chapter 6:

TRANSFUSION AND TRANSPLANTATION

OBJECTIVES

After studying this chapter you should be able to:

- outline the role of the biomedical scientist in the transfusion of blood and blood products;
- describe how blood group systems are named;
- describe the characteristics of the ABO and Rh blood group systems;
- outline the ways in which blood groups are determined;
- discuss some of the adverse effects of transfusion;
- outline how tissue typing is carried out in the laboratory;
- outline the role of the immune system in the rejection of transplants;
- discuss the significance of MHC antigens in determining the rejection of a transplant;
- outline the uses of some of the drugs used in immunosuppression;
- describe situations that give rise to graft versus host reactions;
- explain the uses of bone marrow and stem cell transplants.

6.1 INTRODUCTION

Blood transfusion is the transfer of blood or blood products into the bloodstream of a patient who has lost blood due to injury, disease or an operation. The amount and type of blood or component transfused depends on the needs of the patient. Transfusions of blood and blood products are routine and are generally safe therapeutic procedures that are rarely associated with adverse reactions. However, it is only in the last hundred or so years that the foundations of these procedures have been established. Similarly, the transplantation of organs and solid tissues from one individual to another to

replace diseased or nonfunctional tissue is now well established. However, it is little more than 50 years since the first successful clinical transplant of a kidney in 1954 paved the way for the range of tissue transplants currently available. Advances in the transplantation of bone marrow and stem cells have brought the two fields of transfusion and transplantation closer together.

This chapter will explain the role of the transfusion scientist and will discuss the biochemical and genetic bases of a number of blood group systems and the methods used to determine blood groups. In addition, the adverse reactions of transfusion and the consequences to a fetus of antibodies against fetal erythrocytes being transmitted across the placenta will also be outlined. The evidence for the immunological basis for the rejection of tissue transplants will also be examined, together with the genetics of the HLA system, a series of genes encoding proteins that stimulate rejection of tissues (*Chapter 4*). The chapter will also consider how the rejection of tissues can be prevented by immunosuppressive therapy and will review the set of circumstances which result in **graft versus host disease** (**GVHD**), as well as considering the consequences of GVHD for the relatively new treatments which involve the transplantation of stem cells.

6.2 BLOOD AND BLOOD PRODUCTS FOR TRANSFUSION

The role of the biomedical scientist in the transfusion laboratory is to ensure that the blood and blood products being transfused into a patient are safe. To ensure safety, the blood is tested to determine its blood group and to check that it is not contaminated with harmful microorganisms. In addition, checks are made to ensure that the transfused blood does not contain antibodies that will destroy the erythrocytes of the recipient and cause death.

Blood transfusions are required to replace blood lost as a result of accident or surgery. Surgical procedures which require transfusions include the transplantation of organs, such as the liver and heart, where significant bleeding may occur. Blood may also be given to treat certain diseases, such as anemia. Plasma may also be transfused to treat badly burned patients who have lost significant amounts of fluid or in the treatment of bleeding disorders. Plasma products, such as Factor VIII, to treat hemophilia (*Chapter 13*), or immunoglobulins, to treat certain immunodeficiency disorders (*Chapter 5*), may also be given. Platelet concentrates are also used to treat bleeding disorders (*Figure 6.1*).

In the UK, blood containing leukocytes is no longer transfused for a number of reasons, as shown in *Table 6.1*. Sensitization to Major Histocompatibility Complex (MHC) antigens, which are present on blood leukocytes but not erythrocytes, may have consequences if the recipient later requires a transplant (*Section 6.11*) and GVHD may have a fatal outcome in immunosuppressed individuals. As a consequence, leukocytes are removed from blood, usually within a few hours of collection. This involves filtering the blood through leukocyte-specific filters, which trap the leukocytes but not the smaller erythrocytes or platelets. Such a process is called **leukodepletion** and it reduces the leukocyte count to less than 5×10^6 dm^{-3}. The number of leukocytes left in blood can be assessed by counting in a hemocytometer, or by using a flow cytometer (*Box 6.1*).

Reason	Examples
Possibility of transmitting infectious agents in leukocytes	human immunodeficiency virus (HIV) cytomegalovirus (CMV) prion proteins responsible for variant Creutzfeldt-Jakob disease (vCJD)*
Possibility of immunizing against leukocyte antigens	sensitization to major histocompatibility antigens
Increased risk of graft versus host disease (GVHD) with whole blood	in immunosuppressed or immunodeficient individuals
Possibility of inducing febrile reactions post-transfusion (nonhemolytic febrile reactions)	caused by cytokines released from leukocytes in storage

*In 2003 a recipient of blood taken from a healthy donor who developed vCJD, also died of vCJD 6 years after transfusion. Other evidence since that time suggests that it is possible to transmit vCJD with leukocytes (see also *Chapters 2* and *15*).

