and therefore has a genetic element. Concordance studies also show another very important feature of autoimmune diseases. As mentioned above, if a disease was 100% due to inherited genetic factors the concordance between identical twins would be 100%. The fact that concordance in identical twins is much less than 100%

indicates that environmental factors are also contributing to the development of autoimmune diseases. Other evidence also points to environmental factors contributing to the development of autoimmune disease. The incidence of autoimmune disease has risen dramatically in the past 40–50 years, especially in North America, Europe and Australasia. Although some of this increase may be due to better diagnosis it is clear that there is a real increase and that this has happened too quickly to be explained by genetic changes in the population. Furthermore, populations from countries with a low incidence of certain autoimmune diseases, such as Japan and Papua New Guinea, have seen a rapid rise in the incidence of the autoimmune diseases when they have moved to countries with a higher incidence; again this cannot be explained by genetic factors and must be due to environmental effects.

8. Genetics of autoimmune disease:

Genetic studies have shown that autoimmune diseases are multigenic. This means that many genes contribute to susceptibility to the disease. This is different from diseases, such as cystic fibrosis, which are caused by mutations in a single gene. So what do we mean when we say a gene contributes towards susceptibility to a disease? Many genes are polymorphic; that, is they are present in the population in more than one form. Each form of a particular gene is called an allele. Polymorphism can occur in the part of the gene coding for the protein. This polymorphism can lead to different forms of the protein being made and is called structural polymorphism. The most prominent form of structural polymorphism is in the MHC genes where many different forms of the protein can be made. The second type of polymorphism occurs in the part of the gene not coding for

the protein. This does not affect the structure of the protein but, if the polymorphism is in the promoter/enhancer part of the gene, it can alter the level of expression of the gene and affect how much of the protein is produced. This is called non-structural polymorphism. Polymorphism can therefore affect the biological activity of a protein in two ways: (i) by causing the production of different forms of the protein and (ii) by affecting the amount of protein produced (Figure.7). If it can be shown that people with one allele of a polymorphic gene have a different incidence of a disease than people with a different allele of the gene, the gene is clearly affecting susceptibility to the disease and is called a susceptibility gene. This is presumably due to the difference in amount or activity of the gene product. The degree to which a particular allele of a gene increases susceptibility to a disease can be quantified by calculating what is known as the relative risk, or RR. The RR compares the frequency of a particular allele of a gene in a population with a disease with the frequency of the allele in the population as a whole. Although the calculation is more complicated the relative risk can be seen as the following ratio:

RR =

Frequency of allele in population with autoimmune disease

Frequency of allele in population without autoimmune disease

Modern advances in DNA technology have made it possible to perform genome-wide screening for susceptibility genes in autoimmune diseases.

These screens, combined with animal studies, show that in some autoimmune diseases up to 20 genes may contribute to susceptibility. These

susceptibility genes can be divided into two categories – MHC and non-MHC.

MHC genes:

it was realised many years ago that one of the strongest associations between genes and autoimmunity was with the MHC genes, especially class II MHC. This is perhaps not too surprising given that class II MHC proteins present antigen to CD4 T cells. Autoimmune responses are almost always CD4 T cell-dependent in the same way that nearly all specific immune responses against foreign antigens require CD4 T cells for their generation. The relative risk for some HLA alleles with autoimmune disease is shown in (Table.2). It has been estimated that MHC genes contribute about 50% of the total genetic risk of developing autoimmunity.

Non-MHC genes:

Although the most significant susceptibility gene for nearly all autoimmune diseases is MHC, many other genes also contribute to susceptibility to autoimmunity. One set of genes that affects susceptibility to autoimmunity is the sex-related genes. Many autoimmune diseases are much more frequent in women than men: Hashimoto's thyroiditis is 50 times more frequent in females and SLE occurs 10 times more frequently in women. Although the immunological basis for this is unknown, experimental studies have shown that it is in part due to the effect of sex hormones on the immune system. Other genes are less well characterized. While new DNA technology has made it possible to identify the number of genes and their approximate chromosomal location, identifying the exact susceptibility gene is a time consuming, arduous and expensive business. Some genes have been identified that contribute to autoimmunity in general and are associated with

a number of autoimmune syndromes. People with mutations in the AIRE gene suffer from a number of autoimmune syndromes including thyroiditis and type 1 diabetes. This is thought to be due to the fact that AIRE controls expression of organ-specific proteins by thymic medullary epithelial cells and people with a non-functioning AIRE protein has deficient expression of these genes in their thymus and therefore defective clonal deletion of T cells specific for these autoantigens. Another gene associated with generalized autoimmunity is the CTLA-4 gene. CTLA-4 is a protein that is found on activated CD4 T cells and can also exist in soluble form. CTLA-4 is a negative regulator of T cell function; it binds to CD80 and CD86, as does CD28 but when CTLA-4 binds to CD80/86 it delivers a negative signal to the T cell and inhibits activation. People with defective CTLA-4 function are deficient in their ability to regulate autoreactive T cells and are at increased risk of autoimmune diseases such as thyroiditis, type 1 diabetes and autoimmune Addison's disease, which results in damage to the adrenal glands. In terms of non-MHC genes associated with specific autoimmune diseases, the only non-MHC gene accepted so far is the insulin gene in IDDM. This has a polymorphism in the non-coding part of the gene and different alleles of the insulin gene are associated with different susceptibility to IDDM. Several studies have shown that different alleles of the thyroglobulin gene may affect susceptibility to thyroid autoimmune disease; thyroglobulin is an important autoantigen in thyroid disease. Other genes suspected of being susceptibility genes fall into the category of cytokines and their receptor genes, apoptosis-related genes and genes coding for autoantigens. It is thought that these genes affect susceptibility to

autoimmunity by affecting tolerance induction and the type of autoimmune response generated.

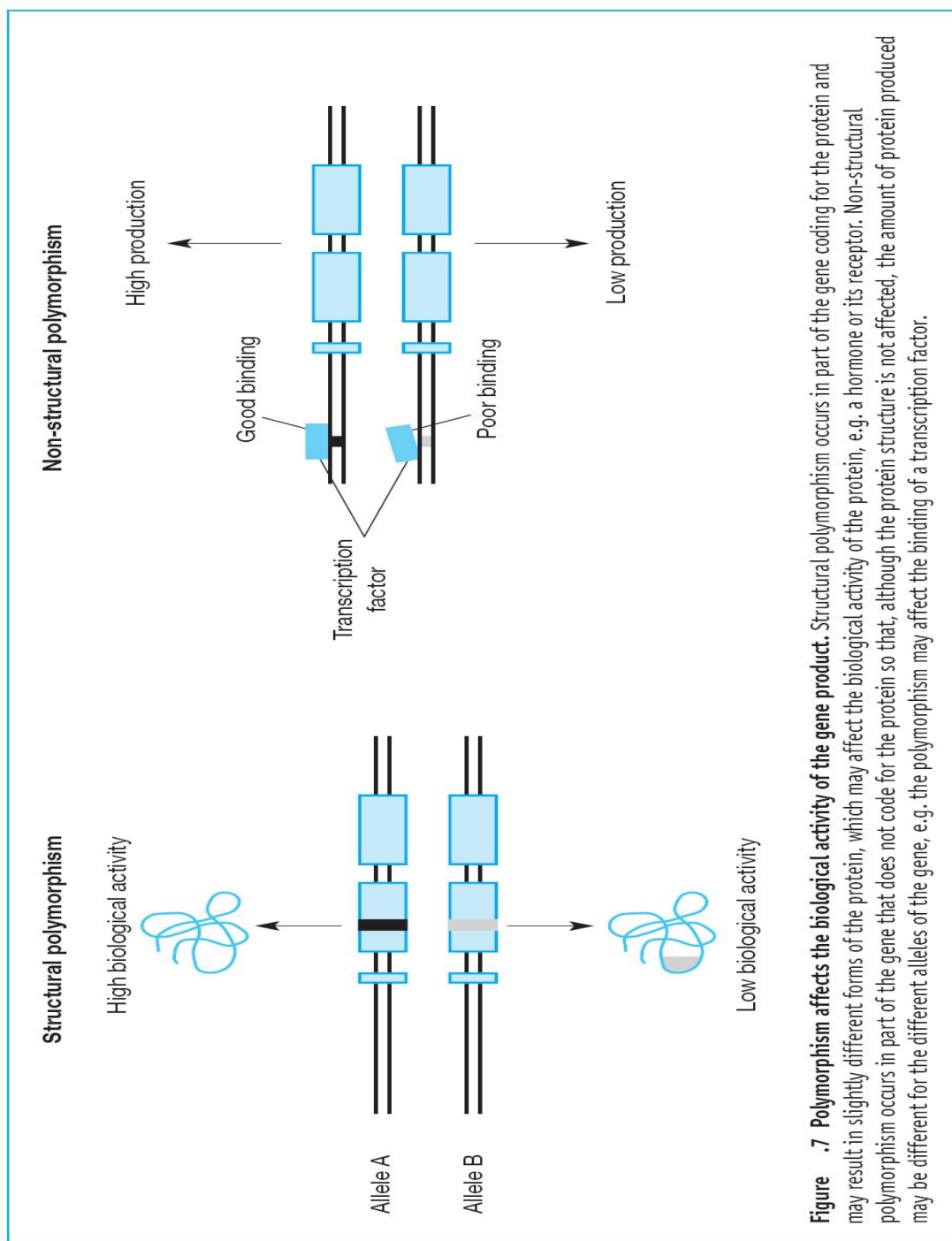


Table .2 MHC association with autoimmune diseases

<i>Disease</i>	<i>MHC association</i>	<i>Relative risk (RR)</i>
Goodpasture's syndrome	DR 2	16
Multiple sclerosis	DR 2	5
SLE	DR 3	6
Myasthenia gravis	DR 3	3
RA	DR 4	7
Hashimoto's thyroiditis	DR 5	3
IDDM	DR 3	3
IDDM	DR 4	3
IDDM	DR 3 + 4	14

9. Environment and autoimmune disease:

Again, while the evidence for environmental effects contributing to the development of autoimmune disease is substantial, it is much more difficult to identify what the environmental influences are. Part of the problem is the chronic nature of autoimmune diseases. An autoimmune response may be initiated by an environmental event and cause damage to tissue for a number of years before any clinical symptoms develop and the person sees a doctor. Therefore it will not be known exactly when the autoimmune response began and it will be difficult, if not impossible, to associate an environmental event with the triggering of an autoimmune response. Furthermore, people may not remember too clearly events that happened to them years ago. However, some behavioral traits are associated with increased incidence of a particular autoimmune disease and allow some

environmental candidates to be identified. The main types of environmental factor associated with autoimmunity include the following:

- **Infectious agents**: The best-known example of infectious agents contributing to autoimmunity is rheumatic fever, where antibodies against Streptococcal M-antigen react against heart myosin, joints and kidney, resulting in arthritis and heart disease.
- **Drugs**: Drugs or their metabolites can bind to self-antigens and make them appear foreign. This can result in the development of autoantibody against the self-antigen itself. Examples of this are penicillin metabolites, which bind to red blood cells, and sedormid, which binds to platelets.
- **Toxins and pollutants**: In an autoimmune disease called Good pasture's syndrome, autoantibodies are produced against type IV collagen, which is present in the basement membranes of the kidney and lung. All individuals with this disease suffer from glomerulonephritis, which leads to impaired kidney function. Cigarette smokers, but not non-smokers, also have a very high incidence of pulmonary haemorrhage, which can be fatal. This is thought to occur because the cigarette smoke damages the lungs, making the basement membrane accessible to the autoantibodies.
- **Food**: There have been a number of reports associating different foods (e.g. cows' milk and diabetes) with autoimmune diseases but none has been substantiated. Because so few environmental agents have been identified it is difficult to know exactly how they contribute to the development of autoimmune disease. The general assumption is that they

contribute to the loss of immunological tolerance that is the central feature of autoimmune diseases.

10. Loss of immunological tolerance:

Autoimmune disease is caused by either the production of high-affinity autoantibodies or the generation of autoreactive T cells. Everyone has B cells with specificity for some self-antigens but they normally do not produce autoantibody because there are no self-antigen-specific Th cells. If autoreactive Th cells are generated because of a loss of T cell tolerance they can help autoreactive B cells to make antibody, and autoimmunity will follow. However, there are ways in which autoreactive B cells can make antibody in the absence of autoreactive Th cells, and these will be described first.

A. Production of autoantibody in the absence of autoreactive Th:

There are two ways in which B cells can make antibody to a self-antigen in the absence of Th cells specific for that self-antigen. One, which is quite simple to understand, is to bypass the need for Th cells. The other, which is more complicated, is due to cross-reactive epitopes between self-antigen and antigens on infectious agents. These processes occur as follows.

a. Th bypass by B cell mitogens:

Bacterial products, such as lipopolysaccharide (LPS), can directly stimulate B cells to proliferate and differentiate into antibody-producing cells without the need for Th. This stimulation is not antigen-specific and the agents are called mitogens. Because any B cell can be stimulated, B cells specific for autoantigens as well as B cells specific for foreign antigens will be stimulated (Figure.8a). This stimulation is transient and once the bacteria

have been eliminated the stimulation of autoreactive B cells and production of autoantibody will stop.

b. Cross-reactive epitopes:

Some proteins on infectious agents have epitopes that are very similar to self-epitopes and this can lead to the production of autoantibody in the following way. Let us take a hypothetical self-antigen that has on it a self-B cell epitope and a self-T cell epitope (Figure.8b). B cells exist that are specific for the self-B cell epitope. They can bind the antigen through the B cell epitope, process the antigen and present the self-T cell epitope on their class II MHC. However, because there are no Th specific for the self-T cell epitope, the B cell will not receive the help it needs to make antibody (Figure.8b). Now imagine an antigen on a pathogen that has the same B cell epitope as the self-antigen but has foreign T cell epitopes. This antigen can also be bound by self-reactive B cells; these B cells process the antigen and now present foreign antigenic peptide on their class II MHC (Figure.8b). Because the antigenic peptide is foreign there will be Th cells present that are specific for the foreign epitope and therefore the B cell will receive the T cell help and can be stimulated to produce autoantibody. The best known example of this is streptococcal M-antigen, which has B cell epitopes that are cross-reactive with cardiac myosin. Following infection with group A streptococci some individuals make antibodies against myosin, resulting in damage to the heart and rheumatic fever. Another scenario is that a drug or environmental chemical can bind to a self-antigen, modifying it and creating a T cell epitope that appears foreign. Again the autoreactive B cell binds to the B cell epitope and presents the foreign-looking T cell epitope to Th cells and autoantibody is produced (Figure.8b). In these situations, where

pathogenic antigens or chemicals provide the foreign T cell epitopes, autoantibody production is usually transient. Once the pathogen has been eliminated or the person has ceased contact with the drug or chemical, the foreign T cell epitope is no longer present and the autoreactive B cell no longer gets T cell help. However, in genetically susceptible individuals, environmental triggers can lead to the activation of Th cells that are specific for a self-antigen; therefore the autoimmune disease becomes self-perpetuating even in the absence of the environmental agent. Why these autoreactive CD4 T cells are present and how they become activated is a subject of much investigation. At present the answer is unclear but a number of mechanisms have been suggested.

B. Activation of autoreactive Th:

The emergence of pathogenic autoimmune Th cells is probably a multistage process that reflects a complex interplay between the many genes and environmental factors described in.

a. Genetic factors:

Originally it was thought that loss of self-tolerance in CD4 T cells occurred because the normal mechanisms of preventing self-tolerance broke down owing to the presence of 'faulty' genes. The reason so many genes were involved in determining susceptibility to autoimmunity was because of the number of different tolerance mechanisms (deletion, anergy and regulation). All of them would have to stop working for autoreactive Th to be generated. This view of the multigenic nature of autoimmune disease has now been modified, mainly because of a surprising set of observations. These studies suggest that there is heterogeneity in how different people maintain tolerance to the same self-antigen. For any given self-antigen some people may

clonally delete CD4 T cells that are specific for the self-antigen but some will not. Although they do not automatically develop autoimmunity, people who do not delete self-reactive CD4 T cells are clearly at more risk of these cells causing an autoimmune response than people who do delete those particular self-reactive CD4 T cells. The mechanisms that different people use to maintain tolerance to a particular autoantigen must be under genetic control and, although they are unknown, the genes controlling tolerance will affect susceptibility to autoimmunity. It is now thought that genetic susceptibility to autoimmunity is due to the inheritance of a specific combination of alleles of many different genes. In addition to controlling self-tolerance these genes may also control other factors associated with an immune response, such as cytokine production or apoptosis. The number of genes is not known but is quite large – 20 different genes contribute to susceptibility to IDDM or SLE. Because you have to have a specific combination of alleles of many genes to be at risk of developing an autoimmune disease, most people will have some susceptibility alleles but not enough to be at risk. Those people with the combination of enough alleles are at risk of getting an autoimmune disease; whether they get it or not depends on environmental factors.

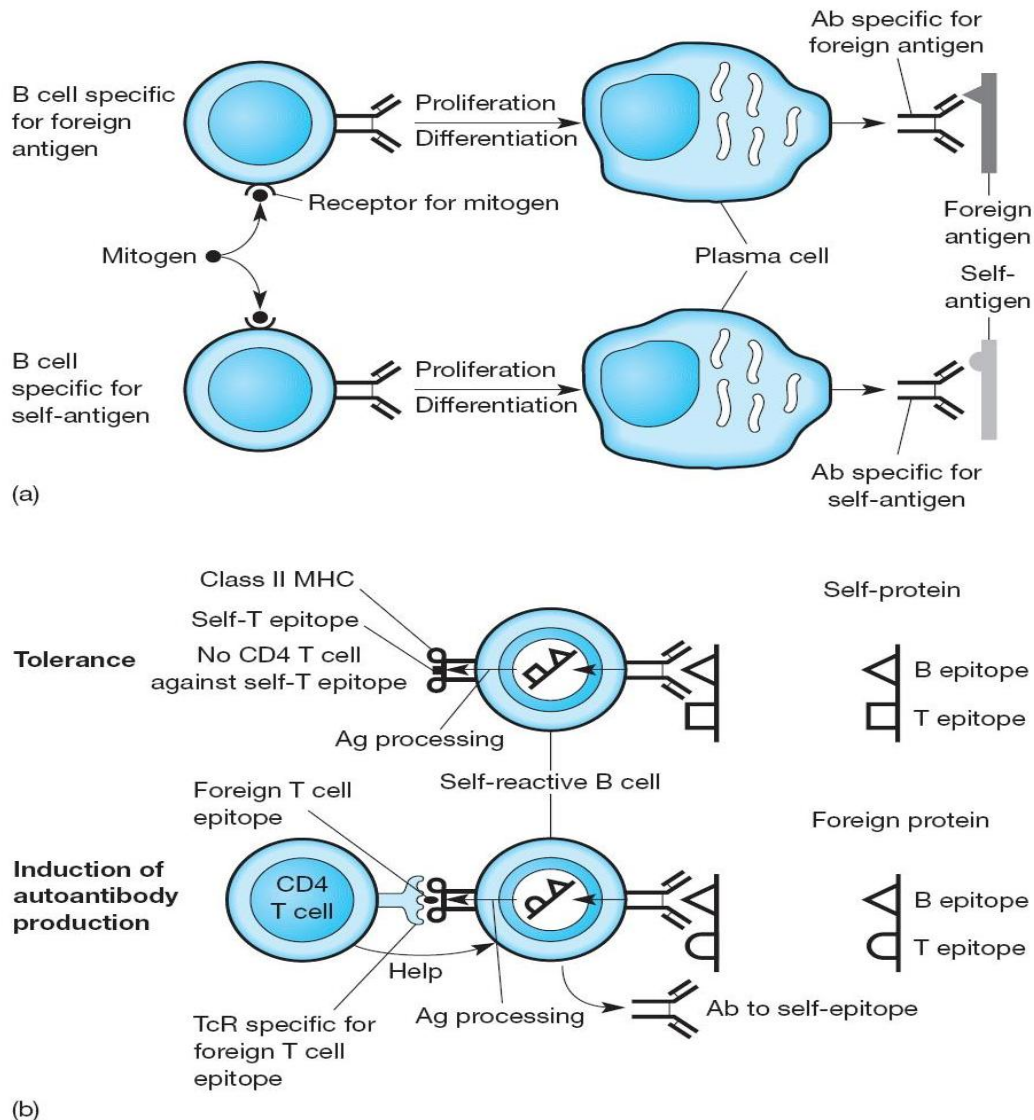


Figure 8. Induction of autoantibody production. (a) Autoantibody production induced by B cell mitogens. B cell mitogens stimulate B cells to proliferate and differentiate into plasma cells irrespective of the antigen specificity of the B cell, leading to production of autoantibody as well as antibody to foreign antigens. (b) Induction of autoantibody by cross-reactive epitopes. In the top example a self-protein is depicted with a self-B cell epitope and self-T cell epitope. Autoreactive B cells with specificity for the B cell epitope may bind the self-protein through the B cell epitope, take up the antigen, process it and present the self-T cell epitope on their class II MHC. However, because there are no CD4 T cells with specificity for the self-T cell epitope, the B cell receives no help and is not activated. In the bottom panel a foreign protein (e.g. from a pathogen) shares the same B cell epitope as the self-protein but has a foreign antigen T cell epitope. The same autoreactive B cell binds to the B cell epitope on the foreign protein and presents the foreign T cell epitope on its class II MHC. Because the T cell epitope is foreign there will be CD4 T cells with specificity for the T cell epitope and the B cell will be activated to produce antibody that reacts with the B cell epitope on both the foreign protein and the self-protein.

b. Environmental activation of autoreactive Th:

Although many experimental procedures can lead to the activation of autoreactive Th in animals it is not clear exactly how environmental factors lead to the activation of autoreactive Th in human autoimmune diseases. One aspect that seems to be very important is the involvement of dendritic cells. Autoreactive CD4 T cells, like those against any antigen, need to be initially activated by antigen presented to them on class II MHC expressed by dendritic cells . Two main mechanisms have been suggested to explain how environmental agents could promote the activation of autoreactive CD4 T cells by dendritic cells (Figure.9):

- **Molecular mimicry:** An environmental agent (such as an infectious organism) will be regarded as foreign by the immune system and dendritic cells will present antigenic peptides derived from the agent on their class II MHC. If one of these antigenic peptides is very similar to a self-antigen peptide, CD4 T cells specific for that peptide will be activated to become Th. These can then react against self-peptides as well as those from the environmental agent and cause autoimmunity.
- **Tissue damage:** Tissue damage, whether caused by a toxic substance or a pathogen, can lead to an inflammatory type of response with the production of cytokines and the release of self-antigens from damaged cells. These two factors can activate dendritic cells to present selfpeptides derived from the self-antigens on their class II MHC, which could then activate self-reactive CD4 T cells.

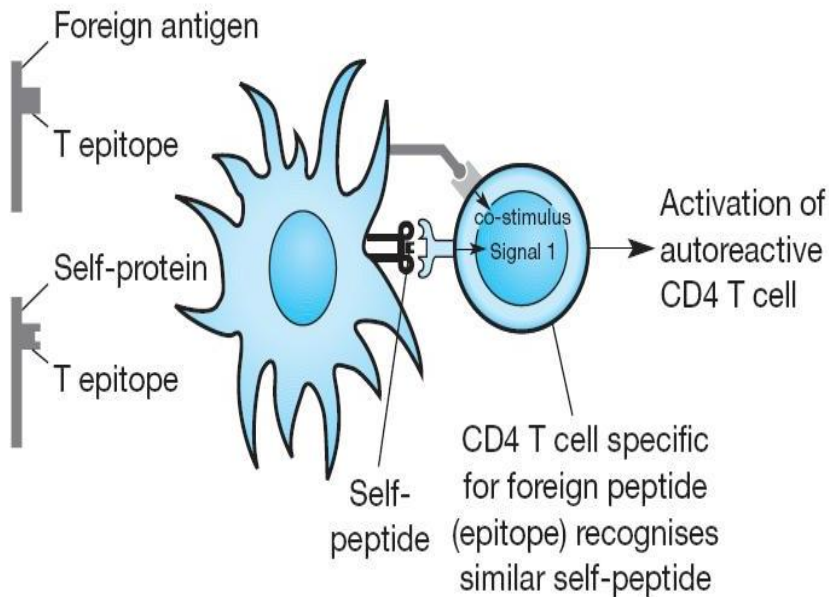
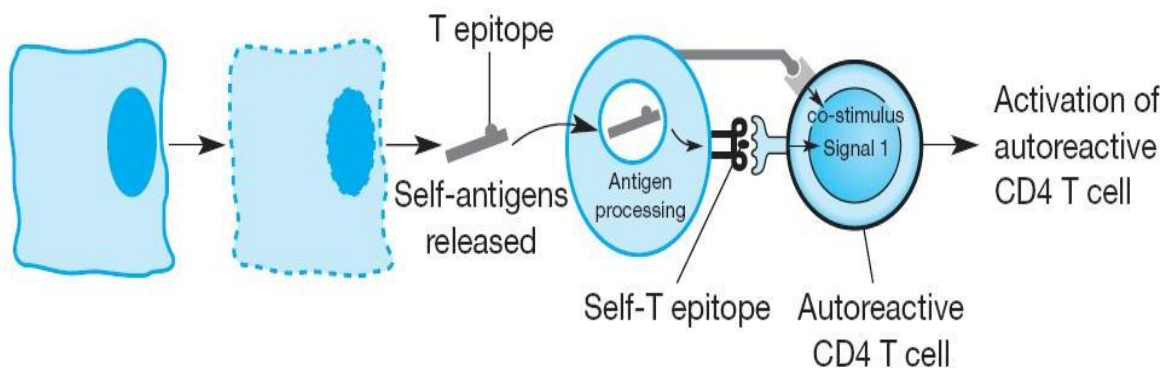
Cross-reactive T cell epitopes**Tissue damage**

Figure .9 Activation of autoreactive Th cells. Activation of autoreactive CD4 T cells can occur due to a pathogen having a very similar epitope to a self so the CD4 T cells activated by the epitope on the pathogen react with the closely related self-epitope (top panel). Alternatively, tissue damage may result in the release of self-antigens, which are taken up and processed by dendritic cells and can stimulate autoreactive CD4 T cells.