Table 6.1 Reasons for leukodepleting blood before transfusion

6.3 THE DISCOVERY OF THE BLOOD GROUP SYSTEMS

It has been known since the seventeenth century that the transfusion of blood between individuals could have rapid and fatal consequences. Fortunately, in 1900 Landsteiner (1868–1943) discovered that individuals could be classified into different groups depending on the characteristics of their erythrocytes and the presence of specific antibodies in their plasma to erythrocyte antigens. These discoveries laid the foundations for the routine and safe therapeutic transfusion of blood. Landsteiner drew blood from a number of individuals and separated the erythrocytes from the plasma. He then mixed together all possible combinations of erythrocytes and plasma from these individuals together and showed that only certain combinations resulted in the clumping or **agglutination** of the erythrocytes (*Figure 6.2*). These patterns of agglutination showed that there were different blood groups, which Landsteiner named A, B and O. In 1902 von Decastelo (1872–1960) and Sturli (1873–1964) discovered a fourth blood group which he called AB. It became clear that fatal blood transfusions resulted from incompatible blood being transfused and

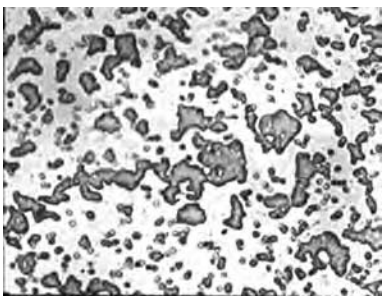
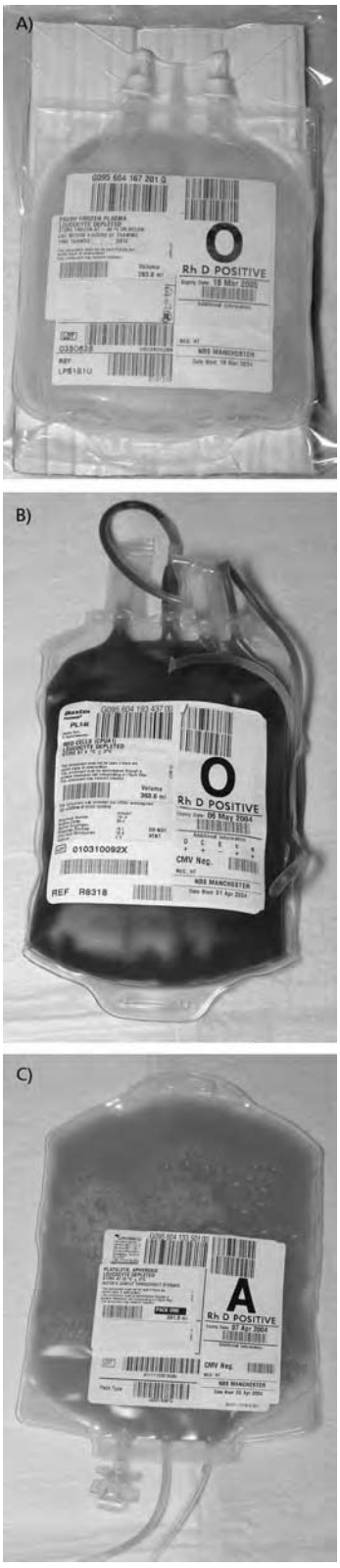


Figure 6.2 The agglutination of erythrocytes by antierythrocyte antibodies.

Figure 6.1 Some blood products: (A) plasma (B) erythrocytes and (C) platelets. Courtesy of the Manchester Blood Transfusion Service, UK.



BOX 6.1 The flow cytometer

The flow cytometer (*Figure 6.3*) is an instrument that can analyze several properties of cells simultaneously in mixed populations. The cells to be analyzed are passed as single cells in a stream past a laser light source. This is usually achieved by having a sheath of fluid passing through an orifice of 50–300 μm. The sample is injected into the sheath fluid as it passes through the orifice. With the right sheath fluid flow rate, the sample and the fluid do not mix (*Figure 6.4*). As the cells are illuminated by the laser beam some of the light is scattered. The scattered light is detected simultaneously by two detectors. One measures side scatter, that is the light deflected 90° from the incident beam. The other detects the light scattered in a forward direction up to 10° from the incident beam. This is the forward scatter. The intensity of the forward and side scatters are related to the size and shape of the cells. The forward scatter is sensitive to the surface characteristics of the cell, whereas side scatter is more sensitive to the granularity of a cell. Thus a mixed population of cells can be analyzed on the basis of these measurements. In addition

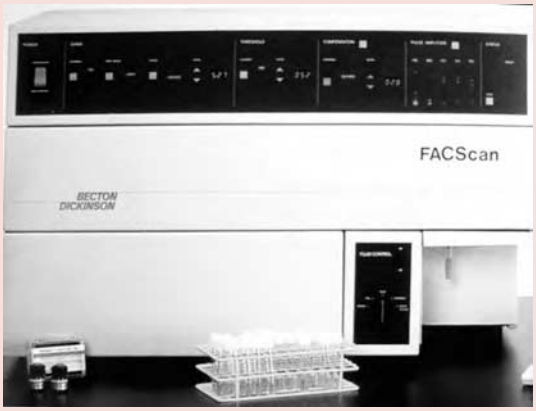


Figure 6.3 A flow cytometer. Obtained from http://flowcyt.cyto.purdue.edu/flowcyt/educate/photos/flowware/fwarepre.htm

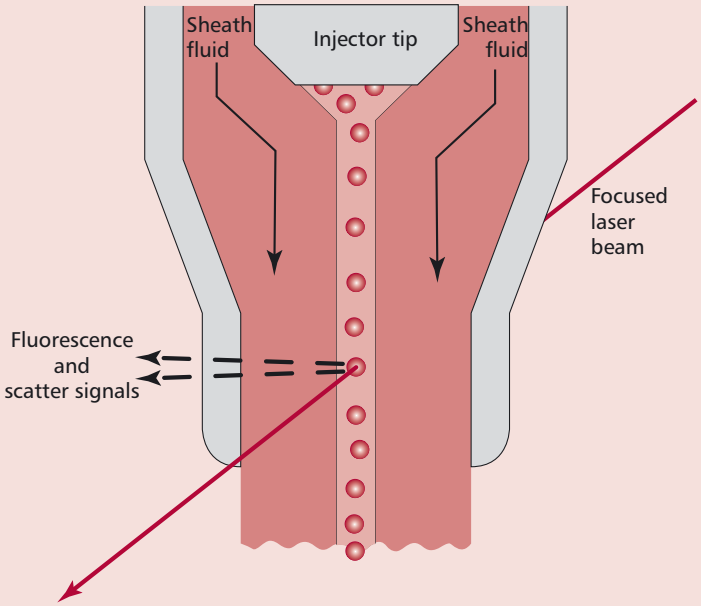


Figure 6.4 Schematic of a Flow Cell. See main text for details.

that these procedures were successful when the blood transfused was of an identical blood group. The discovery of the ABO blood group system led to Landsteiner receiving the Nobel Prize for Physiology and Medicine in 1930. Landsteiner later discovered other blood group systems, including the Rh system. Since then, numerous other systems have been discovered, as shown in

to forward and side scatter, cells can be stained with fluorescent antibodies to proteins that are characteristic of a cell population. For example, in a mixed population of lymphocytes, the T lymphocytes (*Chapter 4*) could be stained with a fluorescent antibody to the CD3 marker. This would allow T lymphocytes to be distinguished from B lymphocytes, which do not have this protein. As the cells are illuminated in the laser beam the T lymphocytes will fluoresce, and this fluorescence is picked up by further detectors, using appropriate filters. The intensity of the fluorescence is related to the amount of CD3 on the surface of the cell (*Figure 6.5*).

It is also possible to distinguish different populations simultaneously if they are stained with antibodies to unique marker proteins and if the antibodies are conjugated to different fluorescent molecules. Thus, helper T lymphocytes (T_H cells) can be stained with a fluorochrome labeled antibody to CD4. At the same time all the T cells can be stained with an antibody to CD3 which is conjugated to a different fluorochrome. Analysis by flow cytometry would reveal four populations of small lymphocytes: those which are CD3– and CD4–, presumably B_C lymphocytes, those that are CD3+ and CD4–, presumably T_C lymphocytes, those that are CD3– and CD4+, a very minor population, and those that are CD3+ and CD4+, that is, the T_H cells (*Figure 6.6*).

The flow cytometer is used in many aspects of pathology science. In transfusion it can be used, for example, to measure the number of fetal erythrocytes in the maternal circulation following a placental bleed. In transplantation it may be used to evaluate the results of a cross match (*Section 6.11*).

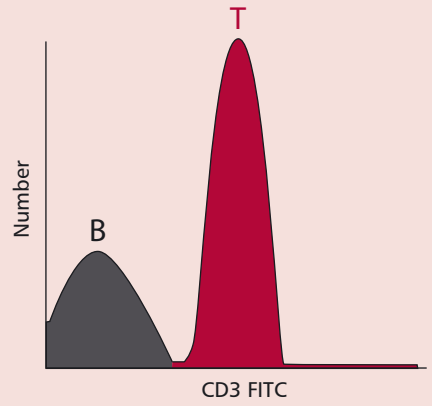


Figure 6.5 Diagram to show how T and B lymphocytes can be distinguished in a flow cytometer.

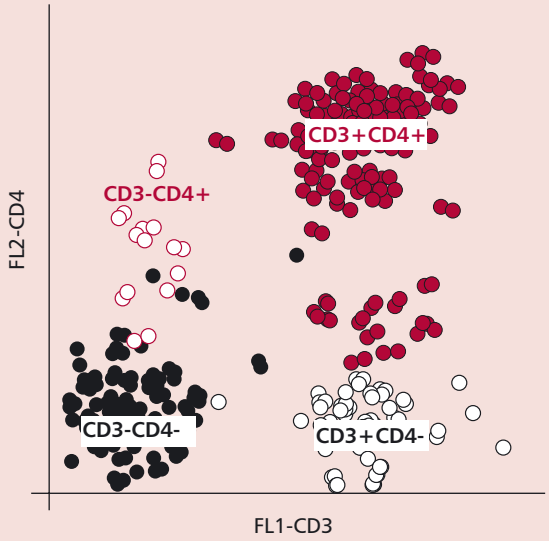


Figure 6.6 Schematic to show how the double staining of lymphocytes can be used to distinguish several populations of cells in the flow cytometer. The cells are represented by the 'dots' on the diagram. See main text for details.

Table 6.2. These blood groups systems have been assigned numbers by the International Society for Blood Transfusion (ISBT) and these, together with the conventional abbreviations, are shown. There is insufficient space in this chapter to discuss all blood groups and only those of greatest clinical significance will be discussed.

ISBT Number	Name	Symbol
001	ABO	ABO
002	MNS	MNS
003	P	P
004	Rh	RH
005	Lutheran	LU
006	Kell	KEL
007	Lewis	LE
008	Duffy	FY
009	Kidd	JK
010	Diego	DI
011	Cartwright	YT
012	XG	XG
013	Scianna	SC
014	Dombrock	DO
015	Colton	CO
016	Landsteiner-Weiner	LW
017	Chido/Rodgers	CH/RG
018	Hh	H
019	Kx	XK
020	Gerbich	GE
021	Cromer	CROM
022	Knops	KN
023	Indian	IN
024	Ok	OK
025	Raph	RAPH
026	John Milton Hagen	JMH

Table 6.2 ISBT Human blood group systems

6.4 THE ABO BLOOD GROUP SYSTEM (ISBT 001)

The ABO blood group system classifies people into one of four major blood groups: A, B, AB and O, according to the different type of antigen present on the surfaces of their erythrocytes. The frequencies of these blood groups vary between different populations (*Table 6.3*).