Summary

- Autoimmune diseases constitute a large group of diseases that are characterized by an immune response against one or more components of the body. Overall they affect 5% of the population in industrialised countries.
- In some autoimmune diseases the pathology is solely due to the production of high-affinity autoantibody against self-antigens. In other diseases there is also an extensive inflammatory infiltrate, consisting of many different cell types in the affected tissue, making it difficult to identify the exact nature of the immune-mediated damage.
- The aetiology of autoimmune diseases is complex, with many genes and environmental factors contributing to susceptibility. One of the major genetic associations is with MHC class II. Few of the other susceptibility genes have been identified.
- The way in which the environment contributes to the development of autoimmune disease is not well understood but it is thought either to provide autoreactive B cells with a source of help for antibody production or to contribute to the activation of autoreactive CD4 T cells.

2. Allergy and other hypersensitivities

Key topics:

- Type I hypersensitivity (allergy)
 - Immunological basis of type I allergic responses
 - Clinical symptoms of allergy
 - Genetics of allergy
 - Environmental factors
 - Treatment
- Type II hypersensitivity
- Type III hypersensitivity reactions
- Contact sensitivity

Introduction:

Although antibody can provide vital protection from disease, there are situations where excessive production of antibody against harmless antigens actually causes disease. This is known as **hypersensitivity** and there are three types of antibody-mediated hypersensitivity. The most common and well known is type I hypersensitivity or allergy. This is mediated by the production of IgE and is commonly seen as an allergic response. Type II hypersensitivity is caused by cytotoxic antibodies against normal or modified tissue components

1- Type I hypersensitivity (allergy):

The most familiar syndrome associated with allergy is asthma. Approximately one in seven children in Britain suffers from asthma and the incidence has risen dramatically over the past 50 years. Until the twentieth

century asthma was a rare condition. Like autoimmunity, asthma rates are highest in more industrialized societies. Asthma is one clinical manifestation of diseases caused by the production of IgE against otherwise innocuous antigens and is an example of an allergic reaction. Antigens that stimulate an allergic response are called **allergens**. Allergens can be derived from several sources:

- a. **Pollens:** Many pollens cause allergy, especially rhinitis and asthma. Pollens from plant, trees and grasses can all cause allergies, with ragweed being a particular problem in North America.
- b. **Insects:** Allergens derived from the house-dust mite are the most common cause of asthma. Cockroaches are also a common source of allergen. Bee and wasp stings also stimulate strong allergic reactions, possibly because they contain pharmacologically active products.
- c. **Animals and birds:** Animal dander (fur, excretory products) and feathers are another major source of allergens. Cats, dogs and rodents are the most common source of animal allergens although this probably reflects the degree of exposure rather than any special feature of these animals.
- d. **Drugs:** Many drugs cause allergic reactions, with penicillin and sulphonamides being among the most common.
- e. **Food:** A number of foods cause allergies with sometimes severe effects. Peanuts, shellfish and milk products are the best known causes of food allergies. Allergic reactions can cause other illnesses apart from asthma: rhinitis (hayfever), eczema (rashes), conjunctivitis and diarrhoea are all examples of allergic reactions. These allergic reactions are localised and

usually reflect the site of exposure. Airborne allergens are more likely to cause asthma and hayfever, while food allergens tend to cause gastric symptoms, although this is not always the case, e.g. food allergens can cause skin reactions. In its severest form an allergic reaction can be systemic, leading to anaphylaxis, an extremely dangerous condition that can be fatal if not treated. The present concern about peanut allergy is due to a number of deaths caused by anaphylactic reactions to foods containing peanut products.

2. Immunological basis of allergy:

The development of allergy occurs in a number of stages, the first of which is **sensitization**.

a- Sensitization:

Sensitization involves the production of IgE in response to exposure to allergen (Figure 1). This IgE production is dependent on CD4 T cells that respond to antigens associated with the allergen and differentiate into Th2 cells. Secretion of IL-4 by the Th2 cells is particularly important in making B cells specific for the allergen switch to IgE. The IgE binds to mast cells, which have receptors, called FcεRs on their surface that bind to the Fc portion of IgE. Mast cells are found in most tissues and there are two types, mucosal mast cells and connective tissue mast cells. Although they are quite similar, the two types of mast cell do differ in a number of respects. IgE can stay bound to mast cells for months and possibly years. The initial exposure to allergen does not usually involve any symptoms.

b- Mast cell activation:

Upon re-exposure to the allergen, the allergen will bind to the allergen specific IgE that is bound to the FcεRs on mast cells. This will cause cross-linking of the FcεRs, resulting in a series of intracellular signaling events that lead to mast cell activation. The immediate consequence of mast cell activation is degranulation and the release of preformed mediators from the mast cell granules. This is followed by the synthesis and release of newly formed mediators (Figure 1). A list of preformed and newly formed mediators, together with their main functions, is given in (Table.1). These mediators cause vasodilatation, increased vascular permeability and smooth muscle contraction, as well as having other effects. The symptoms caused by these events depend on the anatomical site and are described below. These events generally occur within minutes of exposure to the allergen.

c- Late phase reactions:

Activated mast cells secrete TNFα, which activates the endothelium at the site causing expression of adhesion molecules that promote the migration of leucocytes from the blood . Chemotactic factors, including PGD₂, LTB₄, IL-8, MIP1α and eotaxin, are also produced. The net effect of these factors is the later recruitment of eosinophils, basophils, neutrophils and T cells to the site. Activation of these cells produces further inflammation, which is seen as a late phase reaction (Figure 2).

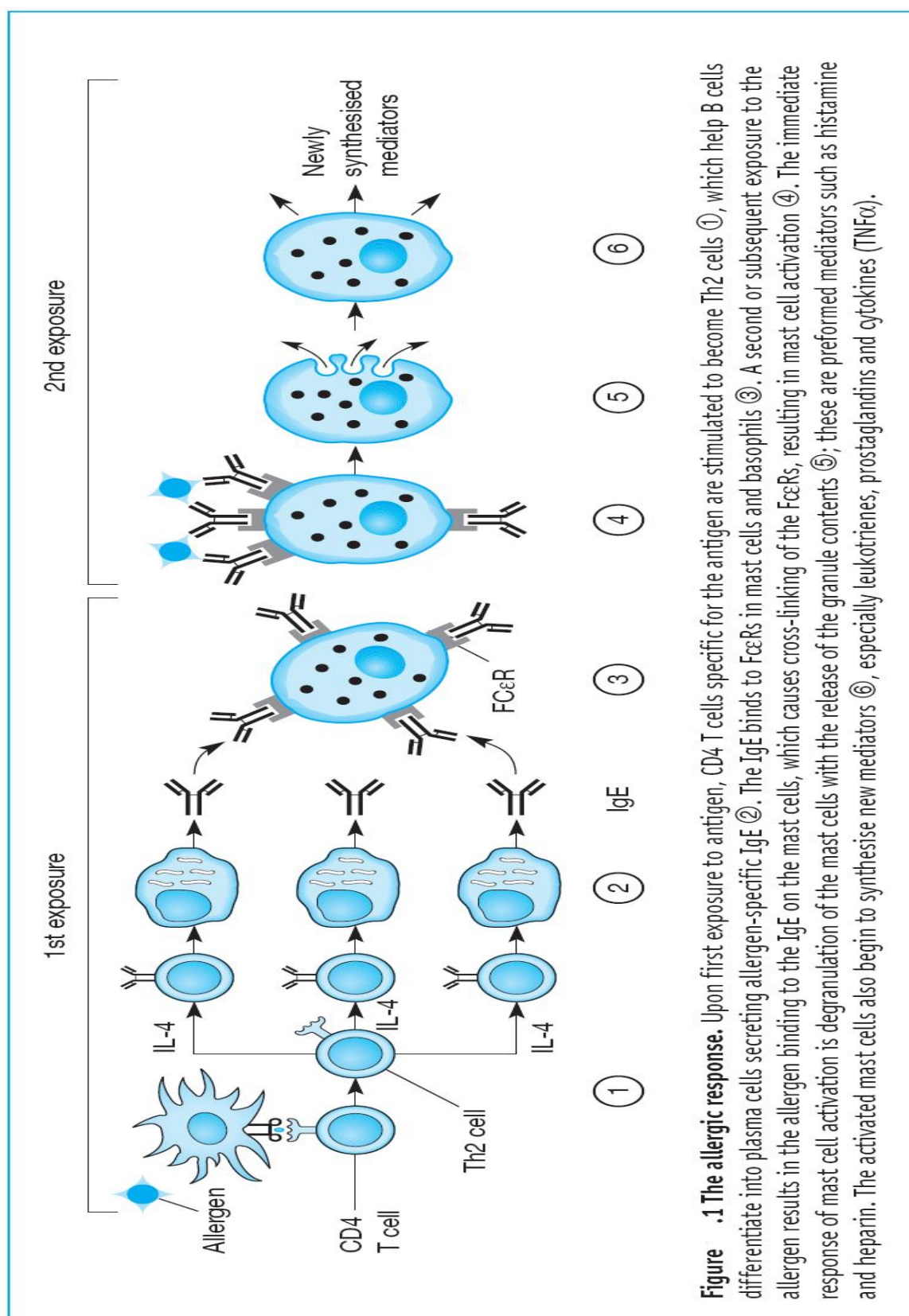
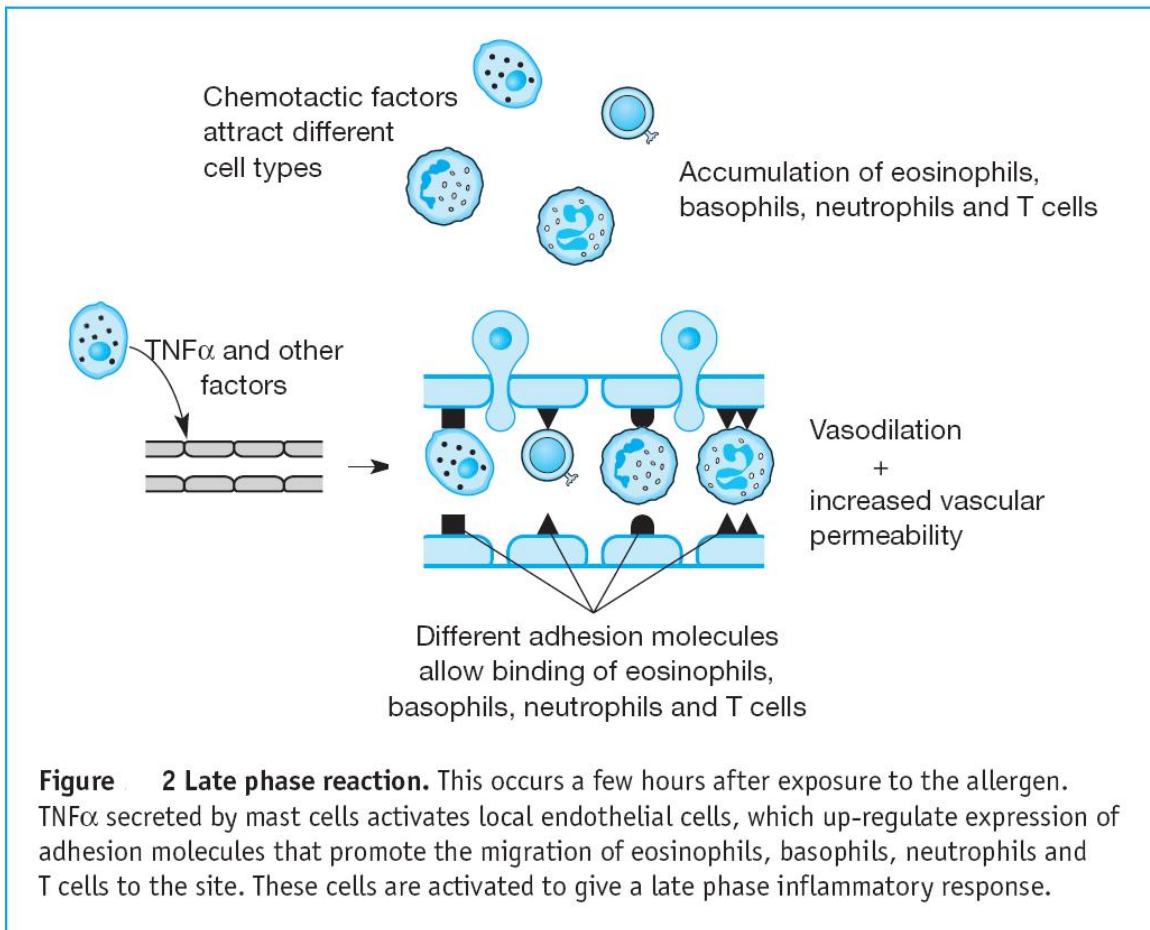


Figure 1 The allergic response. Upon first exposure to antigen, CD4 T cells specific for the antigen are stimulated to become Th2 cells (1), which help B cells differentiate into plasma cells secreting allergen-specific IgE (2). The IgE binds to FcεRs in mast cells and basophils (3). A second or subsequent exposure to the allergen results in the allergen binding to the IgE on the mast cells, which causes cross-linking of the FcεRs, resulting in mast cell activation (4). The immediate response of mast cell activation is degranulation of the mast cells with the release of the granule contents (5); these are preformed mediators such as histamine and heparin. The activated mast cells also begin to synthesise new mediators (6), especially leukotrienes, prostaglandins and cytokines (TNFα).



3. Clinical symptoms of allergy

Allergic responses can be localized to the site of allergen or spread throughout the body and become systemic. The clinical symptoms of localized allergic reactions depend on where the mast cells are localized and can result in asthma, hay fever, diarrhoea/vomiting or skin reactions (eczema and hives). A systemic allergic reaction is called anaphylaxis.

A. Local allergic reactions

- **Asthma**

The main symptom of asthma is shortness of breath. This is caused by narrowing of the large and small airways, a condition called bronchospasm.

The narrowing of the airways can be due to three factors (Figure.3). Histamine, LTC₄ and PGD₂ released by mast cells cause smooth muscle contraction and therefore bronchoconstriction. Histamine and LTC₄ also increase mucus secretion into the airways, which further narrows the available passage for air flow. The late phase reaction results in an inflammatory exudate consisting of eosinophils, neutrophils, basophils, macrophages and platelets, which causes further blockage of the airways.

Prolonged exposure to the allergen can lead to a condition known as bronchial hyper-reactivity. In this condition the bronchi are exquisitely sensitive to histamine and other mediators so that bronchospasm and asthmatic attacks can be triggered by stimuli such as exercise, cold air or passive cigarette smoke without exposure to the allergen. If the individual can avoid the allergen for a period of time this hyper-reactivity may disappear.

- **Rhinitis (hayfever):**

Hayfever is characterised by a blocked and runny nose (rhinorrhoea), coughing, sneezing and itchy eyes and is the result of activation of mast cells in the nose and conjunctivae of the eye. Because the allergen is often pollen, hayfever can be seasonal and occur only during the time of year when a particular pollen is present. The mast cell mediators cause localized vasodilation and increased vascular permeability. This may be accompanied by the entry of mucosal mast cells into the nasal mucosa, where normally only connective tissue mast cells are found. The presence of both types of mast cells can complicate the chemotherapy of rhinitis since neither cell type responds to all anti-allergic drugs.

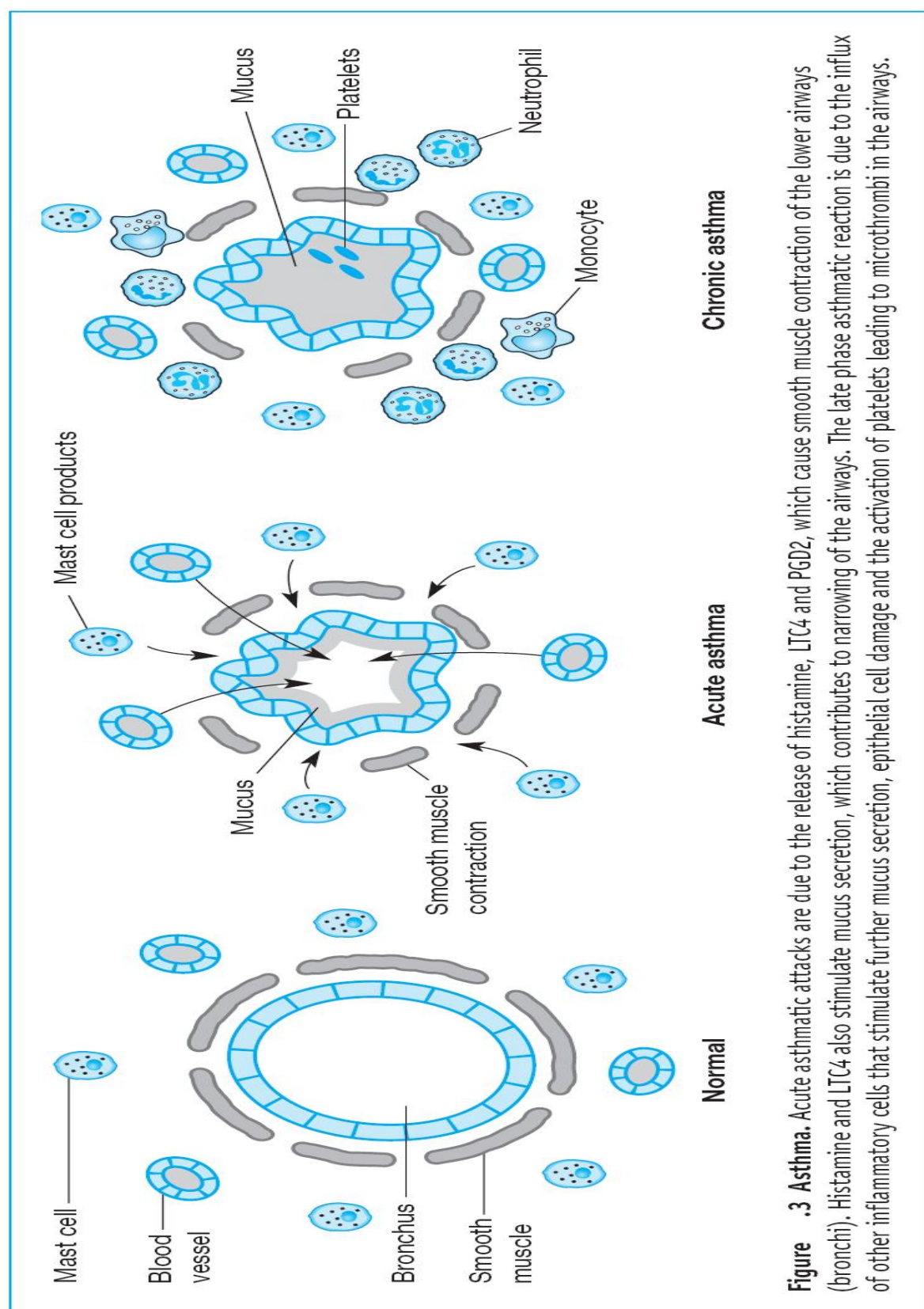


Figure 3 Asthma. Acute asthmatic attacks are due to the release of histamine, LTC₄ and PGD₂, which cause smooth muscle contraction of the lower airways (bronchi). Histamine and LTC₄ also stimulate mucus secretion, which contributes to narrowing of the airways. The late phase asthmatic reaction is due to the influx of other inflammatory cells that stimulate further mucus secretion, epithelial cell damage and the activation of platelets leading to microthrombi in the airways.

- **Food allergies:**

Activation of mast cells in the gut causes smooth muscle contraction, vasodilation, increased secretion of fluids and reduced peristalsis, resulting in vomiting and/or diarrhoea. The inflammation of the mucosa can also allow food allergens to enter the bloodstream, where they can activate mast cells at other sites, causing asthma or skin allergies.

- **Skin allergies:**

Activation of mast cells in the skin results in vasodilation and increased vascular permeability. These cause increased blood flow and oedema, which cause itchy red swellings (called hives or urticaria) that, if chronic, lead to a severe itchy skin rash (eczema).

B. Anaphylaxis:

This is the result of a systemic allergic reaction and can be life-threatening. Depending on its severity it can cause symptoms similar to those seen in localised allergic reactions or it can be a life-threatening condition known as anaphylactic shock. The mildest manifestation of anaphylaxis is hives (urticaria). More severe forms result in swelling of the lips, tongue and larynx, nausea, vomiting and asthma. Anaphylactic shock causes severe swelling of the larynx (laryngeal oedema), severe asthma and/or severe hypotension due to massive loss of fluid from the blood to the tissues, which can lead to circulatory collapse. Death can result from respiratory or circulatory failure.

4. Testing for allergy:

The most common test for allergy is the skin prick test. In this test a minute (A few microlitres) amount of allergen is introduced into the epidermis using

a lancet. If a person is sensitized to the allergen they will give a **wheal and flare** reaction. The basis of the wheal and flare reaction is the same as that for eczema or hives. The **allergen** cross-links the FcεRs on **skin mast cells** causing **mast cell activation**. The mast cell **mediators cause vasodilatation**, which increases blood flow to the area causing **redness** or a **flare**. The mediators also cause **increased vascular permeability**, which makes the blood vessels leaky. The **leakage of blood fluid into the tissue results in edema and swelling, the wheal**. The mast cell mediators also cause itching. The **flare** is seen **within seconds** or **minutes** and the **wheal** occurs **shortly after**. The immediate wheal and flare reaction usually disappears in less than an hour after application of the allergen but **can be followed by the late phase reaction**. This reaction typically occurs **four to six hours** later and is seen as a lump, which may be **accompanied by pain**. The main histological feature is the **accumulation of eosinophils, basophiles and neutrophils** at the site. Skin tests usually give good agreement with allergic status. However, some people with clinical allergy to known allergens fail to give a positive skin test to the allergen and others give a positive skin test to allergens but display no clinical signs of allergy to the same allergen. Total and antigen-specific IgE can be measured by ELISA. Antigen-specific IgE is now considered to be a more reliable predictor of atopy than total IgE.

5. Treatment of allergy:

Treatment of allergy can be divided into three main approaches: **allergen avoidance**, **pharmacological treatment** and **immunological intervention**.

a. Allergen avoidance:

A number of studies have shown that if individuals can avoid contact with allergens their clinical symptoms improve and can disappear. However, it is

not always possible to identify the allergen and, if identified, to avoid it totally. Allergen avoidance does not eliminate the underlying state of allergy, so that exposure to the allergen, even after many years of avoidance, can lead to reappearance of the symptoms.

b. Pharmacological intervention:

Pharmacological treatment of allergy is targeted at preventing mast cell degranulation or inhibiting the inflammatory response associated with allergic reactions. These agents treat the symptoms of allergy but do not change the underlying immune response and therefore do not provide a cure. The most common treatments for allergy are antihistamines, which block the actions of histamine, and inhibitors of mast cell degranulation. Drugs such as sodium cromoglycate and nedocromil inhibit mast cell degranulation but it is not clear whether they work on all mast cell types. More severe allergies are treated with steroids. These may be given locally, i.e. applied to the skin or inhaled, or systemically. They act primarily by inhibiting the production of cytokines or other inflammatory mediators by various cell types, including eosinophils and monocytes. They may also inhibit the migration of inflammatory cells into the site of the allergic reaction. Finally adrenaline is given to treat anaphylactic shock. These agents bind to β -adrenergic receptors on smooth muscle cells and cause smooth muscle relaxation, leading to a reversal of the vasodilatation and increased vascular permeability seen during anaphylaxis. It cannot be used as a long term treatment for allergy.