Phenotype	Antigen	Caucasian	Indian	Black	Overall in UK
A	A	44	24	27	43
B	B	9	38	19	9
AB	A and B	3	15	3	3
O	H	44	22	49	45

Table 6.3 Frequency (as %) of A, B, AB and O blood groups in different ethnic groups and populations

THE ANTIGENS OF THE ABO SYSTEM

The antigens that determine ABO blood groups are oligosaccharide constituents of cell surface glycolipids and glycoprotein. These sugars are added to an existing chain of oligosaccharides which protrudes from the erythrocyte membrane (*Figure 6.7*). The *H* gene, located on chromosome 19, encodes an enzyme, L-fucosyl transferase, which adds L-fucose to the terminal galactose, to form the H antigen. In blood group O individuals, the H antigen is found on their erythrocytes and also on a variety of other cells. People of blood group A and AB possess the *A* gene, encoded on chromosome 9. This gene encodes *N*-acetylgalactosaminyl transferase which adds *N*-acetylgalactosamine to the terminal galactose of the H antigen, to form the A antigen. This antigen is found on the erythrocytes of individuals of blood group A and AB. The *B* gene, which is allelic to the *A* gene, encodes D-galactosyl transferase which adds D-galactose to the terminal galactose of the H antigen, to form the B antigen. This antigen is found on the erythrocytes of blood groups B and AB individuals. The *O* gene, also allelic to the *A* and *B* genes, does not appear to produce a protein, that is, it is a silent gene. The *A*, *B* and *O* genes are inherited in a Mendelian fashion (*Chapter 15*) and A and B are codominant. The genotypes that determine the different phenotypes in the ABO system are shown in *Table 6.4*.

Genotype	Comment	Blood Group (phenotype)
AA	The gene encoding the 'A' glycosyl transferase is present on both copies of chromosome 9	A
AO	The gene encoding the 'A' glycosyl transferase is present only on a single copy of chromosome 9	A
BB	The gene encoding the 'B' glycosyl transferase is present on each copy of chromosome 9	B
BO	The gene encoding the 'B' glycosyl transferase is present on only a single copy of chromosome 9	B
AB	One copy of chromosome 9 has the gene encoding the 'A' enzyme while the other has the gene encoding the 'B' enzyme	AB
OO	Neither the 'A' gene nor the 'B' gene is present on either chromosome 9	O

Table 6.4 Genotypes and phenotypes of the ABO system

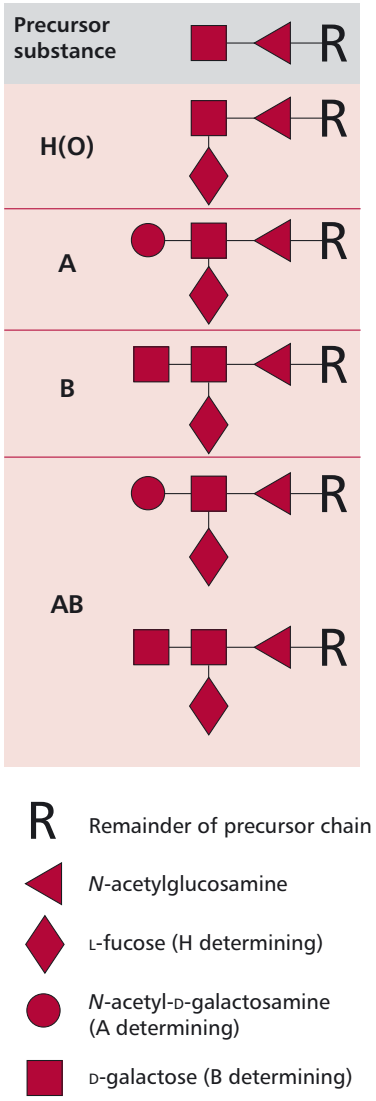


Figure 6.7 Schematic of the antigens of the ABO blood group system. See main text for details.

Approximately 75% of Caucasians attach A, B and H antigens (depending on their blood group) to soluble proteins and secrete them into saliva and other body fluids, and are known as **secretors**. Secretor status is dependent on the expression of a fucosyl transferase, which is encoded by the *SECRETOR* gene on chromosome 19. In the past, the presence or absence of secreted ABH antigens has been used in forensic science for the purpose of identification.

ANTIBODIES OF THE ABO BLOOD GROUP SYSTEM

Transfusion of ABO incompatible blood causes problems because individuals possess plasma antibodies to the complementary antigens. Thus, people of blood group A have antibodies to the B antigen, those with blood group B have antibodies to the A antigen (*Table 6.5*). People of blood group AB have neither antibody, while those of blood group O possess both. These antibodies will agglutinate erythrocytes of the complementary group. The pattern of agglutination when serum and erythrocytes of different ABO types are mixed *in vitro* is shown in *Table 6.6*.

Blood group	Antibodies in plasma
A	anti-B
B	anti-A
AB	neither anti-A nor anti-B
O	anti-A and anti-B

Table 6.5 Antibodies of the ABO system

	Serum			
Cells	A	B	AB	O
A	negative	positive	negative	positive
B	positive	negative	negative	positive
AB	positive	positive	negative	positive
O	negative	negative	negative	negative

Table 6.6 Patterns of hemagglutination when sera and cells of different ABO types are mixed

Antibodies directed against the ABO antigens generally belong to the IgM class though IgG antibodies (*Chapter 4*) may also be found. IgM antibodies are pentameric molecules, which are efficient agglutinators of cells and activators of complement. These antibodies, which are sometimes called isohemagglutinins because they agglutinate erythrocytes, are sometimes also termed 'natural antibodies' because they are present even though people have never been 'immunized' with blood of an inappropriate group. In fact, the blood group antigens are also found on some common bacteria and the antibodies that are produced following infection are capable of cross-reacting with blood group antigens. Of course, people do not make antibodies to antigens that they themselves express, because of immunological tolerance (*Chapter 5*).