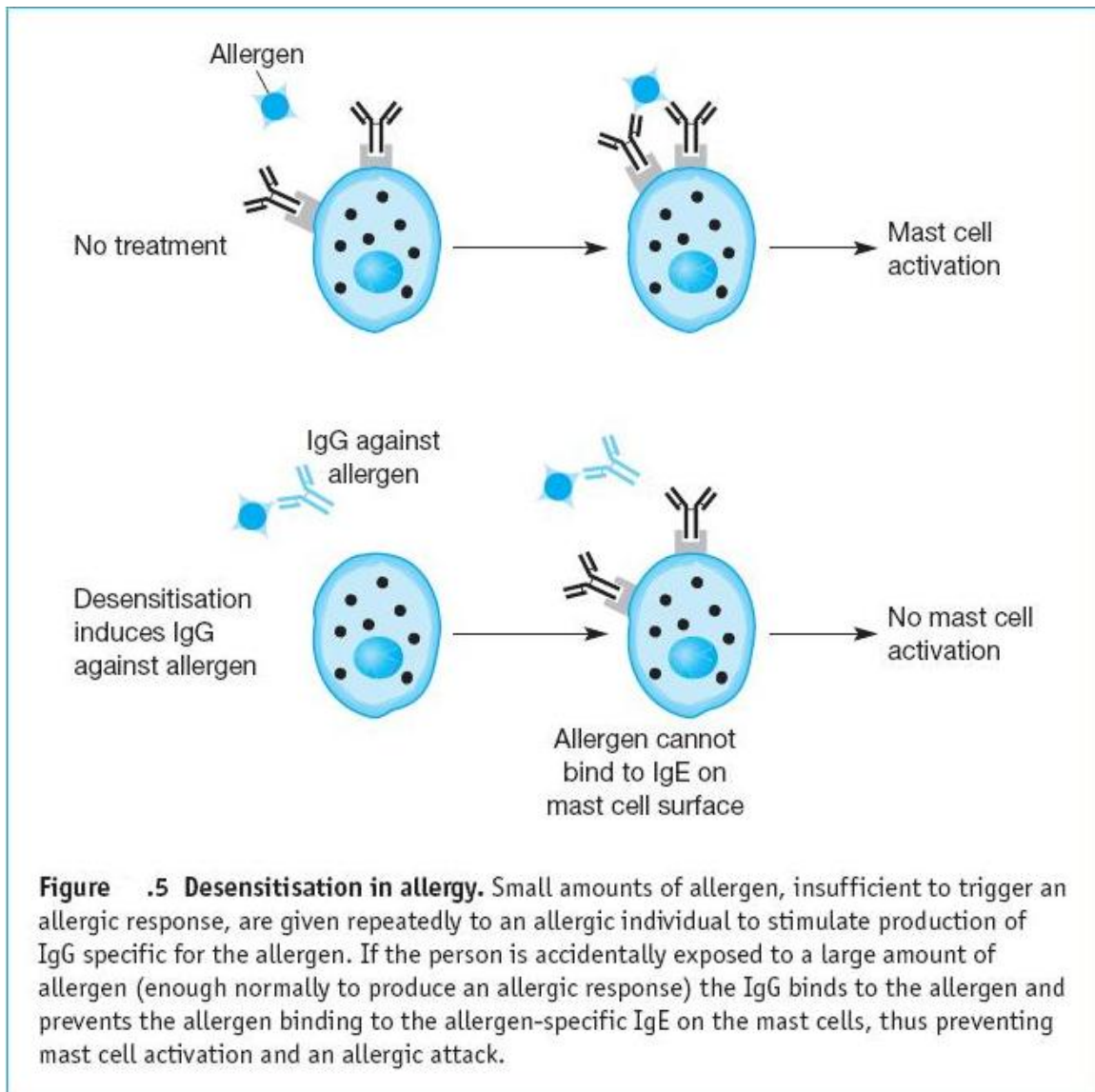
c. Immunological intervention:

In contrast to pharmacological approaches, immunological intervention attempts to modify the immune response against the allergen so that the

production or effects of IgE are negated. A number of different approaches are now being used or developed, some of which are described below.

Desensitization (hyposensitisation) involves **giving small amounts of allergen** to the patient to **induce the production of IgG against the allergen.**

Treatment of allergy If the individual subsequently encounters the allergen, **the IgG binds to the allergen and stops the allergen binding to IgE** on mast cells, thereby **preventing mast cell activation** (Figure.5). Although it can be effective, the procedure has the major drawback that giving too much allergen may trigger a potentially fatal anaphylactic attack. Another approach is to give **anti-IgE antibodies** to **block IgE** from **binding** to the **FcεR on mast cells** and activating them. For reasons that are not understood, it was found that the **anti-IgE antibodies** **inhibited** the **production of IgE** as well as inhibiting its activity; this may be due to anti-IgE binding to IgE on B cells and inhibiting them or causing their opsonisation. This approach has been successful at reducing allergic responses to the house-dust mite in clinical trials.



6. Type II hypersensitivity:

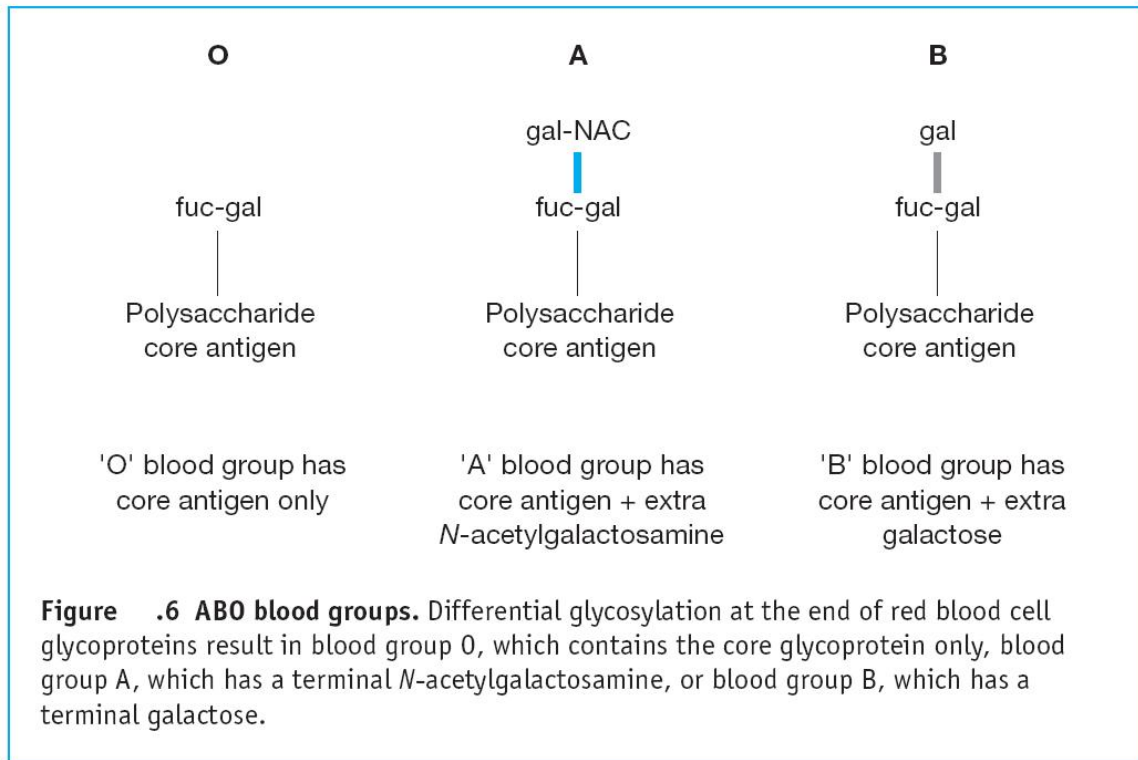
Type II hypersensitivity involves the production of IgG or IgM antibodies that react with antigens on cells or tissues. There are three major syndromes associated with type II hypersensitivity: these are blood transfusion reactions, haemolytic disease of the newborn and drug-induced hypersensitivity. Some autoimmune diseases involve the production of

autoantibodies against cells or tissues, causing a type II hypersensitivity-like reaction.

a. Blood transfusion reactions:

It was appreciated early in the twentieth century that it was not possible to transfuse blood at random between individuals. There are over 20 antigens on erythrocytes, and they vary between individuals. The most important and well known are those that comprise the ABO blood group system. Differences in glycosylation of red cell glycoproteins give rise to three variants of the glycoprotein called A, B or O. The groups are co-expressed so that individuals can be O, A, B or AB (Figure.6). These carbohydrate groups are similar to those seen on bacteria and therefore even if someone has not been exposed to a foreign blood group through blood transfusion, they may develop antibodies against the bacterial antigens that also recognize foreign blood groups. Individuals with blood group O make antibodies to groups A and B; individuals with blood group A make antibodies to B; and those with blood group B make antibodies to A. These antibodies are usually of the IgM class. AB individuals do not make antibodies to A or B and can receive blood of any type. If someone receives an incompatible blood group to which they have antibodies, the antibodies bind to the transfused red blood cells. IgM is very good at fixing complement and complement fixation results in activation of the classical complement pathway and lysis of the transfused red blood cells. This can result in the release of massive amounts of haemoglobin, some of which may be metabolized to bilirubin, which is toxic at high levels. Clinical symptoms include fever, chills, nausea and vomiting, and chest and lower back pains.

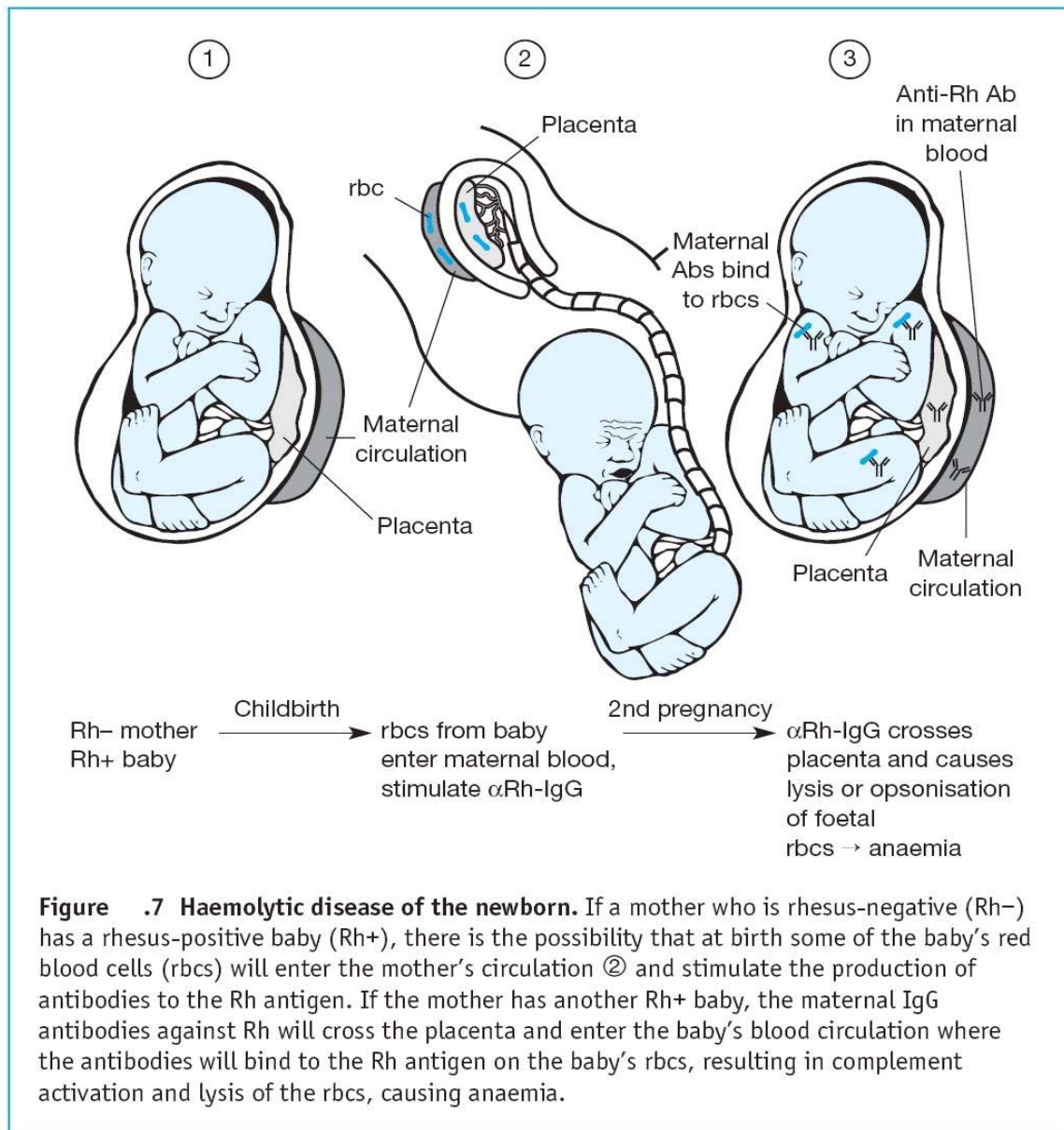
Blood transfusion reactions can also be caused by antibodies to minor blood group antigens. However, these antibodies do not usually arise through cross-reactivity with microbial antigens but instead are the result of repeated transfusions



b. Haemolytic disease of the newborn:

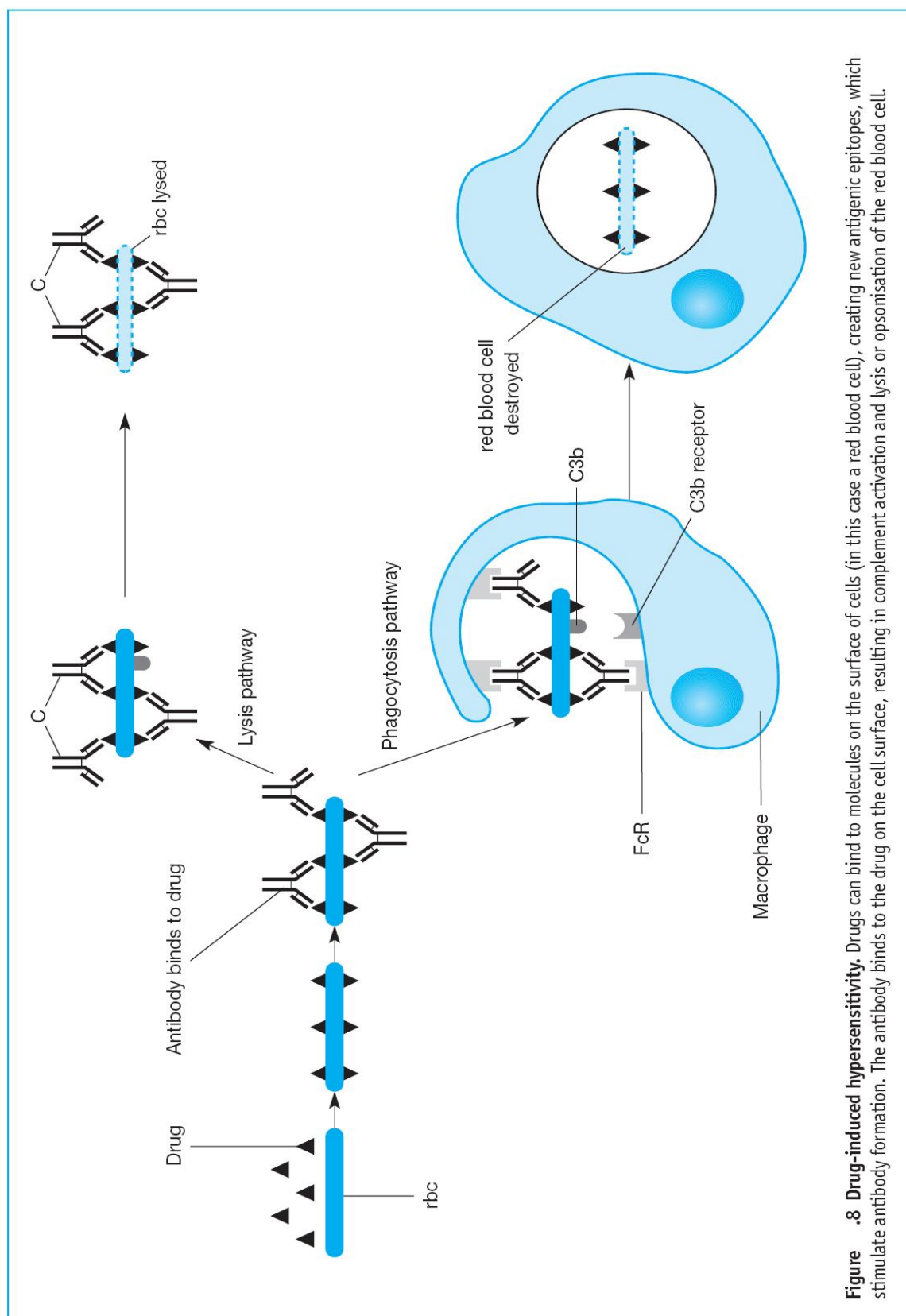
Another antigen on red blood cells is called the rhesus (Rh) antigen. Most individuals are Rh⁺ but a minority lacks the rhesus antigen and is Rh⁻. During childbirth considerable amounts of foetal blood enters the mother's bloodstream. If an Rh⁻ mother has an Rh⁺ baby the woman will be immunized against the Rh⁺ antigen during childbirth and produce IgG antibodies to Rh (Figure.7). If the mother has another Rh⁺ baby, the anti Rh IgG can cross the placenta and bind to the foetus's red blood cells. This can result in opsonisation of the red blood cells and their phagocytosis and

destruction in the liver or spleen. This results in an enlarged spleen and liver and toxicity due to bilirubin. In severe cases the condition can be fatal. Sensitization of the mother can be prevented by giving the mother a preparation of antibodies against the Rh antigen shortly after birth. These antibodies bind to the foetal red blood cells and cause their immediate removal and destruction before they have time to sensitize the mother.



c. Drug-induced hypersensitivities:

Some drugs or their metabolites, can bind to red blood cells or platelets. Although the drugs are too small themselves to stimulate an immune response, by binding to self-proteins the drug-self-protein complex can create a new antigen that appears foreign to the immune system. In a small proportion of people these new antigens stimulate antibody production against the drug. The antibodies bind to the drug and cause either complement- mediated lysis or opsonisation through Fc receptors and C3 receptors on phagocytes (Figure.8). Some drugs, such as penicillin, bind to red blood cells resulting in anemia; quinidine can bind to platelets, resulting in thrombocytopenia. Usually, when the drug is withdrawn the symptoms resolve as non-drug-bound blood cells replace the drug-bound ones.



7. Type III hypersensitivity:

Type III hypersensitivity reactions are caused by immune complexes of antigen and antibody. They are seen in a number of situations and can be local or systemic (Figure.9).

a. Systemic immune complex disease:

The historical example of systemic immune complex disease is serum sickness. Animal serum (usually horse), containing antitoxin antibodies, was given as a treatment for diphtheria or tetanus to neutralise the toxins secreted by the bacteria. Because the serum came from another species, the serum proteins stimulated a strong antibody response. If the person was given a second dose of serum, the antibodies bound to the serum proteins forming immune complexes. These complexes are deposited in blood vessel walls, especially those of the kidney, skin and joints, where they fix complement, leading to an inflammatory response. The resulting symptoms include fever, rashes, arthritis and kidneys malfunction. Although serum sickness can now be avoided, systemic immune complex disease can occur in some chronic infectious diseases, such as leprosy, malaria, hepatitis and streptococcal infection.

b. Localised immune complex disease:

Type III hypersensitivity can also cause a localized disease. This is most commonly seen after repeated inhalation of antigen. The two best-known examples are farmer's lung, caused by the inhalation of mould spores on hay, and pigeon fancier's disease, caused by the inhalation of pigeon antigens in dried fecal particles.

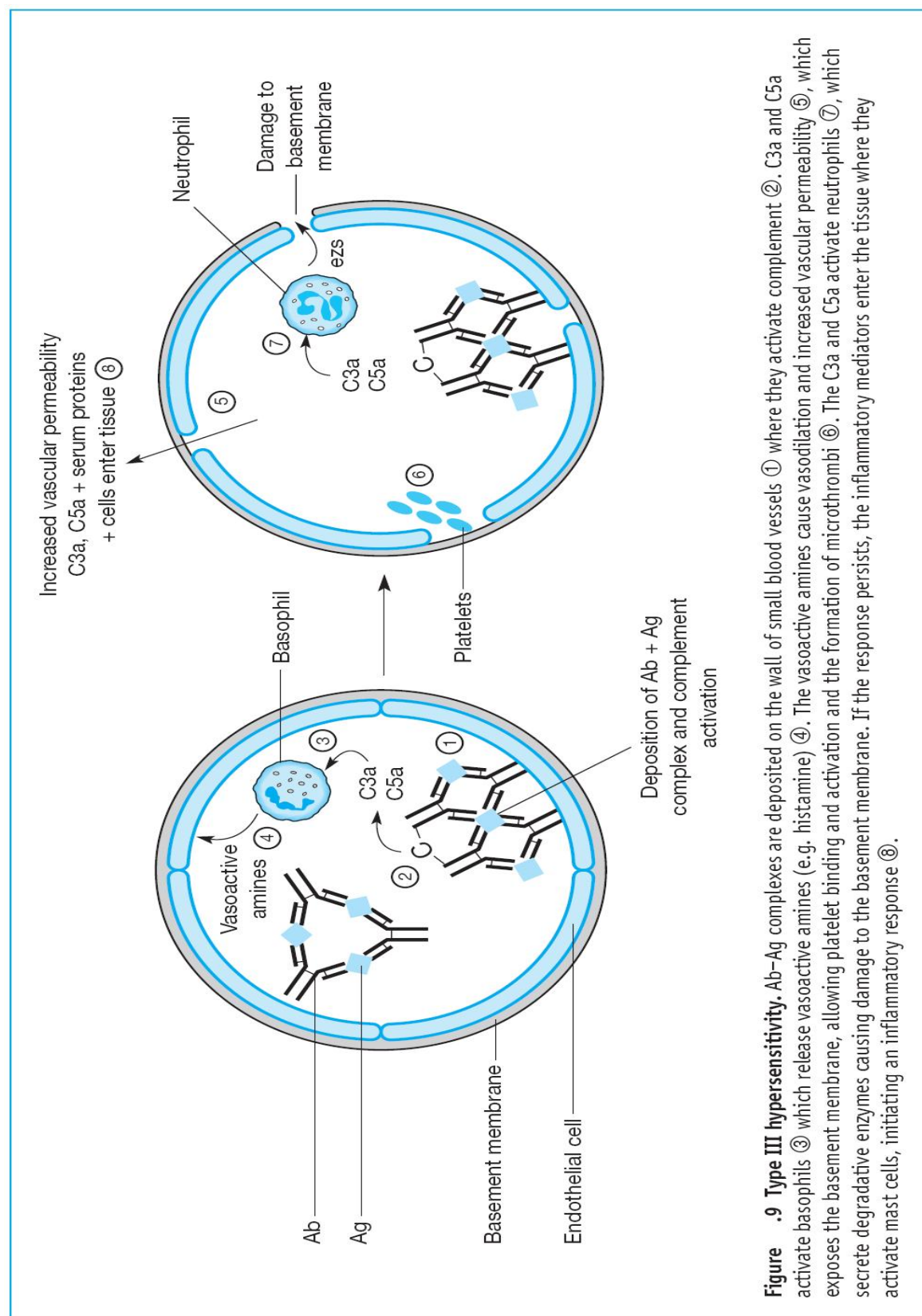


Figure .9 Type III hypersensitivity. Ab-Ag complexes are deposited on the wall of small blood vessels ① where they activate complement ②. C3a and C5a activate basophils ③ which release vasoactive amines (e.g. histamine) ④. The vasoactive amines cause vasodilation and increased vascular permeability ⑤, which exposes the basement membrane, allowing platelet binding and activation and the formation of microthrombi ⑥. The C3a and C5a activate neutrophils ⑦, which secrete degradative enzymes causing damage to the basement membrane. If the response persists, the inflammatory mediators enter the tissue where they activate mast cells, initiating an inflammatory response ⑧.