CONSEQUENCES OF ABO INCOMPATIBLE TRANSFUSIONS

An example of an incompatible blood transfusion would be when a patient of blood group A, the recipient, is given blood from a donor who is blood group B. In this case the anti-B antibodies present in the plasma of the

recipient bind to the donated erythrocytes and activate complement. This results in the simultaneous lysis of billions of erythrocytes in the donated blood. At the same time, the limited amount of anti-A antibodies in the donated blood binds to the recipient's erythrocytes and hemolyzes those too. The lysis of so many erythrocytes, called **acute intravascular hemolysis**, releases so much hemoglobin that acute renal failure and shock result. This has serious clinical consequences and, indeed, is fatal in approximately 10% of cases. In addition to the problems caused by the release of hemoglobin, the fragments of erythrocyte membrane released may also initiate the blood clotting systems, leading to disseminated intravascular coagulation (DIC). The consequences of an incompatible transfusion may vary according to the blood group involved. For example, antibodies to some of the other blood group systems may result in a delayed, extravascular hemolysis. Slower destruction of the donated cells may lead to a decreasing hemoglobin concentration, with the patient suffering a fever and general malaise.

The administration of an incompatible donation is most often due to errors postdonation, and is rarely due to mismatching of bloods.

6.5 THE Rh BLOOD GROUP SYSTEM (ISBT 004)

The Rh blood group system divides people into Rh positive and Rh negative groups depending on whether or not their erythrocytes carry the Rh antigen. Landsteiner and Wiener discovered this system in 1940. They showed that antisera raised in guinea pigs against erythrocytes from rhesus monkeys reacted with 85% of Caucasian blood donors in New York. The Rh system of blood group antigens is often described as if it is a single antigen. However, it consists of a complex series of antigens, which are specified by two genes: *RHD* and *RHCE*. The former encodes the RhD protein which expresses the D antigen while the latter encodes the RhCcEe protein which carries either the C or c antigen together with the E or e antigen. At one time it was thought that another antigen, termed the 'd' antigen, was present when the D antigen was absent. It is now recognized that the d antigen does not exist. However the term is still used to indicate the D negative phenotype.

An individual person inherits a set of genes from each parent, with the possible haplotypes (haploid genotypes) being shown in *Table 6.7*, where the symbol '*d*' is used to express a lack of the *RHD* gene. Under the Fisher system for nomenclature, each Rh haplotype is assigned a code. The commonest genotypes are shown in *Table 6.8*. If an individual has the D antigen, they are said to be RhD positive. Thus, amongst the most common genotypes the only

Haplotype	Fisher system code
DCe	R1
DcE	R2
DCE	Rz
Dce	Ro
dCe	r′
dcE	r′′
dCE	r^y
dce	r

Table 6.7 Haplotypes of the Rh blood group system

Genotype	Frequency in Caucasians / %	Rh status
DCe/dce	33	positive
DcE/dce	11	positive
Dce/dce	2	positive
DCe/DCe	18	positive
DcE/DcE	2	positive
DCe/DcE	14	positive
DCe/Dce	2	positive
dce/dce	15	negative

Table 6.8 Most common genotypes of the Rh blood group system (in Caucasians)

individuals who are RhD negative are those with the genotype dce/dce (or rr using the Fisher code) and these constitute 15% of Caucasians. RhD negative individuals do not normally have antibodies to the D antigen. However, they can become sensitized if transfused with blood from an Rh positive individual. For example, a person with the genotype dce/dce who is transfused with blood from a DCE/DCE individual may make antibodies to C, D and E antigens, although anti-D antibodies are the most common.

HEMOLYTIC DISEASE OF THE NEWBORN

Hemolytic disease of the newborn (HDN) is a serious disease characterized by anemia, splenomegaly, hepatomegaly and edema. The condition is caused by the transfer of maternal anti-erythrocyte antibodies across the placenta. The condition may arise in the offspring of women who are RhD negative. Such women may become sensitized to RhD antigen when they give birth to an RhD positive child. At birth, some of the baby's blood can enter the maternal circulation and the mother will respond by making anti-D antibodies. This does not have any clinical consequence for the child itself, but may cause problems during a subsequent pregnancy, if that fetus is RhD positive. Antibodies to Rh antigens are clinically significant during pregnancy because they are of the IgG class. Thus they can cross the placenta and bind to the fetal erythrocytes. Although Rh antibodies are IgG, they do not appear to activate complement. Instead, the antibody-coated erythrocytes bind to receptors for the Fc portion of IgG (*Chapter 4*) on monocytes and macrophages, a phenomenon known as **immune adherence**. This occurs when blood is traveling through the spleen and liver. The uptake and subsequent destruction of antibody-coated erythrocytes in these organs is known as **extravascular hemolysis**.

The consequences for the second or subsequent child depend on the extent of maternal sensitization and, hence, the amount of circulating antibody. Destruction of fetal erythrocytes *in utero* may lead to fetal anemia and hyperbilirubinemia, an excess of bilirubin in the blood (*Chapter 13*). The concentrations of serum bilirubin indicate the degree of hemolysis. Babies born with significant levels of bilirubin will suffer **kernicterus** or brain damage due to the build up of the lipid soluble bilirubin in the brain. The baby will need phototherapy, which helps to breakdown the bilirubin (*Chapters 11* and *13*).