8. Differences between type II and type III hypersensitivity:

There is often confusion about the differences between type II and type III hypersensitivity, which both involve the same Ig classes (usually IgG) and complement activation leading to inflammation. The crucial difference between type II and type III hypersensitivity is the nature of the antigen. In type II hypersensitivity the antigen is expressed on the target tissue that is affected by the disease, e.g. in haemolytic disease of the newborn the rhesus antigen is on the red blood cells that are removed or destroyed by the antibody. By contrast, in type III hypersensitivity the antigen is not associated with the target tissue. Type III hypersensitivity is the result of the formation of immune complexes of antibody and antigen. It is not the nature of the antigen that determines where type III hypersensitivity reactions occur. The sites of type III hypersensitivity reactions are determined by where the immune complexes are deposited, and this is usually in small blood vessels such as those seen in the joint, skin and kidneys.

9. Contact hypersensitivity:

Contact hypersensitivity reactions (CS) occur in response to encounter of small chemicals with the skin. This results in an eczematous reaction involving swelling, redness and severe itching, which occurs 24–72 hours after contact with the agent in a sensitive individual and is known clinically as contact dermatitis. One of the best-known causes of CS reactions, especially in the USA, is poison ivy. The leaves of the poison ivy plant contain pentadecacatechol, a small chemical that stimulates a very strong CS reaction. Other causes of CS reactions are some metals, such as nickel, gold and chromium, used in jewellery, and chemicals found in rubber. The actual chemicals that stimulate CS reactions are small, often with a molecular mass

of less than 100 DA. These chemicals are too small to stimulate an immune response in their free form and are called haptens. These haptens have the ability to chemically attach themselves to proteins of the host where they modify the proteins, creating new antigenic epitopes that stimulate the CS response. CS reactions are very similar in nature to delayed-type hypersensitivity reactions in that they involve primarily T cells and monocytes but not antibody. However, whereas delayed-type hypersensitivity reactions play a protective role against some intracellular pathogens, CS reactions usually occur against harmless agents and as such are detrimental to the host. Furthermore there seems to be more involvement of CD8 T cells in CS reactions, although their role is not clear. The CS reaction is initiated by haptenated-host proteins being taken up by Langerhans cells in the skin. The Langerhans cells migrate to the draining lymph nodes, where they stimulate hapten-specific CD4 T cells to divide and differentiate into Th and also stimulate hapten-specific CD8 T cells. A week or so after the initial encounter the sensitized CD4 T cells migrate to the site of contact and enter the tissue where they are stimulated by hapten being presented on class II MHC by dendritic cells and tissue macrophages. The Th are stimulated to release cytokines ($\text{IFN}\gamma$, $\text{TNF}\alpha$, $\text{TNF}\beta$, IL-3 and GM-CSF) and chemokines that attract monocytes to the site, resulting in swelling and redness and some tissue damage due to macrophage activation. CD8 T cells also enter the site, although it is not clear whether they contribute to the damage by directly killing hapten-bearing host cells or by secreting cytokines. Upon subsequent encounter with the hapten the reaction is more severe because of the pre-existing sensitized hapten-specific T cells. The response can then involve severe blistering because the extensive

activation of macrophages (and possibly involvement of CD8 T cells) results in more damage and separation of skin cells.

Summary

- There are three types of antibody-mediated hypersensitivities: type I, type II and type III.
- Type I hypersensitivity involves the production of IgE against usually innocuous antigens such as pollen, house-dust mite faeces or food.
- The IgE binds to Fc receptors for IgE (FcεRs) on the surface of mast cells. Upon re-exposure to the allergen, allergen cross-links the FcεRs, leading to mast cell degranulation.
- Depending on the site of mast cell degranulation, allergy can be manifested by asthma, hayfever, vomiting and diarrhoea, hives, eczema or anaphylactic shock.
- Both genetic and environmental factors contribute to the development of allergy. The incidence has increased dramatically in the past 50 years.
- Treatment of allergy involves allergy avoidance, antihistamines, inhibitors of mast cell degranulation, steroids and immunological intervention, such as desensitisation or administration of anti-IgE antibodies.
- Type II hypersensitivities are caused by IgG or IgM antibodies reacting against antigens on cells or tissues, causing opsonisation and complement fixation.
- There are three major types of type II hypersensitivity reactions: blood transfusion reactions, haemolytic disease of the newborn and drug-induced hypersensitivities.

Type III hypersensitivity reactions are caused by the formation of

immune complexes, which are deposited and initiate inflammatory reactions. Antigens may be benign, such as foreign serum proteins (causing serum sickness) and pigeon faecal antigens (causing pigeon fancier's lung), or because of chronic infection with pathogens, such as malaria, leprosy or hepatitis.

- A fourth type of hypersensitivity reaction is contact hypersensitivity, which does not involve antibody but involves CD4 and CD8 T cells and monocyte/macrophages.

3. Acquired immunodeficiency syndrome (AIDS)

Key topics

- History of AIDS
- The human immunodeficiency virus (HIV)
 - Structure
 - Replication cycle
- Clinical course of HIV infection
- Immunology of HIV infection
 - Effects of HIV on the immune system
 - Immune response to HIV
- Chemotherapy of AIDS
- HIV vaccines

1. History and incidence of AIDS:

The story of AIDS began in 1981 when a cluster of unusual diseases was observed in certain groups of people. The two main diseases were pneumonia caused by a yeast, *Pneumocystis carinii*, and an unusual tumor called Kaposi's sarcoma. These diseases were seen initially in homosexual men but later the same symptoms appeared in intravenous drug users and haemophiliacs who were injecting blood-clotting factors. This pattern of disease occurrence suggested that a transmittable agent was responsible for the diseases. The unusual aspect of these diseases was that they are usually only seen in immunosuppressed people and not in people with a fully functioning immune system. The observation that individuals with these

diseases had low numbers of CD4 T cells was consistent with immunosuppression and in 1982 the term **acquired immunodeficiency syndrome**, or AIDS, was used by the Centers for Disease Control in Atlanta to describe the disease. In 1983 the virus that causes AIDS was isolated from the lymph node of an infected individual by Montagnier's group in Paris and was called the **human immunodeficiency virus**, or HIV for short. A second strain of HIV was identified in 1986; this was called HIV-2 and the first strain was renamed HIV-1. HIV-1 and HIV-2 differ in their virulence and geographical location. HIV-2 is less virulent than HIV-1 and is found primarily in western Africa. Genetic studies have shown that both HIV-1 and HIV-2 are natural viruses of primates that have jumped species to infect humans. HIV-1 came from chimpanzees and HIV-2 from the sooty mangabey. Both of these animals are killed for food and it is assumed that it was during this process that the virus initially infected humans. HIV-1 and HIV-2 do not cause immunosuppression in chimpanzees or sooty mangabeys; only when the virus crossed into humans did it cause the profound immunosuppression seen in AIDS.

2. Current incidence of HIV infection and AIDS:

It is estimated that by the end of 2005 over 65 million people worldwide were infected with HIV and over 24 million will have died. The highest rate of increase in HIV infection is seen in sub-Saharan Africa, where an estimated 20–40% of young adults are infected. As a result of this, the life expectancy in sub-Saharan Africa has almost halved; it is now in the 30s–40s instead of approaching 70, which would have been the estimated life expectancy if the AIDS pandemic had not occurred. The pattern of spread in Africa appears to be primarily by heterosexual contact and has a similar

incidence in men and women. By contrast, spread in Europe, the USA and Oceania is still mostly among ‘high-risk’ groups such as homosexuals and intravenous drug users and is much more prevalent in men than women. However, there are signs in the USA that the pattern of spread is changing, with more women being infected.

3. The human immunodeficiency virus:

a. Strains and variants:

Some of the terminology used to describe HIV can be confusing. The two strains of HIV are called HIV-1 and HIV-2. There are also many different subtypes of HIV-1 so vaccines will be needed to protect against all subtypes. Additionally, HIV has a very high mutation rate, giving rise to different forms of the virus known as variants. These variants are important because, as described below, they differ in which cell types they can infect. Although there are different strains, subtypes and variants of HIV, they are very similar in structure and replication and therefore will be described together and referred to collectively as HIV.

b. Structure of HIV:

HIV is a retrovirus (Figure.1) and contains RNA as its genetic material. The HIV genome contains two molecules of single-stranded RNA, each bound by a molecule of reverse transcriptase. Within the genome are also a p10 protease and a p32 integrase. The genome is surrounded by a nucleocapsid consisting of an inner layer of protein called p24 and an outer layer of protein called p17. The outer portion of the virus consists of a lipid envelope derived from the host cell membrane containing two viral proteins gp120 and gp41, which collectively are called viral **envelope proteins**.

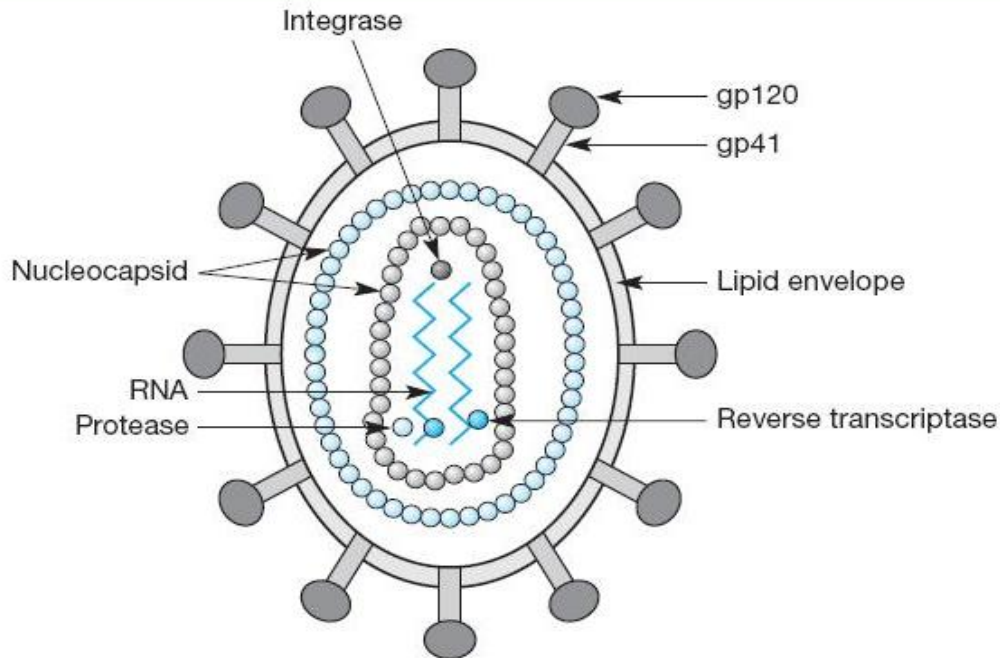


Figure .1 Diagram of an HIV particle. HIV is a retrovirus and has two single-stranded RNA molecules as its genetic material. The RNA is associated with reverse transcriptase, integrase and polymerase enzymes, which are necessary for viral DNA and RNA synthesis. Surrounding this is the nucleocapsid, which consists of an inner layer of p24 protein and an outer layer of p17 protein. The outer portion of the virus consists of a lipid layer derived from the host cell into which is inserted the viral gp41 envelope protein. Each gp41 protein molecule is associated with a molecule of the gp120 envelope protein.

c. Replication cycle of HIV:

Like any other virus, HIV must infect a host cell before replicating with the viral progeny, leaving the cell to infect others.

• Infection:

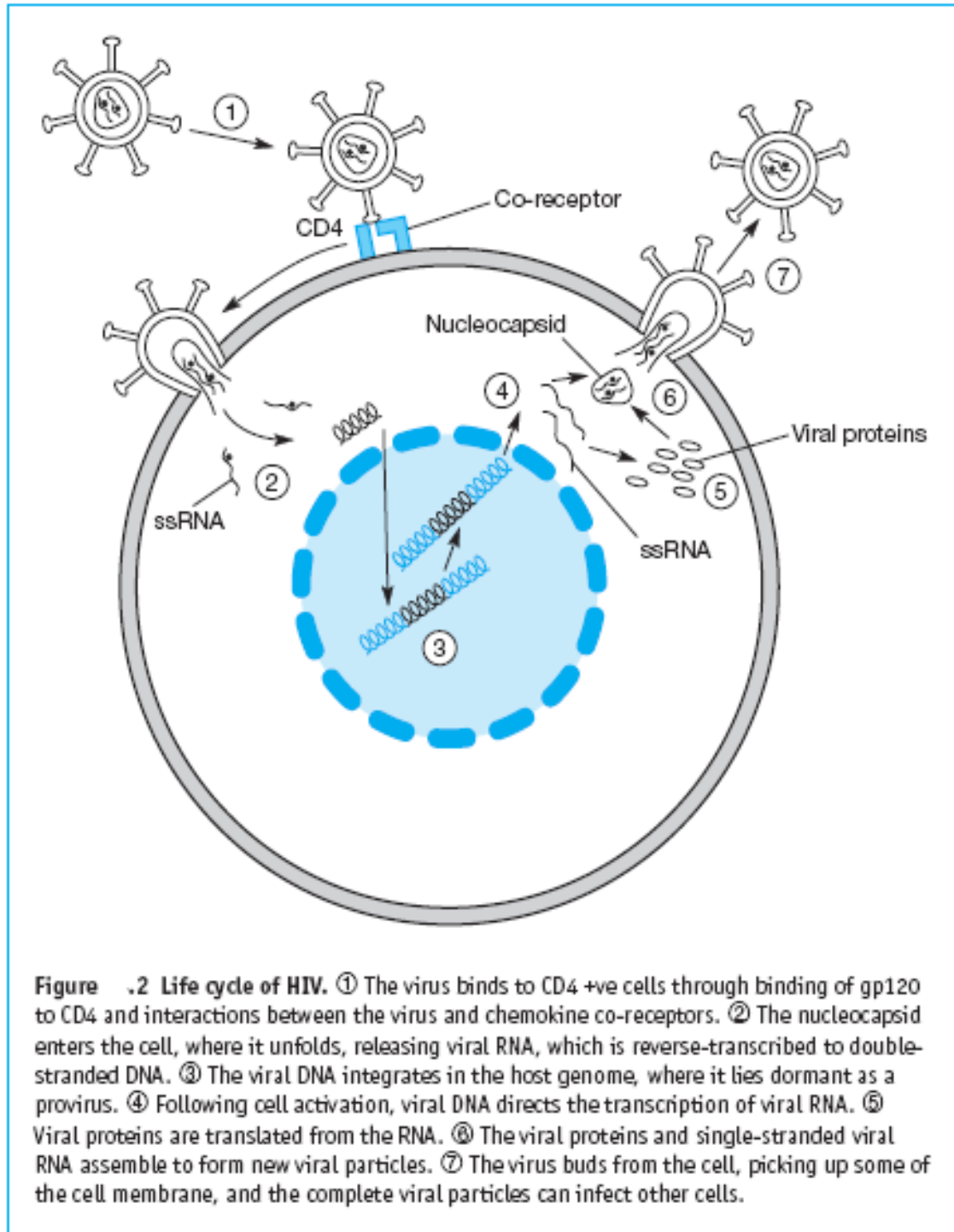
HIV infects CD4⁺ cells. In addition to CD4 T cells, monocytes and dendritic cells also express CD4, although at lower levels than T cells. There are two stages to viral infection: binding to the host cell, and fusion with the cell membrane to allow the virus to enter the cell (Figure.2). The initial binding of HIV to the host cell involves the gp120 protein on the surface of the HIV particle binding to CD4 on the host cell surface. However, binding of gp120

to CD4 is not enough for HIV to infect the cell. The gp41 component of the envelope protein binds to a second protein on the cell surface. This second protein differs between variants of HIV. In some variants the gp41 binds to the chemokine receptor CCR-5, which is on the surface of CD4 T cells, monocytes and dendritic cells. These variants can therefore infect all of these cell types and have been called M-tropic. Other HIV variants bind to another chemokine receptor called CXCR-4, which is present on CD4 T cells but not on monocytes or dendritic cells. These variants can only infect T cells and are called T-tropic. The CCR-5 gene has two alleles, one of which results in a non-functional form of the CCR-5 protein. Approximately 1% of Caucasians are homozygous for the null allele and therefore express no functional CCR-5 protein. These individuals are resistant to infection with HIV. Since their expression of CXCR-4 is normal, this suggests that initial infection is by M-tropic variants using the CCR-5R. Indeed isolates of HIV in recently infected individuals are predominantly M-tropic. The emergence of T-tropic variants is often a sign of rapid progression to AIDS. Following binding of Gp120 to CD4 and the relevant chemokine receptor, gp41 mediates fusion between the viral envelope and host cell membrane, allowing the viral nucleocapsid to enter the cell.

- **Replication:**

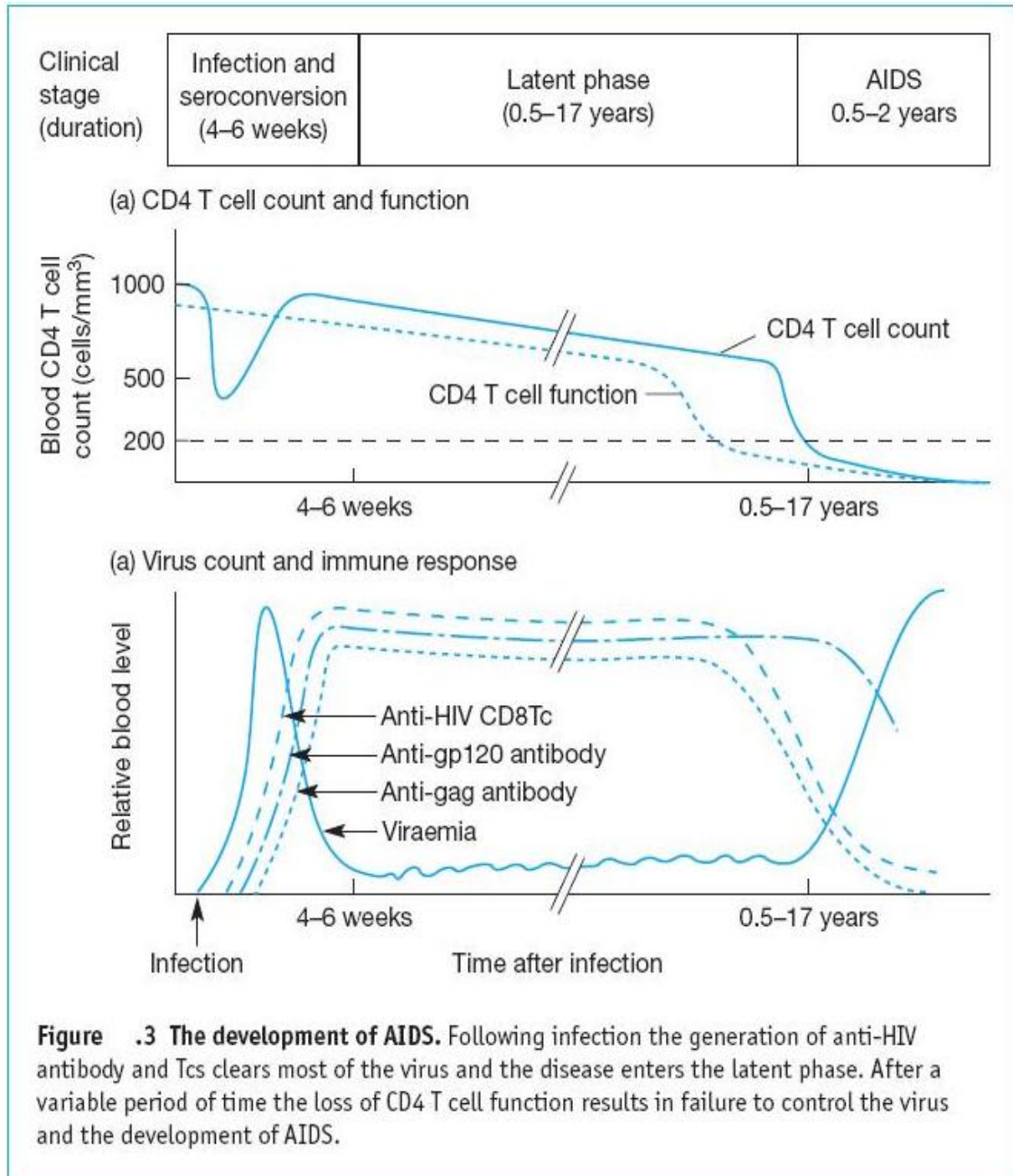
Once inside the cell the nucleocapsid of the virus is removed and the reverse transcriptase copies the RNA into double-stranded DNA. The DNA integrates into the host cell DNA, where it is known as a provirus. This stage of viral infection is known as the **latent** stage and the virus can lie dormant for a long time. When the infected cell is activated, viral RNA is transcribed from the proviral DNA and viral proteins are translated using host cell

protein synthesis machinery. The viral proteins and RNA assemble into particles that bud from the cell, taking some of the host cell membrane to form the viral envelope. These particles can now infect other cells.



4. Clinical course of HIV infection:

The clinical progression of HIV infection can be divided into three stages: infection, the latent stage and development of AIDS (Figure.3).



a. Infection:

Most people show no symptoms immediately after infection but about 15% demonstrate symptoms that are reminiscent of influenza: these include fever, malaise, aching muscles, sore throat and swollen lymph nodes. Some people also develop swollen lymph nodes without any other clinical symptoms.

Following infection, antibodies to HIV antigens are produced, a process called **seroconversion**. The detection of antibodies to HIV is used to test for infection.

b. Latency:

This period is generally asymptomatic although about 33% of infected people will have swollen lymph nodes. The average time from infection to the development of AIDS is about 10 years but the length of the latency period is extremely variable, lasting less than a year in some people and upwards of 15 years in others. It is not clear whether everyone infected with HIV will inevitably develop AIDS.

c. Development of AIDS:

The end of the latency period is accompanied by the emergence of various symptoms that indicate progression to AIDS without treatment. These symptoms include weight loss, night sweats, fever and diarrhoea. There are also infections such as oral candidiasis, herpes simplex and shingles caused by minor opportunistic infectious agents. AIDS is defined clinically by the appearance of major opportunistic infections or by a drop in the CD4 T cell count to below 200 cells/ μ l of blood. The opportunistic infections are caused by all categories of pathogens as shown in (Table.1). Without treatment AIDS invariably leads to death. Death is caused by combinations of the infections described in (Table.1) although some infections are more

prominent according to geographical location. In Europe, the USA and Oceania the commonest infection is pneumonia caused by *Pneumocystis carinii*. In Africa and Asia,

Table .1 Opportunistic infections in AIDS

<i>Pathogen</i>	<i>Type of organism</i>	<i>Disease</i>
<i>Candida albicans</i>	Fungus	Thrush, disseminated mucocandidiasis
<i>Pneumocystis carinii</i>	Fungus	Pneumonia
<i>Cryptococcus neoformans</i>	Fungus	Meningitis
Herpes simplex	Virus	Pneumonia
Varicella-zoster (chicken pox)	Virus	Shingles, pneumonia
Cytomegalovirus	Virus	Pneumonia
<i>Mycobacterium tuberculosis</i>	Bacterium	Pneumonia
<i>Salmonella</i> spp.	Bacterium	Diarrhoea, septicaemia
<i>Cryptosporidia</i>	Protozoan	Diarrhoea
<i>Toxoplasma gondii</i>	Protozoan	Encephalitis

5. Immunological events associated with HIV infection:

HIV has one of the most complicated relationships with the immune system of any infectious agent. Because of the nature of the cells it infects and the molecules that it binds to, HIV has a dramatic effect on the immune system, eventually causing profound immunosuppression. Before that stage, and contrary to earlier beliefs, the immune system, far from failing to mount a significant response against the virus, generates a very powerful response

against HIV. With almost any other virus this response would be enough to eliminate the pathogen but because of the special features of HIV the virus is able to survive and eventually destroy the immune system.

6. Changes in the immune system during HIV infection:

The hallmark of HIV infection is the gradual decrease in CD4 T cell numbers. However, there are a number of other changes to the immune system that occur following HIV infection.

a. Changes in lymph nodes:

Many HIV+ individuals develop swollen lymph nodes during initial infection and this can persist even during the clinically latent phase. Histological examination of the lymph nodes shows increasing disruption of the normal lymph node architecture, an influx of CD8 T cells and eventual loss of germinal centres.

b. Loss of CD4 T cell function:

As the infection progresses HIV+ individuals show a loss of CD4 T cell function that cannot be explained simply on the basis of reduced CD4 T cell numbers and is somehow associated with the effects of the virus (Figure.3). Transplantation recipients taking immunosuppressive drugs can show drops in CD4 T cell numbers similar to those seen in HIV+ individuals but the transplant recipients demonstrate much better CD4 T cell function. The reasons for the loss of CD4 T cell function are not clear. One consequence of the loss of Th activity is the reduced ability to mount a delayed-type hypersensitivity reaction and as the infection progresses the ability to generate antibody responses is also lost.

c. Antibody abnormalities:

Somewhat paradoxically, HIV+ individuals can demonstrate increased total

levels of serum Ig despite the impaired ability to generate specific antibody responses. Again the basis for this is not clear but it is thought to reflect abnormalities in the normal regulation of immune responses caused by the virus. Possibly also related to generalized immune dysfunction, HIV+ individuals can show increased production of autoantibodies to antigens such as red blood cells, spermatozoa or myelin (a component of nerve sheaths) and they may suffer from flare-ups of allergies such as eczema.