If the anemia is severe, the fetus may die of heart failure or *hydrops fetalis*, which is an extreme edema of the entire body of the fetus. This may lead to

spontaneous abortion. A longer term child may be stillborn. The considerably enlarged spleen and liver in a child born alive with HDN is associated with erythrocyte destruction in these organs. The baby may also have a facial rash indicative of hemorrhages due to impaired platelet function.

The commonest cause of HDN is antibody to the RhD antigen although it may also be caused by antibodies to other blood group antigens, for example, antibodies to blood group A or B antigens, if the antibodies are of the IgG class. In addition, antibodies to the Rhc antigen, and to the Kell blood group antigen (*Section 6.6*), may also be involved.

All pregnant women in the UK and other developed countries now have their ABO and Rh status checked at their initial hospital booking, which is usually at 12–16 weeks of pregnancy. They are also checked for anti-D, anti-c or anti-Kell (*Section 6.6*) and, if these are present, the concentrations will be monitored regularly throughout the second trimester of pregnancy. If the levels of clinically significant antibodies start to rise, clinical intervention may be necessary. Concentrations of anti-D below 4 international units (IU) cm^{-3} are unlikely to cause HDN, those between 4–15 IU cm^{-3} have a moderate risk of HDN, while values above 15 IU cm^{-3} are associated with a high risk of HDN.

Margin Note 6.1 HDN and the ABO system

Hemolytic disease of the newborn does not occur within the ABO system if Anti-A and Anti-B are of the IgM class. This is because IgM does not cross the placenta. In addition, a woman who is blood group A, RhD negative, is unlikely to become sensitized to a fetus who is blood group B, RhD positive because, when the fetal red cells enter her circulation following the birth, her anti-B antibodies will destroy the fetal erythrocytes before sensitization to RhD can occur.

HDN prophylaxis

Since the 1970s, a prophylactic treatment to prevent Rh sensitization has been available which has greatly reduced the incidence of HDN due to RhD sensitization. The treatment involves the intramuscular injection of at least 500 IU of anti-D immunoglobulin within 72 h of the birth of a RhD positive baby. The administered antibodies bind to any erythrocytes from the baby which have entered the maternal circulation and destroy them, preventing the mother from making antibodies.

In 2002, the National Institute for Clinical Excellence (NICE) in the UK recommended that antenatal anti-D prophylaxis should be routinely offered to any pregnant Rh negative woman who has not made anti-D antibodies to prevent sensitization predelivery caused by, for example, a placental bleed.

Direct antiglobulin testing and Kleihauer testing

The direct antiglobulin test (DAT), formerly known as the Direct Coombs test, is undertaken to see if maternal antibodies are present on the baby's erythrocytes. If they are present, then a sample of the erythrocytes from the baby will be agglutinated by an antibody to IgG, known as antihuman globulin (*Figure 6.8*).

The Kleihauer test uses the Kleihauer-Betke stain and is a method of assessing the volume of fetal blood that has entered the maternal circulation. In most cases the volume that has entered will be less than 4 cm^3, and 500 IU of Anti-D is sufficient to remove the erythrocytes in this volume. However, for less than 1% of women, the volume of fetal blood is larger and the mother consequently needs more than this amount of anti-D. The Kleihauer test is carried out on a sample of maternal blood 2 h after delivery. The principle of the test is that the hemoglobin in adult erythrocytes can be eluted with acid, whereas the hemoglobin of fetal erythrocytes is resistant to acid elution. A smear of maternal blood is placed in a solution of hematoxylin and hydrochloric acid, pH 1.5, and then is counterstained with eosin. The maternal red cells appear as pale 'ghosts' whereas the fetal erythrocytes stain pink with eosin, while the leukocytes stain blue (*Figure 6.9*). The ratio of fetal to maternal cells is an indicator of the volume of blood that has entered the circulation. This test can also be carried out during pregnancy if a placental bleed is suspected.

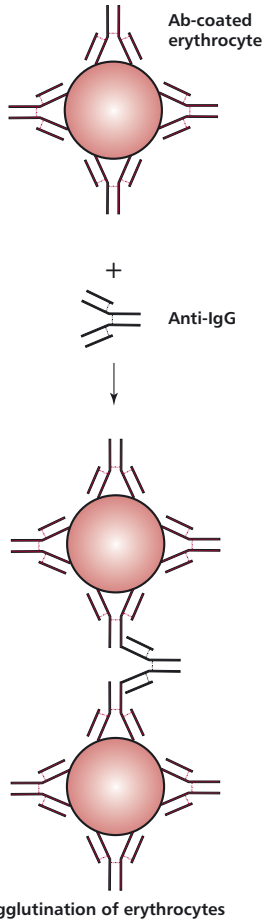


Figure 6.8 Schematic to show the direct antiglobulin test. See main text for details.

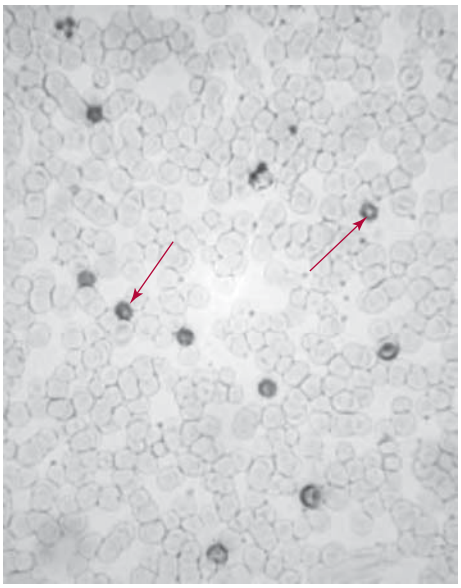


Figure 6.9 Results of Kleihauer test. The darker staining erythrocytes are fetal cells.

6.6 OTHER BLOOD GROUP SYSTEMS

The Lewis blood group system (ISBT 007, symbol Le) is related the Lewis antigens Le^a and Le^b present on erythrocytes. However, these antigens are not integral parts of the membrane but are soluble plasma proteins which become reversibly adsorbed onto erythrocyte membranes. The levels of bound antigens therefore vary, although the erythrocytes of children 2 years old and above have approximately adult levels.

The Le^a and Le^b antigens are not the products of different forms of a single gene, but arise from different actions of a fucosyl transferase that attaches fucose residues to an oligosaccharide known as type-1 precursor oligosaccharide. If the fucose is added to a subterminal position it produces the Le^a antigen, whereas attachment to the terminal position gives the Le^b antigen. Approximately 72% of white populations are Le(a–b+), that is, they lack the Le^a but have the Le^b, 22% are Le(a+b–), and 6% lack both antigens. Antibodies to the Le antigens are usually of the IgM class and, as such, do not cause HDN since they do not cross the placenta.

The Duffy system of blood group antigens (ISBT 008, symbol FY) is comprised of six antigens, of which Fy^a and Fy^b are the most significant in transfusion reactions. These antigens are expressed on an erythrocyte membrane glycoprotein and also form the site of attachment for malarial parasites (*Chapter 2*). Thus a Fy(a–b–) individual, who does not express the blood group antigens, has a selective advantage in a malarial area. Indeed, 68% of blacks of African descent are of this phenotype, which is rare in whites. Antibodies to Duffy antigens belong to the IgG class and may cause HDN.

The Kidd antigens (ISBT 009, symbol JK) are expressed on a membrane glycoprotein, which is associated with urea transport. The Jk^a and Jk^b antigens result from the expression of a codominant pair of genes. Approximately 27% of whites, and 57% of blacks are Jk(a+ b–) while 50% of whites and 34% of blacks are Jk(a+ b+). The Jk(a–b+) phenotype is found in 23% of whites and

only 9% of blacks, while the Jk(a–b–) phenotype is rare in both populations. Antibodies to Jk^a and Jk^b belong to the IgM or the IgG class and may cause a mild form of HDN.

The Kell blood group system (ISBT 006, symbol KEL) is formed from 24 antigens expressed on a glycoprotein of the erythrocyte membrane. The antigen K (formerly Kell) is highly immunogenic and IgM or IgG antibodies to it are common in transfused patients. Similarly, antibodies to its allele, designated *k* (formerly Cellano), can also cause HDN, although specific antibodies are rare in transfused patients.

6.7 LABORATORY DETERMINATION OF BLOOD GROUPS

Traditional methods for determining blood groups rely on the agglutination of erythrocytes by antibodies, usually referred to as hemagglutination. Hemagglutination can be carried out on glass microscopy slides or in microtiter plates in which agglutination patterns are easily distinguished from the settling of erythrocytes. Recent years have seen increasing use of the Diamed typing system to detect hemagglutination. This is a system which uses monoclonal typing antibodies, distributed in a gel, contained in individual tubes set in plastic 'cards'. Cells are added to the antibodies and the cards are centrifuged. Where agglutination has occurred, the agglutinates remain on top of the gel, whereas nonagglutinated cells settle through the gel to the bottom (*Figure 6.10*). Most transfusion laboratories now use gel technology for blood grouping and compatibility testing.

Whichever technique is used, a blood group, such as the ABO grouping, is determined by incubating the individual's erythrocytes with antibodies to known antigens (anti-A and anti-B in this case) and also mixing the individual's plasma with erythrocytes of known A, B, AB or O blood groups. The pattern of hemagglutination shown will enable the determination of the blood group.

Hemagglutination occurs when antibodies to an erythrocyte antigen cross-link the cells, forming visible aggregates. The extent of hemagglutination depends on the temperature, pH and the ionic strength of the medium. Agglutination is favored in low ionic strength saline (LISS). Erythrocytes have a net electronegative charge and repulsive forces normally keep them about 20 nm apart. When antibodies bind to the erythrocyte, the reduced surface charge allows the cells to agglutinate. This is most effectively achieved with IgM antibodies, which can cause direct agglutination of erythrocytes. To obtain a direct agglutination with IgG antibodies, it is usually necessary to include bovine serum albumin in the medium, which masks the charges on the erythrocytes and allows them to come closer together. Another method of reducing the negative charge is to use proteolytic enzymes to remove surface proteins that carry the charge. The enzyme can be added to the erythrocytes prior to the addition of the antibody, or all the components can be added together. Polycationic polymers such as polybrene will also reduce the negative charge on erythrocytes.

The antiglobulin test *(Section 6.5)* uses the ability of antihuman globulin (AHG) to agglutinate erythrocytes coated with nonagglutinating erythrocyte-specific IgG. This can be used to detect erythrocytes already coated with anti-erythrocyte IgG in the direct antiglobulin test (DAT), or can be used on cells which have been incubated with antibody *in vitro* (see *Figure 6.8*).

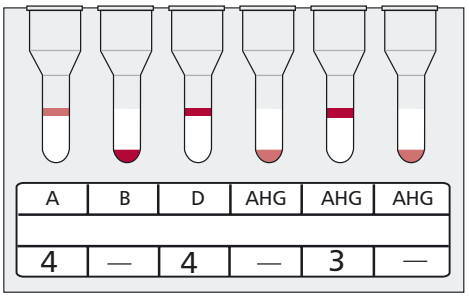


Figure 6.10 The Diamed gel card system for determination of blood group. Agglutinated cells do not penetrate the gel. The blood group indicated here is A Rh+, as indicated by the first three tubes where agglutination has occurred in tubes 1 and 3. AHG is antihuman globulin (***Section 6.5***).