7. Immune response to HIV:

Immediately following infection with HIV there is a period of rapid viral replication resulting in high levels of virus in the blood (viraemia) (Figure.3). HIV is an intracellular pathogen and as such would be expected to stimulate a strong CD8 cytotoxic T cell response and possibly a delayed type hypersensitivity response, as well as the production of antibody. In fact strong antibody responses to gp120 envelope protein and the p24 protein of the nucleocapsid (also known as gag) are seen soon after viral infection and there is also a powerful CD8 cytotoxic T cell against gp120, p24 and some of the proteins that make up the reverse transcriptase (pol antigens). The combined antibody and cytotoxic T cell response is extremely effective and eliminates more than 99% of the virus. Following this strong response the disease usually enters the latent stage. It was initially thought that the latent stage of the disease was one in which the virus was largely in the proviral stage and therefore inactive and that very few CD4 T cells were being destroyed as a consequence of being infected by the virus. These conclusions were based primarily on studies examining the number of productively infected (meaning that whole virus could be cultured from the cells) CD4 T cells and overall CD4 T cell numbers in the blood of HIV

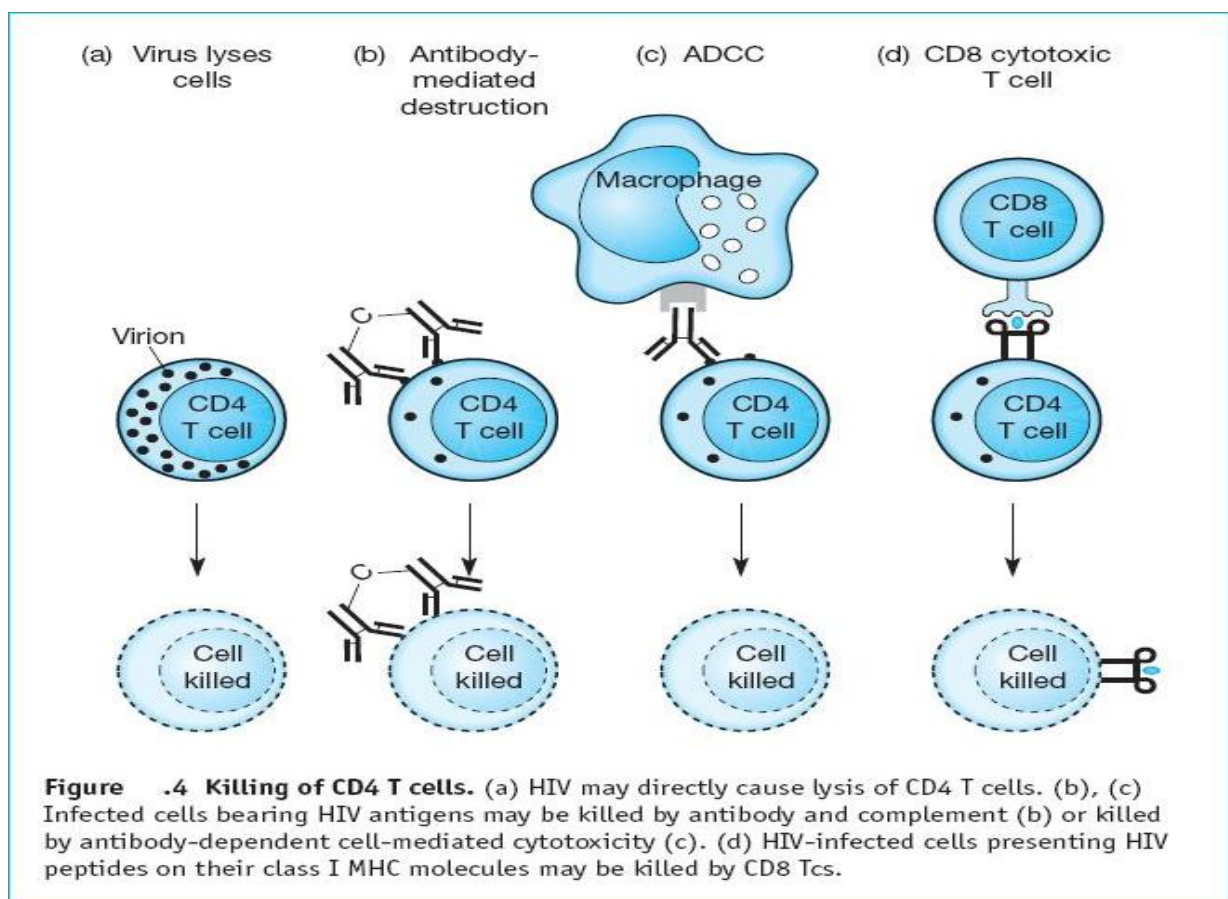
infected individuals. A number of recent studies suggest that this picture obtained from blood was not accurate. Newer techniques to measure viral RNA, a sign that the virus is replicating, have shown that there is a persistent level of active viral replication in the blood even during the latent period. The level of this viraemia is a good prognostic indicator of the progression of the disease: individuals with a low level of viraemia progress more slowly to AIDS than those with a higher level of viraemia. Examination of lymph nodes has shown 10–100 times more productively infected CD4 T cells than seen in blood. Furthermore, lymph nodes of HIV+ people in the latent period show structural abnormalities that become more severe as the disease progresses. Also during HIV infection the relative proportion of the total number of CD4 T cells in the blood increases so that the actual loss of CD4 T cells is greater than that reflected in the blood. Treatment of infected individuals with drugs that prevented the virus from infecting new cells showed that (i) the immune system was eliminating up to 30% of the total viral load *every day* but that in the absence of these drugs the virus replicated at an enormous rate and the eliminated virus was replaced, and (ii) up to 2×10^9 CD4 T cells were being destroyed every day but most of these were replaced by the immune system so that the overall CD4 levels in the blood declined only very slowly. It now appears that the latent stage of HIV infection may be clinically latent but actually represents a very dynamic situation with high viral destruction and replication and extensive CD4 T cell destruction and replacement. Two questions occur as a consequence of this picture: why does the immune response not clear the virus, and what is the cause of the extensive CD4 T cell destruction? In terms of why the

immune system does not clear the virus, HIV has a number of features that enable it to survive in the face of a powerful immunological onslaught:

- It has a very high replication rate. This is the main reason why the virus is not totally cleared.
- It can hide as a provirus where it is not detectable by the immune system.
- It has a very high mutation rate, so that antigens to which antibody and CD8 Tcs have been made mutate and are no longer recognised by the immune system. Antibodies and CD8 Tcs are made against the mutated antigen but this can mutate again and so the process of evading the immune response goes on and on. The cause of CD4 T cell loss is thought to be a combination of direct killing by the virus and destruction of virally infected cells by the immune system (Figure.4). CD4 cells that are infected by the virus express viral antigens on their surface. These may be viral peptides presented by class I MHC or they may be soluble gp120 bound to CD4 on the T cells. HIV+ individuals can have large amounts of soluble gp120 in their blood and lymph, which will bind to CD4. Because of the expression of viral antigen, CD4 T cells can be killed in a number of ways (Figure.4):

- **Antibody + complement.** Anti-gp120 binds to the gp120 bound to CD4, resulting in complement fixation and activation and cell lysis.
- **Antibody-dependent cell-mediated cytotoxicity.** Macrophages and NK cells have Fc receptors on their surface. This enables them to bind to the Fc portion of the anti-gp120 antibody bound to the CD4 T cell and kill the cell.

• **CD8 cytotoxic T cells** kill virally infected cells expressing HIV antigenic peptides in association with their class I MHC. By generating these responses the immune system is actually doing what it is supposed to; it is generating effector mechanisms that eliminate virally infected cells and thereby the virus. Unfortunately the main type of infected cell is the CD4 T cell and therefore the virus kills the very cells needed to generate an immune response against it. Although the immune system makes a valiant effort to replace the CD4 T cells that are destroyed every day, the production of CD4 T cells never quite matches the destruction and here is a gradual drop in CD4 T cell numbers. Eventually the CD4 T cell numbers become too low to maintain normal immune responsiveness and immunosuppression ensues.

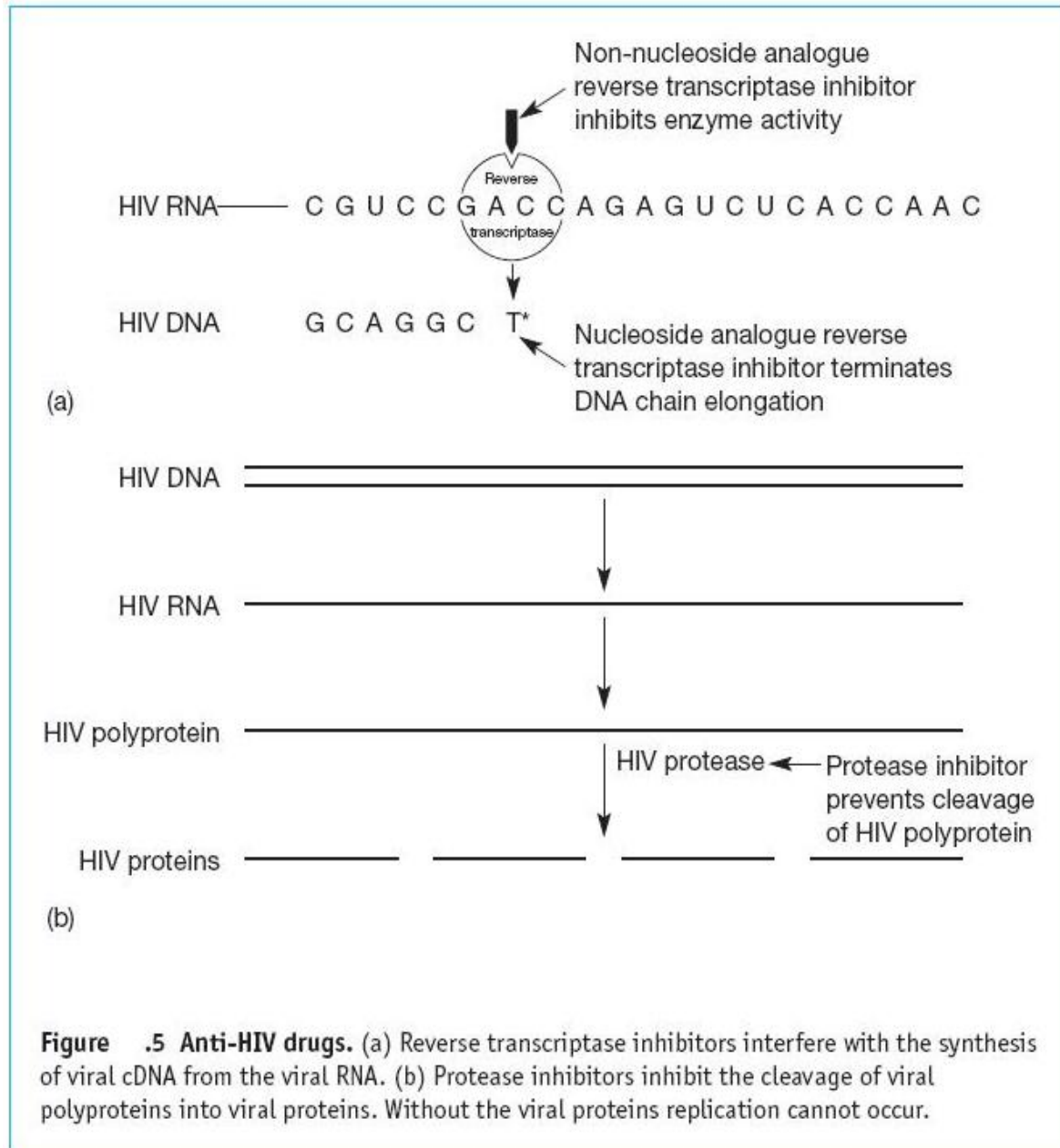


8. Chemotherapy of HIV:

The high mutation rate of HIV is not only a problem for the immune system. It is also a problem in trying to treat HIV+ individuals with antiviral drugs. The first anti-HIV drug was zidovudine (AZT), a reverse transcriptase inhibitor (Figure.5) introduced in 1987. Although it inhibited replication of HIV, the virus very quickly mutated and became resistant to the drug.

There are now three types of anti-HIV drug in use clinically and combinations of two, or preferably three, drugs are of longer clinical benefit because there is less chance of the virus mutating to become resistant to three drugs at once. The three main categories of drug are nucleoside analogue reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and HIV protease inhibitors (Figure.5). The two types of reverse transcriptase inhibitor act in different ways to inhibit the reverse transcriptase and therefore complement each other's action. The protease inhibitors inhibit HIV protease, an enzyme used to cleave a number of viral precursor polyproteins to produce many HIV proteins and enzymes (Figure.5). Without protease activity the virus cannot replicate. Usually combination chemotherapy consists of two reverse transcriptase inhibitors and a protease inhibitor. This can be very effective at lowering levels of virus and raising CD4 T cell levels and results in significant clinical improvement so that many AIDS patients are able to leave the AIDS clinic and return home. The new therapy is also thought to be responsible for the sharp drop in death from AIDS seen in the USA since 1996. However, there are problems with combination therapy. There is considerable toxicity, especially to the bone marrow and gut, which means some people cannot take the drugs. The regime for taking the drugs is complicated and intrusive

to a normal lifestyle. Some of the drugs have to be taken with food and some without, and some drugs cannot be taken within a certain time period of each other. Finally the drug treatment is very expensive – about \$15,000 a year – so that it is not readily available in the countries that need it the most.



9. HIV vaccines:

Attempts to generate vaccines for HIV have fluctuated in intensity over the years for a variety of different reasons, both economic and scientific. Initially two types of vaccines were considered:

- **Prophylactic vaccines:** The aim of these vaccines was to provide protection to individuals who were not infected with the virus; in this respect the aim was the same as for vaccines against other infectious agents such as measles, mumps, polio, smallpox, etc.
- **Therapeutic vaccines:** Because AIDS initially was considered a disease of immunosuppression where HIV-infected people did not make a very good response against the virus; it was thought it might be possible to boost the immune response of HIV-infected people so that they could mount a response and clear the virus. With the current appreciation that most HIV-infected people make a very strong response against the virus it is not so clear how effective this approach might be. The development of prophylactic vaccines against HIV has considerable difficulties of both an immunological and a logistical nature. The main immunological issues concerning development of an HIV vaccine are which of the many different current approaches to vaccine development to use, given the many subtypes and variants and the mutation rate of the virus, and what type of immune response to try and stimulate. Additionally there are no good animal models of HIV. The main logistical problems are in testing the vaccines. Traditionally vaccines were developed against acute diseases that often occurred in waves or epidemics (e.g. measles or mumps) and had a low mortality rate. It was therefore comparatively straightforward to vaccinate a section of the population and see whether these people were protected from

the disease compared with non-vaccinated individuals. It was possible this way to get an answer to the effectiveness of the vaccine in a relatively short time. Because AIDS is a chronically progressive disease the conventional approach would take many years to give an answer – too long for most people to wait. There are a number of vaccines in clinical trials at present using DNA, recombinant and antigen/peptide approaches, although none has demonstrated convincing protection so far.

Summary

- AIDS as a disease was first identified in 1981 in groups of high-risk individuals: homosexual men, intravenous drug users and haemophiliacs. Currently it is estimated that 30 million people are infected with the AIDS virus.
- The virus causing AIDS was isolated in 1983 and called the human immunodeficiency virus (HIV). A second strain, called HIV-2, was identified in 1986.
- HIV is a retrovirus, i.e. its genetic material consists of RNA. It infects CD4+ cells, mainly CD4 T cells but also monocytes and dendritic cells.
- Clinically HIV infection is characterised by the development of opportunistic infections, especially with the yeast *Pneumocystis carinii*, and the development of a rare tumour, Kaposi's sarcoma.
- The development of AIDS can take over 15 years from infection. Following infection and seroconversion there is a latent period that is largely aclinical.
- Although the latent period is aclinical there is an extensive amount of viral destruction and replication and also destruction and replacement of CD4 T cells. However, there is a gradual net loss of CD4 T cells, leading eventually

to immunosuppression and the development of opportunistic infections, which are fatal without treatment.

- The most successful chemotherapy for HIV is triple therapy with a combination of reverse transcriptase inhibitors and viral protease inhibitors. However, this therapy has significant side-effects, is difficult to comply with and is very expensive.
- HIV vaccines are faced with the problem of the high mutation rate of the virus and logistical problems of testing the efficacy of a vaccine against a chronic infection.

4. Manipulating the immune system: (transplantation and tumours)

Key topics:

- Transplantation
 - Types of transplant
 - Transplantation antigens
 - Immune responses to transplants
 - Preventing graft rejection
- Tumour immunology
 - Tumour antigens
 - Antibody-based tumour therapy
 - Tumour vaccines

1. Introduction:

Part of the reason for understanding as much as possible about how the immune system functions is to aid in the development of treatments that can manipulate the immune system to prevent or treat diseases in which the immune system is involved. One method of manipulating the immune system, that of vaccination against infectious disease has prevented millions of deaths and has removed the need for billions of children to go through the misery and danger of many common childhood illnesses such as measles, mumps, polio and diphtheria. However, manipulation of the immune system does not stop at vaccination. In situations such as transplantation and cancer

it is also desirable to alter the way the immune system behaves, either to prevent graft rejection or to harness the power of the immune system to attack tumour cells. This chapter will cover some aspects of immune manipulation in the fields of transplantation and cancer.

2. Transplantation:

Transplantation involves the transfer of tissue, cells or organs from one anatomical site to another. These two anatomical sites may be different sites on the same person, e.g. with a skin transplant for burns, or they may be equivalent anatomical sites in different people, e.g. a heart transplant from one individual to another. Transplantation of a variety of tissues, cells and organs is now an accepted medical procedure for treating a wide variety of diseases and further treatments using transplants are being developed (see Table.1).

Table .1 Types of clinical transplant

<i>Transplant</i>	<i>Disease treated</i>
Kidney	Nephritis, diabetic complications
Heart	Heart failure
Lung/heart + lung	Cystic fibrosis
Pancreas/islets of Langerhans	Insulin-dependent diabetes mellitus
Liver	Congenital defects of the liver, hepatitis, cirrhosis
Cornea	Cataracts
Skin (usually autologous)	Burns
Bone marrow	Congenital haematopoietic defects (e.g. thalassaemia, severe combined immunodeficiency), leukaemia, lymphoma

3. Categories of transplant:

As with so many other areas of immunology, transplantation has its own jargon. Terms have been used to describe the relationship between donor and recipient in transplantation:

- a. Autograft:** A transplant of tissue from one site to another in the same individual is called an autograft. The most common instance is the transplantation of healthy skin to the burn area of burn victims to try to prevent infection and dehydration and promote wound healing.
- b. Isograft:** An isograft is a transplant performed between genetically identical individuals, e.g. identical twins. Like autografts, isografts will normally survive without any immunosuppression.
- c. Allograft:** Transplants between non-genetically identical individuals of the same species are called allografts. This is the most common category of transplant and the graft will be rejected in the absence of immunosuppressive therapy.
- d. Xenograft:** This involves transplanting tissue from one species to another, e.g. baboon to human or pig to human. As might be expected, xenografts are subject to the strongest rejection responses and pose extra problems in preventing their rejection.

4. Transplantation antigens:

Transplants between non-identical donors and recipients (allografts or xenografts) are rejected because the immune system recognises foreign antigens on the graft and does what it is supposed to: mount a specific immune response to eliminate the foreign organ. The antigens that stimulate graft rejection are called, unsurprisingly, transplantation antigens. The

transplantation antigens that stimulate rejection of allografts fall into two categories: major transplantation antigens and minor transplantation antigens.

a. Major transplantation antigens:

The major transplantation antigens are the class I and class II MHC molecules, HLA-A, B and C and HLA-DP, DQ and DR. As described in Chapter 4, MHC genes are highly polymorphic so that the donor and recipient will almost certainly have different alleles of at least some of the MHC loci. The cells of the graft will therefore have MHC molecules on their surface that are foreign to the recipient. Foreign (allogeneic) MHC antigens stimulate a particularly vigorous immune response (Figure.1). The reasons for this are not totally clear. Class I MHC alloantigens stimulate strong antibody and CD8 cytotoxic T cell responses. Class II MHC alloantigens stimulate CD4 T cells to become effector Th and also stimulate antibody responses. The Th help in the generation of antibody against class I and class II MHC, the generation of CD8 cytotoxic T cells, and the development of delayedtype hypersensitivity reactions (Figure.1).

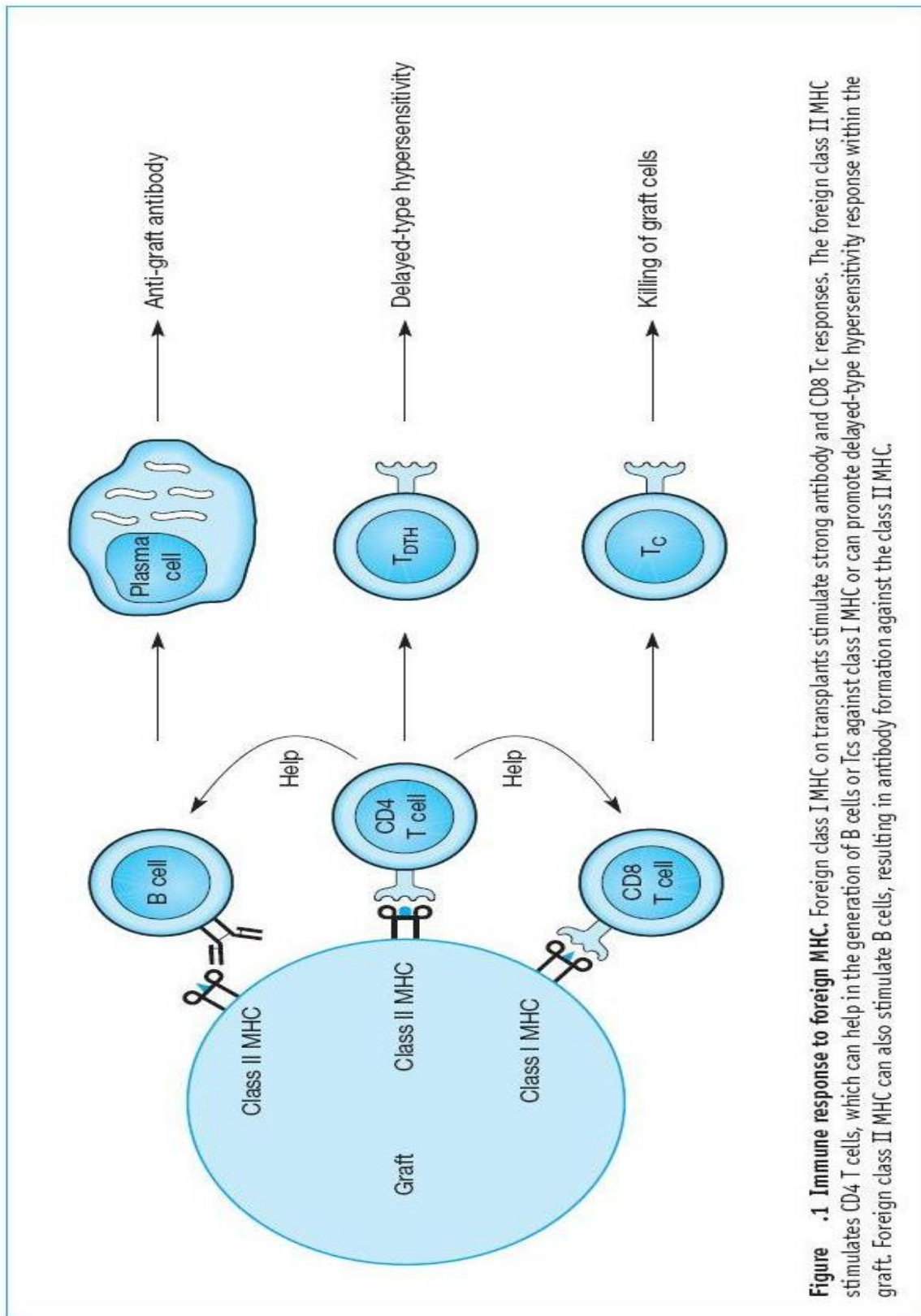
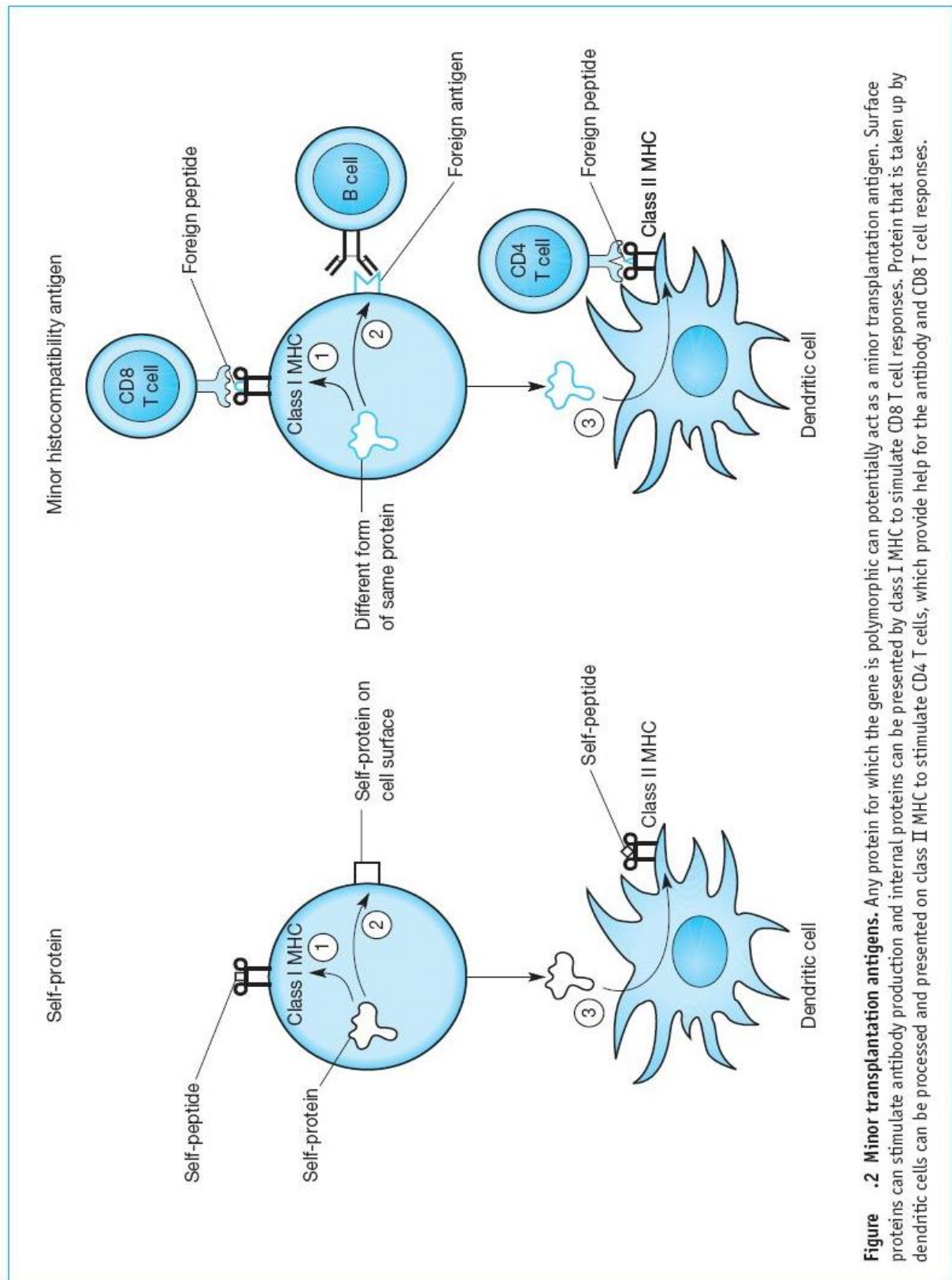


Figure .1 Immune response to foreign MHC. Foreign class I MHC on transplants stimulate strong antibody and CD8 Tc responses. The foreign class II MHC stimulates CD4 T cells, which can help in the generation of B cells or Tcs against class I MHC or can promote delayed-type hypersensitivity response within the graft. Foreign class II MHC can also stimulate B cells, resulting in antibody formation against the class II MHC.

b. Minor transplantation antigens:

Proteins other than MHC are polymorphic, although to a much lesser degree than class I and II MHC. The graft donor and recipient will have different forms of many of these proteins which can act as antigens. Because they do not stimulate as strong a rejection response as MHC they are called minor histocompatibility antigens. Minor histocompatibility antigens are recognized by the recipient's T cells in association with self-MHC in the same way as any antigen from a pathogen (Figure.2). The self-MHC can be on the recipient's cells or, if the graft and recipient are matched for any MHC molecules, on the graft cells – in this case self- and graft MHC are shared. There are many minor transplantation antigens (probably more than 70), although few have been identified. Most stimulate only T cells although antibody is also made to antigens other than MHC on a graft.



5. The immune response against transplants:

Graft rejection responses have been categorised into three main types: hyperacute, acute and chronic.

- a. Hyperacute rejection:** occurs if the recipient has pre-existing antibody against the graft. The antibody bind to antigens on the graft, resulting in complement fixation and activation of the clotting pathways. The graft essentially turns into a massive blood clot within minutes or hours of transplantation. Hyperacute rejection should be prevented by crossmatching .
- b. Acute rejection:** is the equivalent of a primary immune response against the graft. All kinds of effector mechanism can be generated against the graft: antibody, cytotoxic CD8 T cells and delayed-type hypersensitivity responses. Which of these occurs depends on a number of factors, including the degree of matching. They act against the graft in the same way as they act against pathogens, Without immunosuppression the graft would be rejected in 7–20 days.
- c. Chronic rejection:** is a process that usually starts after the graft has been in place for more than a year. One of the main pathological changes seen is thickening of the graft's arterial walls, which can lead to occlusion of the artery and ischaemia. Eventually a large enough part of the graft is without an adequate blood supply and the graft stops functioning.

6. Preventing graft rejection:

There are three ways in which steps are taken to prevent graft rejection (Figure.3). First of all the donor and recipient are blood typed so that they

are compatible for blood groups. They are also cross-matched to check that the recipient does not have pre-existing antibody to the donor. Crossmatching involves taking some serum from the recipient and adding it to leucocytes from the donor together with some complement. If the recipient has anti-donor antibodies they will bind to the donor leucocytes and fix complement, resulting in lysis of the leucocytes. The second step to improving successful transplantation is matching the recipient and donor for HLA by tissue typing. Unless the donor is a family member there will be almost no chance of matching the donor and recipient at all six HLA loci, especially taking into account that MHC molecules are co-expressed and therefore people may well express two different alleles at one or more of the MHC loci. However, the more HLA matches there are between donor and recipient, the better the chance of a successful transplant. Matching at *HLA-DR* (Figure.4) has been shown to have the most beneficial effect but matching at *HLA-A* and *HLA-B* is also of benefit. The third step in preventing graft rejection, and the one that made clinical transplantation feasible, is the use of immunosuppression. Antigen-specific immunosuppression is the suppression of the immune response to the graft without affecting the ability to respond to other antigens, including pathogens. This is still at the experimental stage with respect to transplantation. Non-antigen-specific immunosuppression involves the use of drugs or antibodies that inhibit immune responses against any antigen. Therefore, although the response to the graft is inhibited, so is the response to pathogens. This is the type of immunosuppression used clinically and transplant recipients are consequently at increased risk of infection and also of the development of some types of tumours.

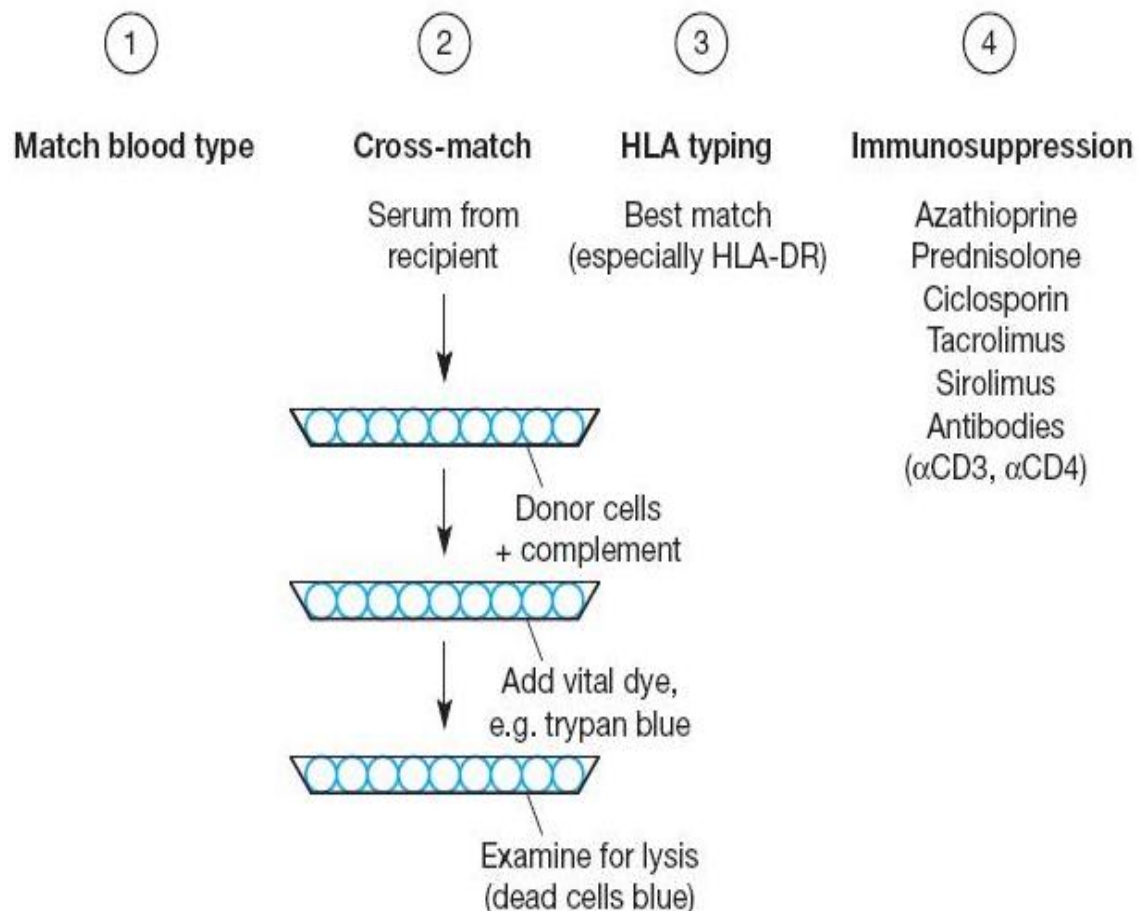
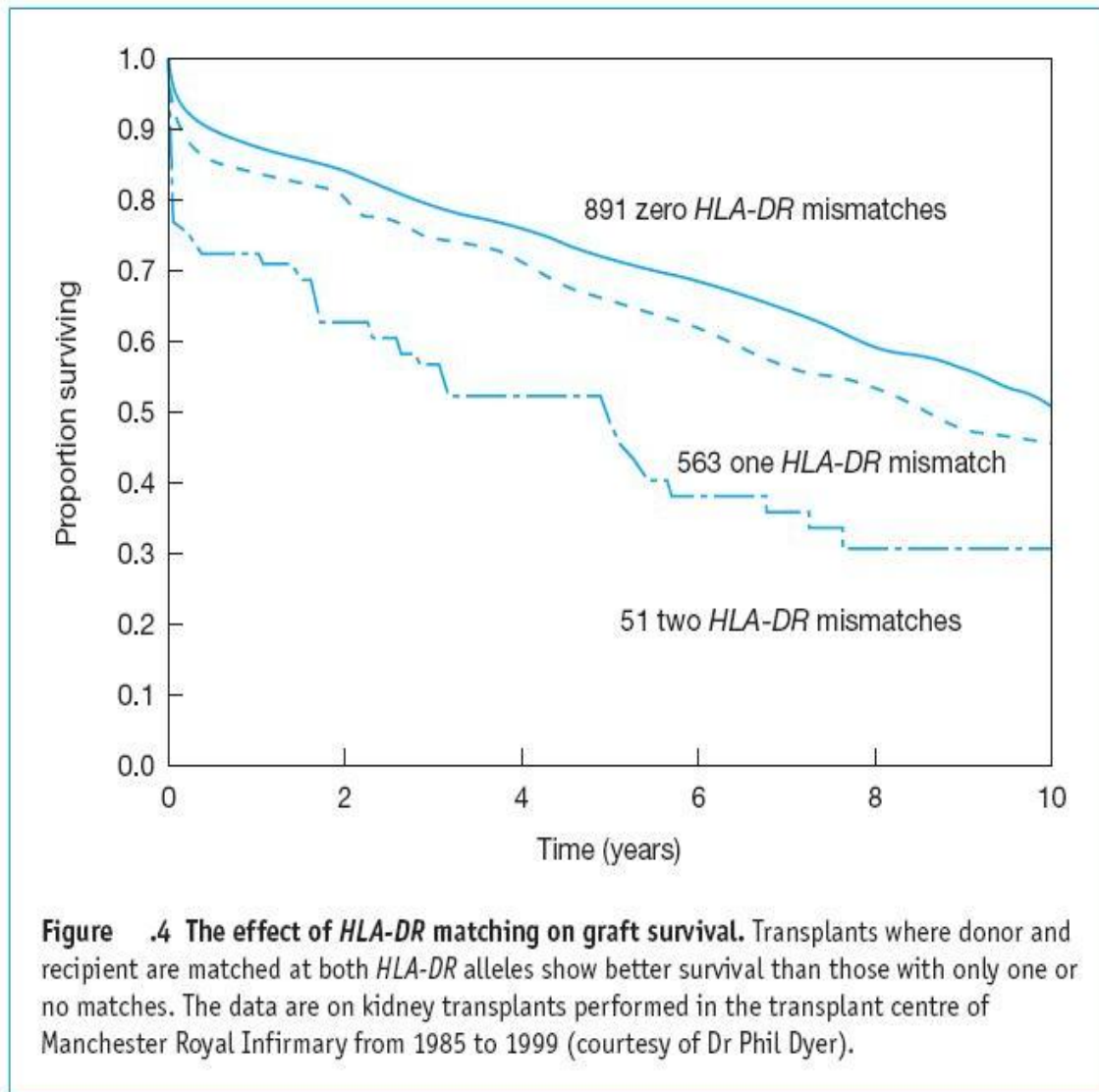


Figure .3 Steps in preventing transplant rejection. To prevent hyper-acute rejection caused by pre-existing antibody against donor antigens, the recipient and donor are matched for blood type and also cross-matched. Cross-matching involves incubating serum from the recipient with white blood cells from the prospective donor in the presence of complement. If the recipient has antibodies against donor antigens, the antibodies will bind to the donor cells and fix complement, resulting in lysis of the cells. The lysis can be detected by adding a vital dye such as trypan blue, which stains dead cells blue, while live cells are seen as white under a microscope. Potential donors and recipient are HLA-typed (see Chapter 4) and the donor with the best match is chosen for the transplant. Finally immunosuppressive agents are given to the recipient to try to prevent the anti-graft immune response.



7. Clinical immunosuppression:

The first class of immunosuppressive drugs was the anti-mitotic agents.

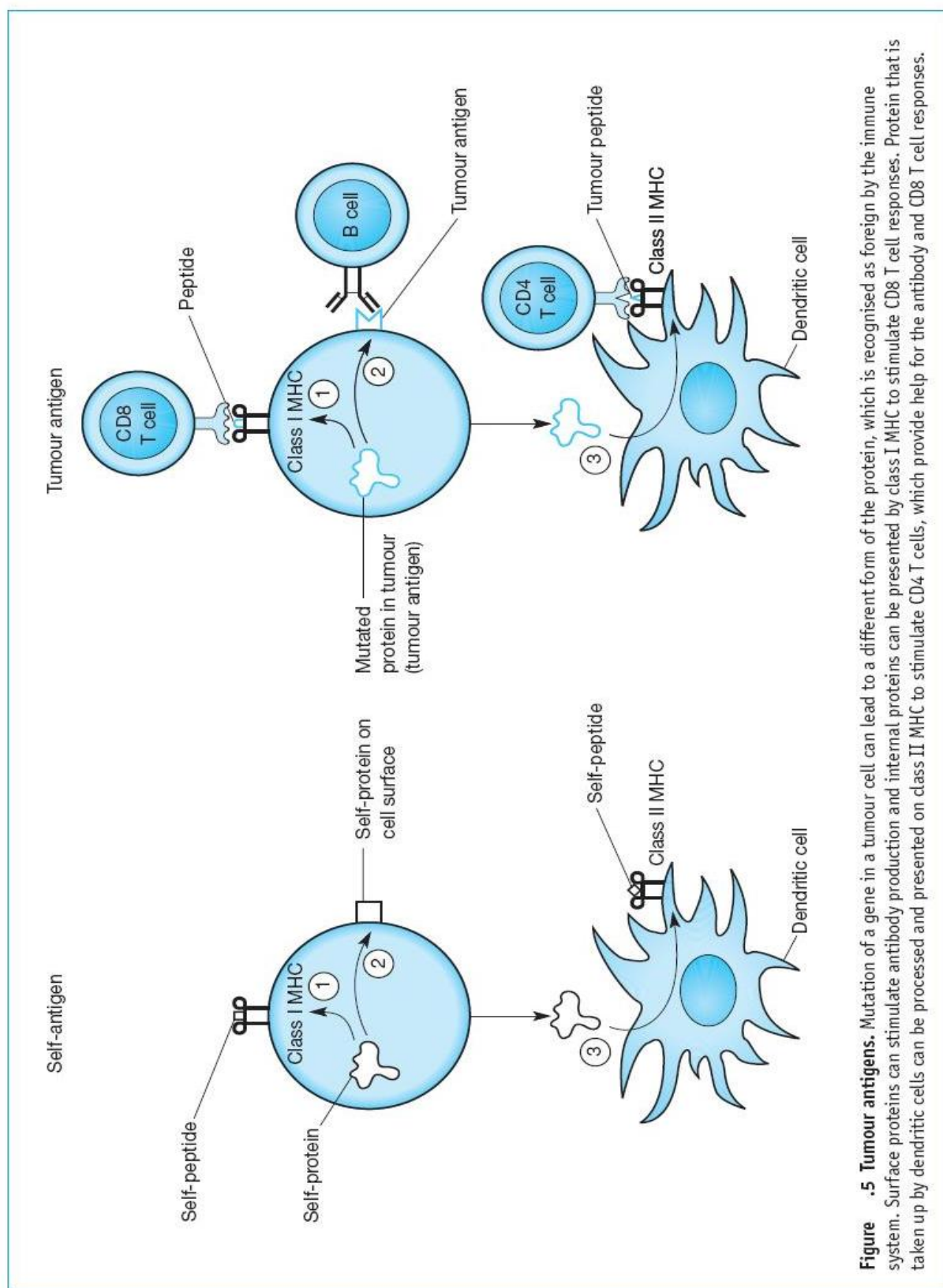
They were able to inhibit the proliferation of lymphocytes required during a specific immune response. The most commonly used drug in transplantation is azathioprine. One problem is that other tissues containing rapidly dividing cells, such as bone marrow and gut epithelium, are also affected. Corticosteroids affect the transcription of many genes in lymphocytes and

other cells. A consequence of this is that steroids affect lymphocyte migration, resulting in fewer cells entering the graft. They also inhibit macrophage and monocyte function and have general anti-inflammatory effects. The most commonly used steroid in transplantation is prednisone.

Up until the 1980s the immunosuppressive regimen of choice was a combination of azathioprine and prednisone. This could be quite effective and in some centres one-year kidney graft survival rates exceeded 80%. In the 1980s the first of a new breed of immunosuppressive drugs was introduced into clinical transplantation with dramatic effects on graft survival rates. This was **Ciclosporin**, a fungal product, which like the later members, tacrolimus and sirolimus, specifically inhibits CD4 T cell function. The drugs differ in the molecular details of their action but a major effect they have in common is to inhibit the **IL-2 pathway**. Ciclosporin and tacrolimus inhibit the production of IL-2, and sirolimus inhibits signalling through the IL-2 receptor. Because IL-2 plays a vital role in the proliferation of CD4 T cells, inhibition of the pathway provides a powerful immunosuppressive effect. Finally antibodies can be used in transplantation to inhibit T cell function. These include polyclonal antithymocyte globulins from the serum of sheep or goats immunised with human thymocytes, or monoclonal antibodies to T cell antigens such as CD3 or CD4. These antibodies act by causing the destruction of T cells or interfering with their function. They cause profound immunosuppression and therefore are used primarily in the period immediately after transplantation and to treat acute rejection episodes where the conventional immunosuppression is failing to prevent the rejection response.

8. Using the immune system against tumours:

Tumour immunology has had a chequered past. In the nineteenth century it was noticed that some individuals with cancer showed a temporary remission (i.e. improvement) in the clinical status of their tumour during bacterial infection. Treatments were practised that involved deliberate infection of cancer victims with bacteria, a procedure that probably stood more chance of killing the patients than helping them. In the second half of the twentieth century the genetics of cancer became better understood and it was realised that cancer involved mutation of normal genes and that these mutated genes could give rise to altered proteins that could act as new antigens that were foreign to the individual (Figure.5). The concept of immune surveillance was born. This idea was that the immune system constantly patrolled the body looking for cancerous cells that expressed mutant antigens, which would be recognised as foreign. An immune response would then be generated against the mutant antigen leading to the destruction of the tumour cells. This led to a series of naive approaches in which it was thought that simply boosting the immune system would somehow increase the response against tumour cells and 'cure' cancer. These attempts were unsuccessful and brought the field of tumour immunology into a modicum of disrepute. Today much more is known both about the immune system and the biology of cancer. This has led to a renewed interest in harnessing the immune response to provide protection against cancer. There are two main approaches that utilise the immune system to try to destroy tumour cells. These are the use of antibodies against tumour cells and the development of tumour vaccines.



9. Antibodies can be used to kill tumour cells:

The approach of using antibodies against tumours is to make monoclonal antibodies that are specific for tumour antigens and to inject them into patients. Two variations have been considered. One is to use unmodified antibodies in the hope that they will bind to the tumour cells and recruit other components of the immune system to kill the tumour cells. This could be through complement fixation and lysis or the involvement of antibody-dependent cell-mediated cytotoxicity (Figure.6). A number of monoclonal antibodies have now been licensed for clinical use and more are in clinical trials (Table.2). A second approach with monoclonal antibodies is to attach them to toxic agents, which may be radioactive isotopes or toxins (Figure.6). These agents are too toxic to use directly but the theory is that the antibody will bind to antigens on the tumour cell and be internalised, leading to delivery of the toxic agent specifically to the tumour cells. Specific agents that have been considered for use include ¹³¹I, bacterial toxins such as diphtheria toxin, and plant toxins including ricin, a very powerful toxin obtained from the castor bean. A number of these immunotoxins have been used in clinical trials but the overall results have been somewhat disappointing.

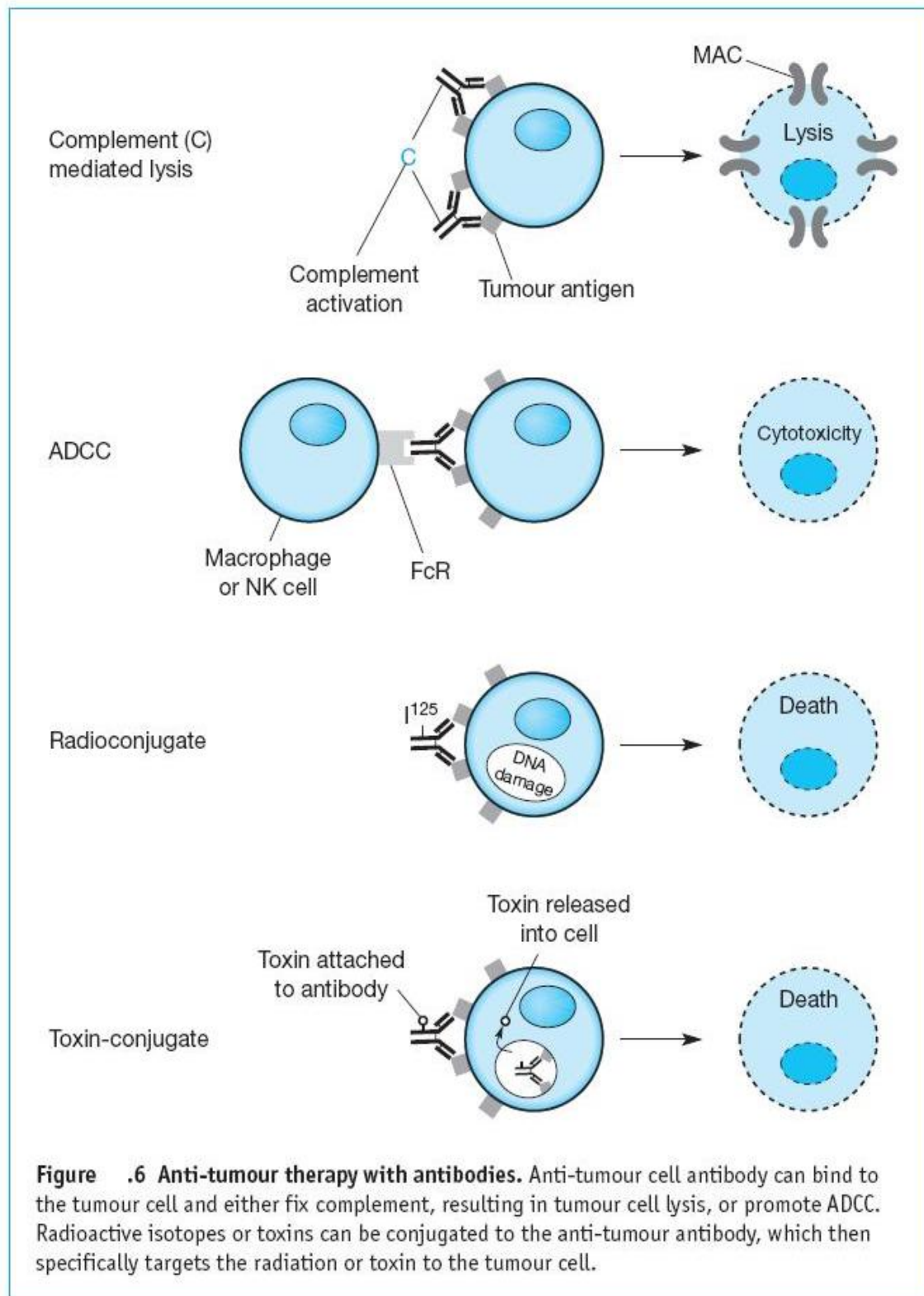


Table .2 Antibodies being used as anti-tumour agents

<i>Antibody</i>	<i>Target antigen</i>	<i>Tumour type</i>	<i>Status</i>
Rituximab	CD20	Non-Hodgkin lymphoma	Licensed
Cetuximab	EGF-R	Colorectal	Licensed
Trastuzimab	Her2 (EGF-like receptor)	Breast cancer	Licensed
HuJ591	PSA	Prostate cancer	In clinical trials
Bevacizumab	VEGF	Colorectal (metastatic)	Licensed
¹³¹ I-tositumomab (Radio-Ab)	CD20	Non-Hodgkin lymphoma	Licensed
Gemtuzumab-Ozogamicin (Toxin-Ab)	CD33	Acute myeloid leukaemia	Licensed

EGF-R, epidermal-growth-factor receptor

PSA, prostate-specific membrane antigen

VEGF, vascular endothelial growth factor

10. Tumour vaccines:

Tumour vaccines represent an approach to try to stimulate immune responses to tumours in the same way that microbial vaccines are used to stimulate responses against pathogens. Experimental evidence has shown that many tumours are antigenic; that is, they express antigens that can be the target of an immune response. There are, however, a number of features about tumours that enable them to avoid stimulating an immune response. Many tumours downregulate MHC molecules, enabling them to avoid recognition by T cells. Some tumours secrete immunosuppressive substances such as transforming growth factor- β . The aim of tumour vaccines is

therefore to overcome the lack of immune responses to tumours and many approaches have been tried for this.

a. Peptide and DNA based vaccines:

Many tumour antigens have been identified and in some cases peptides representing the T cell epitopes have also been characterized (Table.3). Tumour antigens can be unique to individual tumours as a result of random mutations in the genes encoding the antigens or they can be shared tumour antigens. Shared tumour antigens are those that are expressed by tumours but not normal tissue or they are expressed in a quantitatively different way in tumour cells compared to normal. These have formed the basis of vaccines involving the injection of antigens or peptides with adjuvants (Figure.7), or the use of DNA vaccines. There are two conventional adjuvants licensed for clinical use: alum and a squalene–oil–water emulsion. However, other agents, especially cytokines such as interleukin-2, granulocyte/ macrophage colony-stimulating factor (GM-CSF) and interleukin-12, have been incorporated into tumour vaccines to try to boost the immune response. Many of these vaccines have been tested in clinical trials, especially for melanoma and breast cancer patients, using a number of antigens/peptides including the MAGE family and MUC-1. DNA-based tumour vaccines use the same approach as with infectious agents involving injection of DNA-encoding tumour antigens or peptides intra-muscularly or dermally via gene guns. It has been shown that the bacterial DNA acts as an adjuvant because the unmethylated CpG motifs are recognised by TLRs on dendritic cells and stimulate dendritic cells to become good stimulators of T cells.

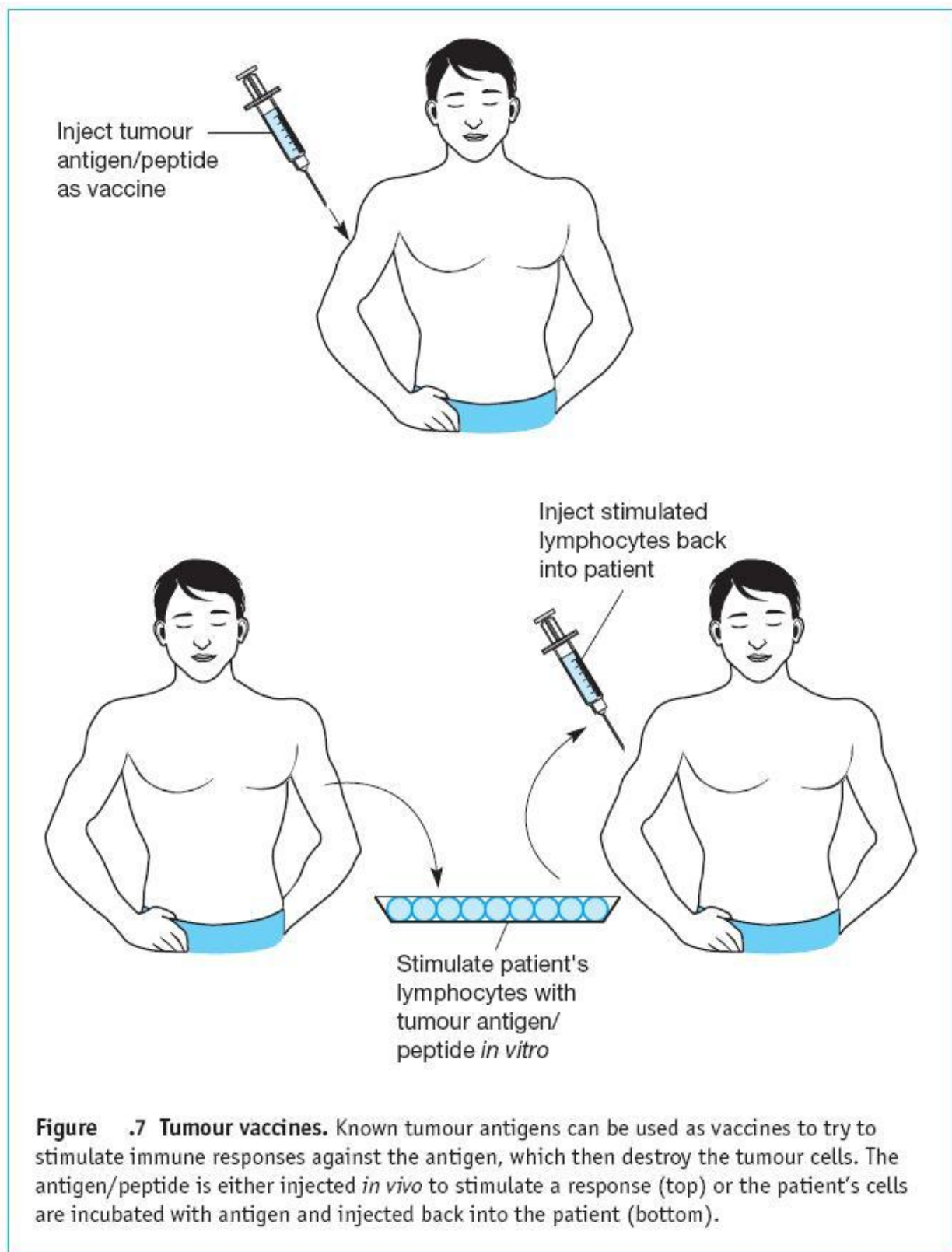


Table .3 Some examples of tumour antigens

<i>Antigen</i>	<i>Type of antigen</i>	<i>Expression in tumour cells</i>	<i>Expression in normal cells</i>
Antibody antigens			
Carcinoembryonic antigen (CEA)	Foetal	Colon + others	Yes
MUC-1	Aberrantly glycosylated mucin	Breast	Yes
p53	Mutated form of protein	Many	No
T-cell antigens			
MAGE-1, MAGE-3	Foetal	Melanoma, breast	Testis
ras	Mutated form of protein	Many	No
Tyrosinase	Differentiated cell product	Melanoma	Yes
Human papilloma virus E6 and E7 proteins	Viral gene product	Cervical carcinoma	Yes

b. Whole-cell based vaccines:

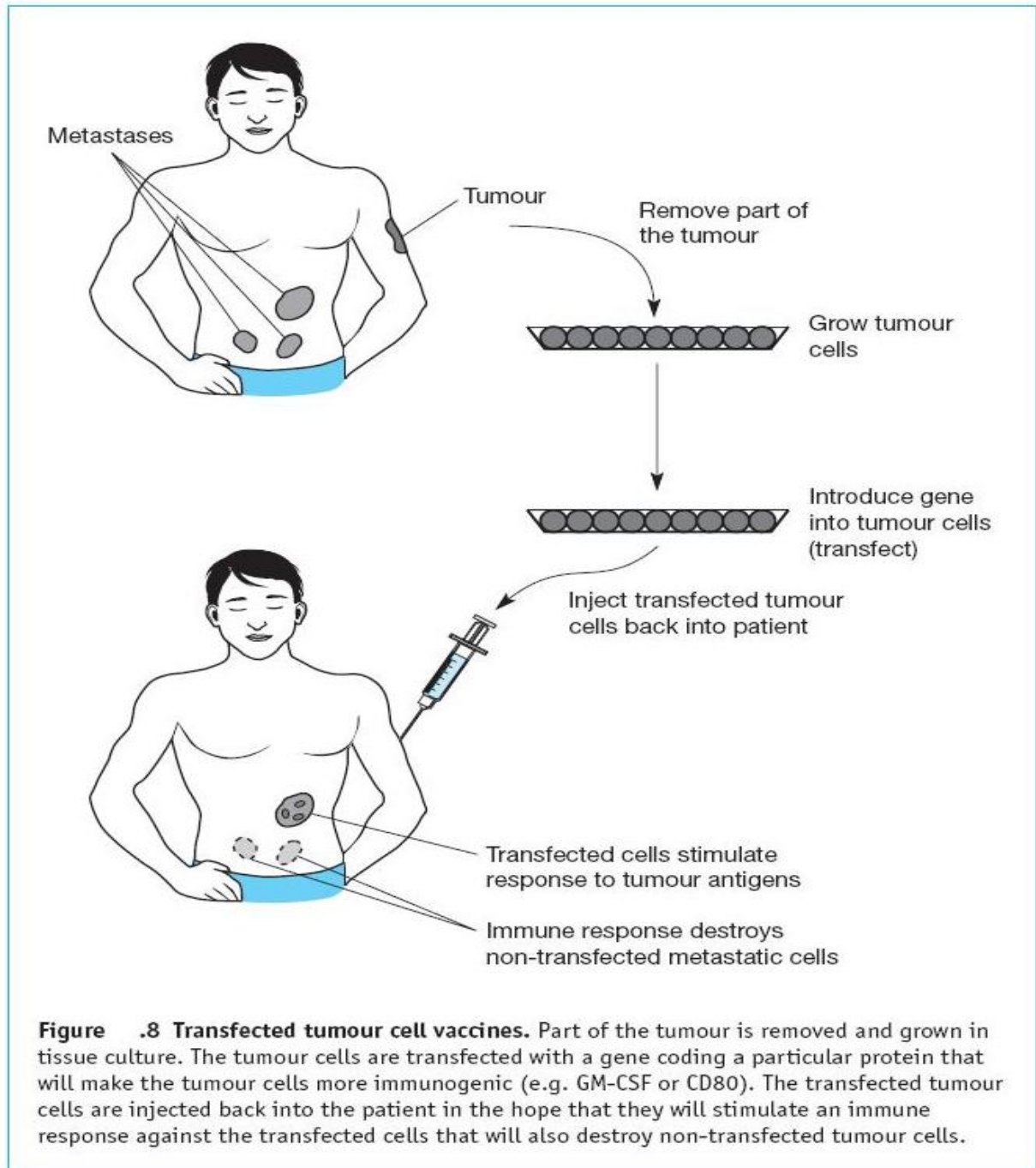
One of the problems with the antigen/peptide approach is that for many tumors no tumour specific antigens have been identified and even some of the known tumour antigens are not expressed by tumors in all individuals. Another approach has been to use irradiated tumour cells or tumour cell lysates as vaccines. These have the advantage that both unique tumour and unknown shared antigens will have the potential to stimulate an immune response. Whole vaccines can be based on either cells derived from the tumors of the patient themselves or on tumor cell lines, although the latter, being derived from other individuals' tumors, will not stimulate responses against the patient's unique tumour antigens. Generally they are also given

with an adjuvant. Whole vaccines have been tried in clinical trials for melanoma and pancreatic cancer, among others.

c. Transfected tumour cell vaccines:

One of the findings from experimental and clinical trials with tumour cells as vaccines was that on their own they did not stimulate a very good immune response. An alternative approach to the use of adjuvants in whole vaccines is to transfect tumour cells. This approach has the advantage that it does not require knowledge of the particular tumour antigens but instead involves manipulation of the tumour cells so that they directly or indirectly stimulate immune responses. A typical approach is to take out some tumour cells and transfect them with genes that will make the tumour immunogenic or stimulate an inflammatory response (Figure.8). Tumour cells have been transfected with co-stimulatory molecules (CD80 or CD86) so that they can directly stimulate CD4 T cells. In experimental situations the transfected tumour cells stimulated a response that protected against both transfected and non-transfected tumours. The most successful experimental approach has been to transfect tumour cells with granulocyte macrophage colony-stimulating factor (GM-CSF). When injected back into animals the transfected tumour cells secrete GM-CSF, which attracts dendritic cells to the area. These dendritic cells process tumour antigens and carry them to draining lymph nodes, where they present antigen on their class II MHC, activated tumour-specific CD4 T cells and generate antitumor responses. These GM-CSF transfected tumour cells have also been shown to provide protection against both transfected and non-transfected tumors. It is vital that the transfected tumors also protect against nontransfected tumors because in the clinical situation you will be taking only a small proportion of the

tumour cells for transfection and the transfected cells must be able to stimulate an anti-tumour response that is effective against non-transfected tumour cells.



d. Dendritic cell-based vaccines:

One of the ways in which GM-CSF is thought to increase the immunogenicity of tumour cells is to activate dendritic cells so that they are better able to present tumour antigens and stimulate CD4 T cells. It is now possible to grow DCs in tissue culture by incubating peripheral blood monocytes with GM-CSF and IL-4. These dendritic cells can then be incubated with tumour cell lysates or tumour-specific antigens/peptides so that they take up antigen and present it on their class II MHC molecules. The dendritic cells are then injected back into the patients in the hope they will stimulate tumour-specific T cells. This approach has been tested in clinical trials for melanoma and gastro-intestinal cancers.

e. Cytokines, antigen-specific T cells and natural killer cells:

Cytokines have been given systemically to try to boost immune responses, with limited success for some tumors. Although not strictly a vaccine, cytokines are being given with vaccines to increase immunogenicity (see above). Another use of cytokines, particularly IL-2, is to enable tumour specific T cells to be grown *in vitro*. Lymphocytes extracted from tumors, so-called tumour-infiltrating lymphocytes (TILs), can be grown in tissue culture with IL-2 alone to expand the number of T-cells that have recently been activated with tumour antigen *in vivo*. Alternatively TILs or blood derived T cells can be cultured with tumour antigen/peptide and IL-2 so that tumour antigen-specific T cells are stimulated by antigen and grow under the influence of IL-2. The cells can then be injected back into the patient to exert anti-tumour activity. A final use of cytokines is in improving natural killer cell activity. Natural killer cells from blood can be grown in tissue culture and proliferate in the presence of high concentrations of IL-2 to become

what are known as lymphokine-activated killer (LAK) cells. These LAK cells have enhanced tumour-killing activity and can be injected back into patients in the hope they will kill tumour cells. It is evident from these examples that many innovative approaches are being used to induce or increase anti-tumour immune responses. Although they have been used in clinical trials, results have not been spectacular. Part of the problem is that the early clinical trials were in terminally ill patients with widespread untreatable tumours and the trials were not controlled. Results of the trials in terms of partial or complete tumour remissions were compared with historical remission rates for the particular tumours. However, results in some of the trials were encouraging enough for the approaches to be taken forward to larger controlled trials. This, combined with ever increasing knowledge of the interaction between the immune system and tumours, should lead to the development of effective tumour vaccines that can result in the elimination of established tumours.

Summary

- The most common form of transplant is an allograft, which is between two non-genetically related individuals of the same species. There is also considerable interest in xenografting tissue from other species to humans.
- Allografts stimulate strong immune responses against foreign MHC and non-MHC antigens. These can result in the generation of antibody, CD8 T cells and delayed-hypersensitivity responses against the graft.
- Graft rejection is prevented by immunosuppressive drugs, which are effective but can leave the recipient at increased risk of infection and the development of certain tumours.

- Immunotherapy of tumours involves two main approaches: the use of anti-tumour antibodies to destroy tumour cells and the development of tumour vaccines.
- Anti-tumour antibodies can be given in unmodified form or attached to radioactive or toxic agents. As yet, neither form has been very successful in clinical trials.
- Because tumours do not stimulate very good immune responses, two main types of tumour vaccine are being tested to try to stimulate antitumour immune responses. One type involves immunisation with identified tumour antigens to stimulate responses against the antigen. The other type involves transfecting tumour cells with genes that will make tumour cells stimulate an immune response that will be directed against both transfected and non-transfected tumour cells.

5. Immunological memory and vaccination

Key topics:

- Immunological memory
 - Memory for antibody production
 - T cell memory
- Vaccines
 - Properties of an ideal vaccine
 - Current vaccines
 - Future vaccines

1. **Immunological memory:**

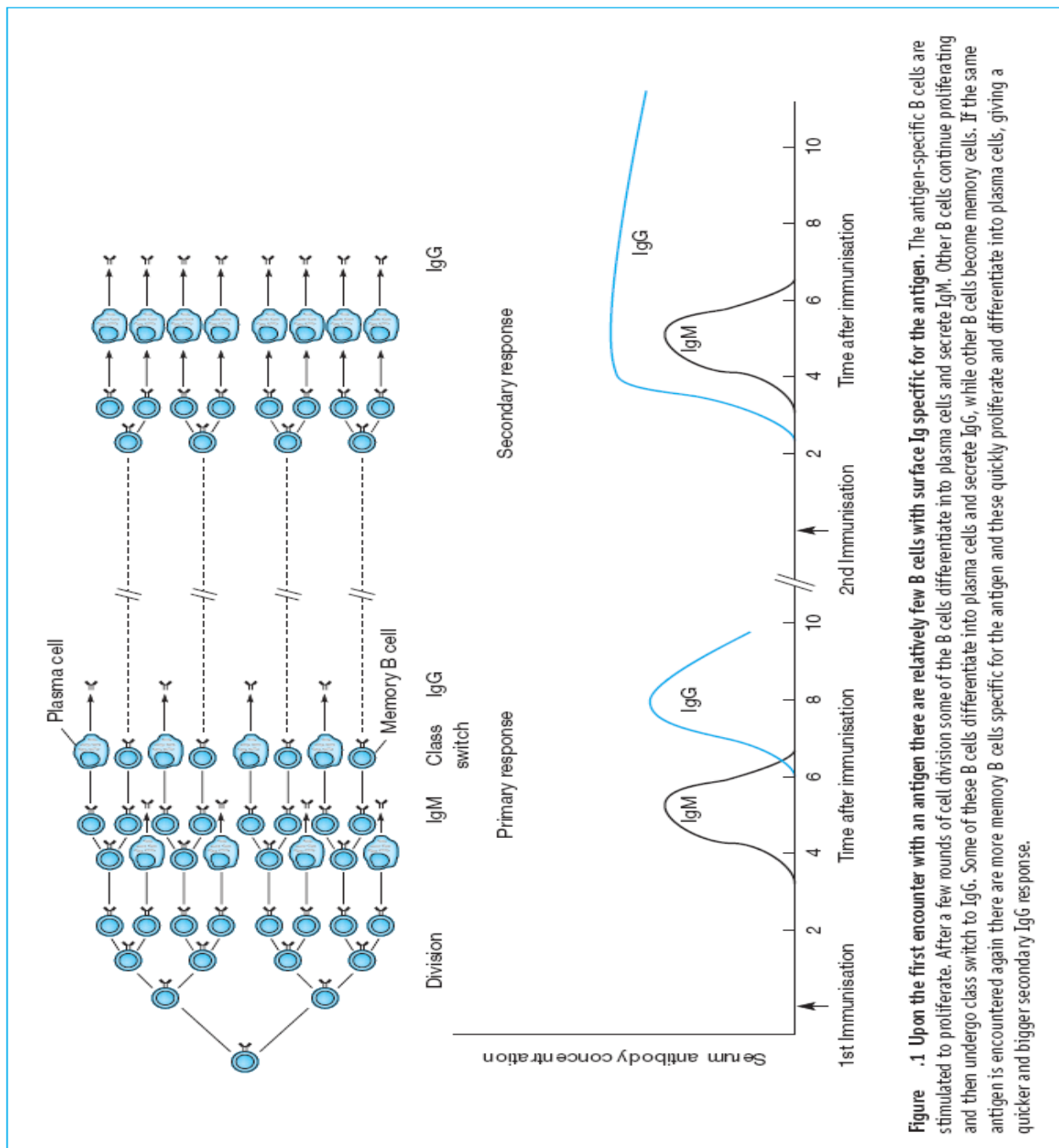
One of the features of specific immune responses is known as immunological memory, but what exactly is meant by immunological memory? Immunological memory can best be illustrated by examining antibody responses after a first and second injection with antigen (Figure.1). IgM is made typically within a week after the first immunization. IgG production follows after IgM. If you look at antibody production following a second exposure to the same antigen the IgM response is essentially the same. However, the IgG response shows dramatic differences from the primary injection. The IgG response is **faster**, **bigger** and, although not depicted in (Figure.1), **better**. The response to the first immunization with antigen is also called the **primary** response and that to the second immunisation the **secondary** response. Another feature of immunological memory is that it is specific for the particular antigen. In the example in (Figure.1) you only get a bigger response to a second immunization with the

same antigen; if the second immunization was with a different antigen to the first you would get a primary response to the second antigen. Immunological memory is a feature of all specific immune responses and therefore involves B cells, CD4 T cells and CD8 T cells.

a. B cell memory:

B cell memory is the best understood feature of immunological memory. Memory B cells are generated only in germinal centres and are B cells that have undergone proliferation, class switch and affinity maturation (Figure .1). Because antigen-specific B cells have undergone proliferation before becoming memory B cells, there will be more antigen-specific memory B cells than there were B cells before the encounter with antigen. This will contribute to a bigger and faster antibody response. Memory B cells have already undergone antibody class switch, and therefore have IgG, IgA or IgE on their surface, which enables them to be distinguished from naive B cells that have not seen antigen before and that express IgM plus IgD on their surface. It also means that memory B cells do not have to undergo class switch when re-stimulated by antigen, which speeds up the secondary response. The fact that memory B cells have undergone affinity maturation means that the secondary response will be better, i.e. higher-affinity antibody production, than the primary response. Memory B cells still have to proliferate and differentiate into plasma cells before they secrete antibody and this process requires help from CD4 T cells. Memory T cells circulate through the lymphatic and blood system and the way in which they interact with CD4 T cells in lymphoid tissue is thought to be the same. However, there are some differences in stimulation of memory B cells, one of them being that soluble antigen is good at stimulating memory B cells while

antigen needs to be presented on the surface of a macrophage to stimulate naive B cells. Not only is the differentiation of memory B cells into plasma cells dependent of CD4 T cells but also the actual development of memory B cells is dependent on CD4 T cells.



b. CD4 T cell memory:

This is the least well understood facet of immunological memory, a somewhat paradoxical situation given the central role of CD4 T cells in regulating specific immune responses. One of the main problems is that there are no definitive ways of identifying quiescent memory CD4 T cells in the same way that surface IgG, IgA or IgE can be used to identify memory B cells. One of the questions for which there is evidence both for and against is whether memory CD4 T cells are **functionally** different from naive CD4 T cells or whether CD4 T cell memory is simply quantitative, i.e. upon first encounter with antigen, antigen-specific CD4 T cells proliferate so that when you encounter the antigen again you have more CD4 T cells specific for that antigen so that they can generate more Th faster than in a primary response. There is evidence that memory CD4 T cells have less requirement for co-stimulation to be activated than do naive CD4 T cells, suggesting that memory CD4 T cells are functionally different.

c. Memory CD8 T cells:

During a primary Tc response, the proliferation of CD8 T cells generates large numbers of antigen-specific CD8 T cells, about 90% of which die after the infection is cleared. The remaining 10% of cells provide CD8 memory T cells. These are able to respond more quickly to subsequent infection by providing more cells that can differentiate more quickly into Tcs than naive CD8 T cells. Memory CD8 T cells also produce more cytokines, especially IFN γ , after antigen stimulation than do naive CD8 T cells. Although the generation of Tcs in a primary response is not necessarily dependent on CD4 T cells, the generation of memory CD8 T cells does not occur in the absence of CD4 T cells.

2. Vaccines:

The understanding that the immune system could protect against further infection with a pathogen provides the basis for vaccination. It has been known since ancient times that there are many diseases that people usually get only once in their lives. These include diseases such as smallpox, measles and mumps. Although nothing was known about the mechanisms (indeed, up until the past few centuries, nothing was known about infectious pathogens) this state of protection against further infection was known as **immunity**. It was also known that immunity was specific – if you got smallpox and survived you would be protected against getting smallpox again but not against getting other diseases. Vaccination was the process of trying to induce immunity without going through the disease process, and some forms of vaccination have been practiced since ancient times . It is now appreciated that immunity is brought about by immunological memory.

3. Properties of an ideal vaccine:

Vaccination is a way of manipulating the immune system to provide protection from disease caused by a pathogen without subjecting the person or animal to the disease. To be effective, a vaccine should stimulate the right type of immune response in the right anatomical site. Depending on the pathogen, antibody, CD8 T cells, delayed-type hypersensitivity (DTH) or a combination of these would be appropriate. For many pathogens the induction of secretory IgA at the appropriate mucosal site may protect from infection. There are also other properties that an ideal vaccine should have:

- **Safety.** There should be no side-effects from the vaccine and no risk of procedural errors in vaccine manufacture exposing individuals to pathogens or their toxic products.

- **Price.** Cheapness is obviously desirable and almost essential in many parts of the world with high rates of endemic disease and few economic resources.
- **Stability.** Ideally a vaccine should be able to be stored in high ambient temperatures so that it is available in hot climates with limited refrigeration facilities.
- **Ease of administration.** Children, the main target of vaccination, do not like having needles stuck in them and therefore oral vaccines are the most suitable form for administration and also can cut out the cost of materials for injection. Vaccines have become safer. Variolation, used to induce protection against small pox carried a 1% death rate, which would now be considered unacceptable. The first true vaccine, using cowpox to vaccinate against smallpox was much safer. Many of the vaccines that have been developed and are still in use today are quite safe and effective, despite being developed when relatively little was understood about the immune system. Vaccines are now available for a wide variety of pathogens (see Table.1). However, there are still problems. Some vaccines carry a small risk of side-effects. Although the actual risk is small there can be a knock-on effect, which is the loss in a population of a phenomenon called **herd immunity**. Herd immunity occurs when the proportion of people in a population that is immune to a pathogen is so high that the pathogen cannot find enough susceptible hosts to infect and cannot survive in the population. Therefore even the minority of individuals who are not immune to the pathogen do not get infected. However, if people stop being vaccinated, or having their children vaccinated, the proportion of susceptible people increases to a level where the pathogen can become re-established in the

population and herd immunity is lost. A second issue with vaccines is that there are still many diseases for which no effective vaccine exists. These diseases, including malaria, tuberculosis and parasitic worms, kill in excess of ten million people annually and some estimates suggest 30% of infant mortality could be prevented by successful vaccination. There is still no vaccine for AIDS.

Table .1 Some of the current available vaccines

<i>Organism</i>	<i>Type of vaccine</i>
Viruses	
Measles	Attenuated virus
Mumps	Attenuated virus
Rubella (German measles)	Attenuated virus
Polio	Attenuated, killed virus
Chickenpox	Attenuated virus
Rotavirus (infant diarrhoea)	Recombinant vaccine
Influenza	Inactivated virus
Hepatitis (A and B)	Recombinant vaccine
Bacteria	
Diphtheria	Toxoid
Pertussis (whooping cough)	Killed bacteria, subunit vaccine
Meningitis – <i>Neissera meningitidis</i>	Capsular polysaccharide
– <i>Haemophilus influenzae</i>	Polysaccharide-conjugate
Tetanus	Toxoid
<i>Streptococcus pneumoniae</i>	Capsular polysaccharide
Cholera	Killed bacteria

4. Vaccines – the past and the present:

The situation with cowpox and smallpox is unusual. Other pathogens do not have naturally occurring harmless equivalents in other species that provide a ready-made source of vaccine. Therefore other approaches were needed to develop vaccines, many of which are still in use today:

a. Killed or inactivated pathogen:

This approach, used primarily for viruses, involves taking the virus and treating it with heat or chemicals so that it is no longer infectious. These vaccines stimulate good antibody responses but, because the virus is no longer infectious, do not stimulate cell-mediated immunity. This approach has been used for influenza, rabies, polio (Salk vaccine) and some bacteria including cholera. One problem is the possibility of some pathogen particles surviving the killing or inactivation process; this happened with the polio vaccine in the 1950s.

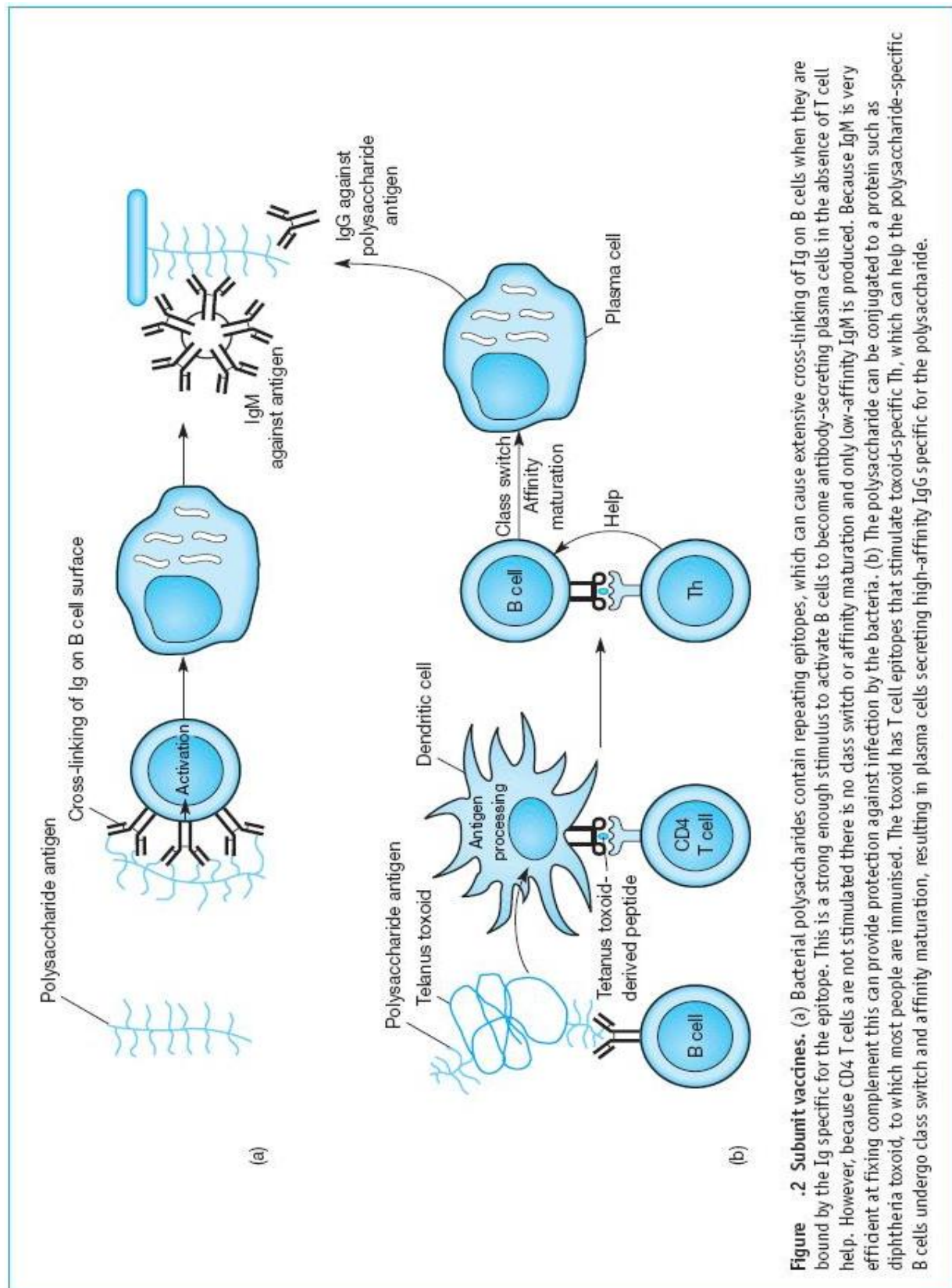
b. Attenuated pathogen:

Attenuated pathogens are still viable and cause infection but do not cause disease. Attenuation is usually achieved by growing the organism in cells of another species so that the pathogen becomes adapted to cells of the other species and grows poorly in human cells. Because they can infect cells they stimulate cell-mediated immunity as well as antibody. These vaccines have been developed for polio (Sabin vaccine), measles, mumps and tuberculosis, among others. A problem is the possibility of reversion to full pathogenicity, either in the vaccine stock or in the vaccinated individual.

c. Subunit vaccines:

In some situations an antibody response against a particular component of the pathogen is sufficient to provide immunity. Many bacteria produce a

polysaccharide coat that prevents phagocytosis in the absence of antibody. Vaccination with the polysaccharide induces antibody, which is enough to provide immunity. This approach has been used against *Haemophilus influenzae*, which causes 'flu, and *Neisseria meningitides*, a cause of potentially fatal meningitis. One problem with these vaccines is that the polysaccharide antigen does not stimulate Th cells and therefore only IgM is produced. To overcome this, the polysaccharide can be conjugated to a protein such as tetanus toxoid. The tetanus toxoid stimulates Th cells, which can help the B cells specific for the polysaccharide to switch to other antibody classes and make a bigger antibody response (Figure .2). Subunit vaccines can also be proteins as in the case of the vaccine for hepatitis B, where immunisation with the major surface antigen of the virus, called the HbsAg, induces the production of protective antibodies.



d. Toxoids:

Where pathogens cause disease almost solely through the production of toxins it is possible to vaccinate just against the toxin. This prevents disease upon infection and the immune system can then mount a response against the pathogen and eliminate it. To prevent the toxic effects of the toxin upon vaccination, the toxin is treated chemically so that it loses toxicity but retains antigenicity. Examples of toxoid vaccines are those for tetanus and diphtheria.

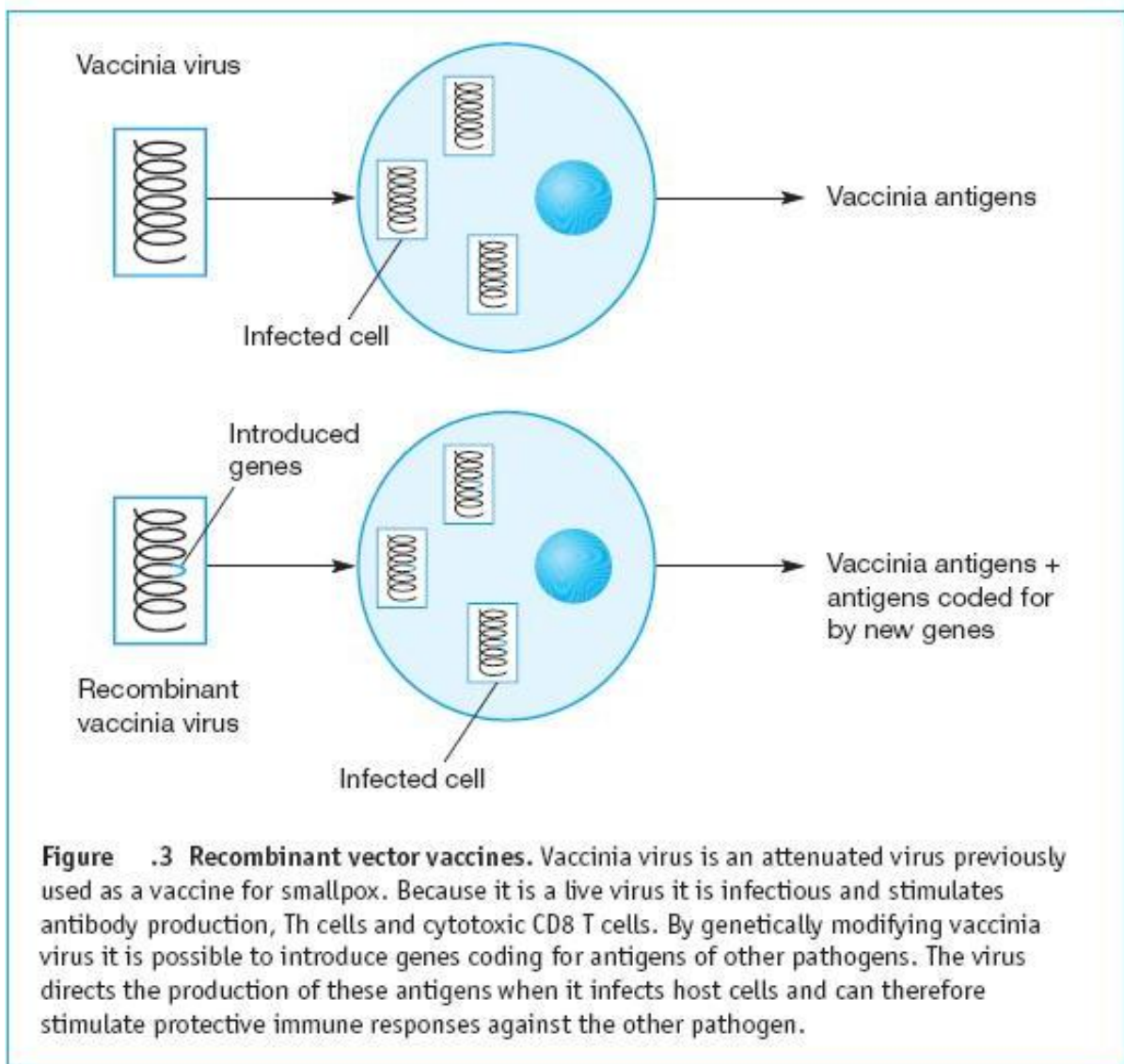
5. Vaccines – the present and the future:

The development of genetic engineering holds great promise for the future of vaccinations. Already some of the subunit and toxoid vaccines that used to have to be purified from bulk cultures of the relevant organisms can be produced using recombinant DNA technology so that the relevant genes are introduced into bacteria or yeast that produce large quantities of the protein. However, other applications of DNA technology raise the prospect of developing vaccines that are safer, cheaper and more stable than many of the present vaccines. These aspects are all important, especially for the use of vaccines in countries where refrigerated storage conditions are not widely available. The new styles of vaccine being developed for trial include the following:

a. Recombinant vector vaccines:

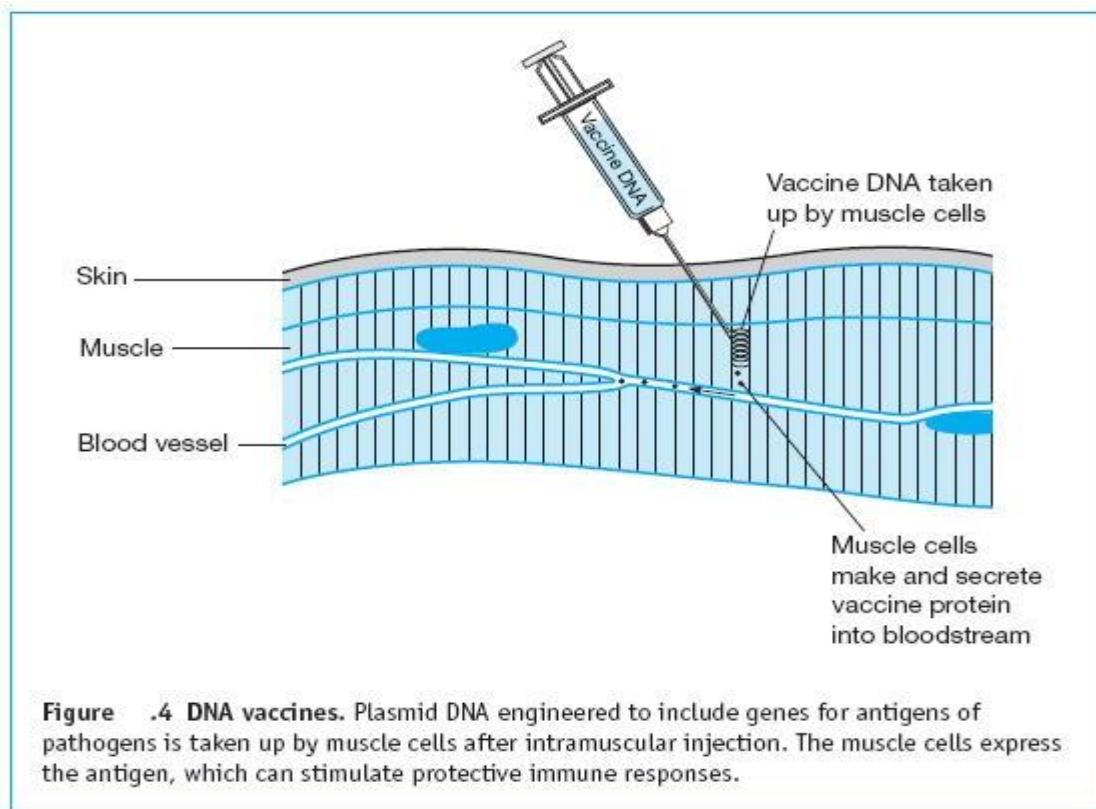
These are vaccines in which the genes encoding important antigens for a pathogen are introduced into the genome of attenuated viruses or bacteria (Figure.3). An example would be to introduce the gene for a pathogenic antigen into the genome of the vaccinia virus, which previously was used for

immunization against smallpox. The vaccinia virus, in addition to directing the expression of its own antigens, would also cause expression of the pathogen's antigen and stimulate immunity against smallpox (which is not necessary because natural infection with smallpox should not occur) and the pathogen. Because the vaccinia virus can infect cells it should stimulate good antibody responses and cell-mediated immunity against the pathogen's antigen.



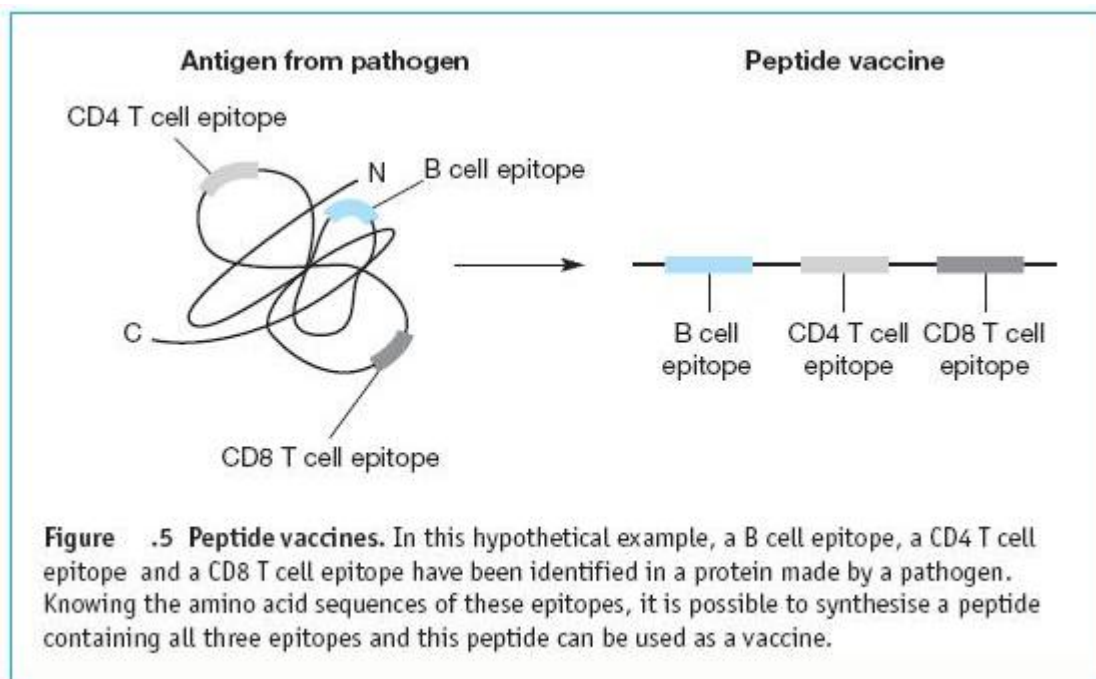
b. DNA vaccines:

One fairly recent finding is that when muscle cells are exposed to DNA, they can take up the DNA and express the proteins coded for by the DNA. This happens *in vivo* and provides a mechanism for inducing the production of a wide variety of proteins including pathogenic antigens. In practice the DNA is in the form of a plasmid (Figure .4). This means that promoters can be introduced into the plasmid, which causes high production of the pathogen's protein. Experimental (DNA) plasmid vaccines induce both antibody- and cell-mediated immunity and have been shown to provide immunity against infection with the pathogen from which the antigen DNA was derived. Clinical trials are under way with several DNA vaccines. One major advantage of DNA vaccines is that DNA is very stable and does not require refrigerated storage.



c. Peptide vaccines:

The type of vaccine that has the potential to be the safest, cheapest and easiest to store is the peptide vaccine. This consists of a synthetic peptide that contains a CD4 T cell epitope and, depending on the pathogen, a B cell epitope and/or CD8 T cell epitope (Figure.5). They can be produced chemically and therefore, in bulk, cheaply and, because they do not involve DNA or the inactivation of toxins, there should be no risk of accidental exposure to toxins or virulent organisms. The main drawbacks to peptide vaccines have been identifying appropriate epitopes and making the peptides immunogenic. Their development has therefore been slower than originally hoped. Overall vaccination has been a tremendously successful form of manipulating the immune system by stimulating specific immune responses against antigens on pathogens and has led to a significant reduction in mortality and morbidity.



Summary

- Immunological memory is a mechanism for making immune responses to a second, or subsequent, exposure to an antigen faster and bigger.
- Memory for antibody responses is based on memory B cells produced in germinal centers during a primary antibody response. Because memory B cells have already switched antibody class to IgG, IgA or IgE and have undergone affinity maturation, they are able to differentiate rapidly into high-affinity antibody-secreting plasma cells, providing a quicker response. There are also more memory B cells, providing a bigger memory response.
- Memory CD8 T cells are also presents in higher numbers than prior to initial exposure to antigen and are able to differentiate more rapidly into Tcs, which also secrete more cytokines.
- Immunological memory provides the basis for vaccination and there are many different vaccines which are in use or being developed.
- For vaccines to be effective they must stimulate the right type of response and be safe. Ideally they should also be cheap, stable and easy to administer.
- Current vaccines are attenuated or heat-killed versions of the pathogen, subunit vaccines that are antigens from the pathogen, or toxoids that are inactivated toxins. They have provided protection against many illnesses with considerable mortality or morbidity but there are many infections for which no vaccines are available.
- Vaccines under development or being introduced for clinical use are recombinant vector vaccines, DNA vaccines or peptide vaccines. They have the advantages that they may be safer, cheaper and easier to store than many current vaccines.