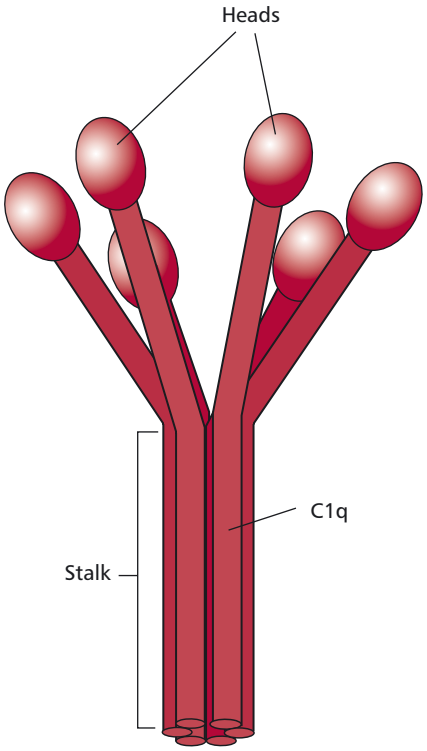


Figure 6.11 A diagram of the C1q molecule to show the six sites ('heads'), each of which can bind to an Fc region of an IgG or an IgM molecule.

6.8 ROLE OF COMPLEMENT IN TRANSFUSION REACTIONS

In *Chapter 4*, the 'beneficial' role of complement in eliminating immunogens was discussed. The activation of complement is also involved in some forms of immunological hypersensitivity and can cause some of the problems associated with autoimmune disease (*Chapter 5*). However, given that it amplifies the actions of antibodies, complement can cause many of the problems associated with transfusion reactions. Thus, complement causes lysis of sensitized erythrocytes, that is, erythrocytes coated with anti-erythrocyte antibodies.

The classical pathway for complement activation is initiated when IgG or IgM binds to an epitope, in this case on the erythrocyte's membrane. The antibody could be IgM, as is usually the case with Anti-A or Anti-B, or IgG, as is the case with antibodies to Rh antigens. The binding of antibody to the epitope induces a conformational change in the Fc region of IgG or IgM, allowing the binding of C1 protein. The C1 is comprised of three loosely associated proteins called C1q, C1r and C1s. The C1q is a large protein and has several binding sites allowing it to bind to multiple Fc regions of antibodies (*Figure 6.11*). It requires at least two of these sites to bind to adjacent Fc regions on the surface of a cell to activate complement. For this reason IgM, which has several Fc regions per molecule, is more efficient than IgG at activating complement. Indeed, a single molecule of IgM can activate complement, whereas it takes about 1000 molecules of IgG to achieve the density required for activation. The binding of C1q activates C1r and this, in turn, activates C1s which acquires proteolytic activity (*Figure 6.12*). C1s has two substrates: C4 and C2 each of which are hydrolyzed to two fragments: C4a, C4b and C2a and C2b respectively. Proteins C4b and C2a are the larger fragments in each case and they combine to form a new proteolytic enzyme, C4b2a. A single enzyme molecule can generate a number of product molecules. Thus for a limited number of antibody molecules, many molecules of C4b2a are formed, because enzymic steps allow amplification to occur. C4b2a is the classical pathway **C3 convertase** (*Chapter 4*) which cleaves C3 into two fragments: a larger C3b molecule and a C3a. The former binds to the target cell membrane where it may bind a molecule of the C3 convertase to form a **C5 convertase**, which catalyzes the hydrolysis of C5 into C5a and C5b. C5b binds to the cell membrane and forms a site for the build up of the **Membrane Attack Complex** (**MAC**). This is a large, cylindrical, hydrophobic structure constructed from single molecules of C5b, C6, C7, C8 and several molecules of C9. When it inserts into the membrane it forms a pore of approximately 10 nm diameter. Since amplification has occurred at each enzymic step, the target cell membrane may be covered with MACs (*Figure 6.13*). The MACs allow small ions to equilibrate across the cell membrane, increasing the osmotic pressure within the cell so that water moves across the membrane into the cell causing it to lyze. *In vitro* this can be seen as a sudden clearing of the cloudy suspension of erythrocytes. *In vivo*, several regulatory proteins may prevent direct lysis of the erythrocytes. Instead, the cells are lyzed by phagocytic cells that have receptors for C3b and other complement proteins on their membranes. *Table 6.9* lists some of the receptors involved in the clearance of sensitized erythrocytes. In the transfusion laboratory it is much easier to look for complement proteins on erythrocytes than to look for antibodies, since a small amount of antibody may result in large amounts of complement on the cells. Thus, the presence of complement proteins on cells is used as an indicator of the presence of complement binding antibodies.

It is essential for the transfusion scientist to be able to detect hemolytic antibodies. Such antibodies may be present due to transfusion reactions, to HDN or they may be autoantibodies to erythrocytes, as happens in autoimmune hemolytic anemia. The presence of relevant antibodies may be detected in

Receptor	Distribution on cells involved in clearance of sensitized erythrocytes	Protein bound
CR1	erythrocytes, neutrophils, eosinophils, monocytes, macrophages	C3b, C4b, C3bi (an inactive form of C3b)
CR3	neutrophils, large granular lymphocytes, macrophages	C3bi
CR4	neutrophils, monocytes, macrophages	C3bi

Table 6.9 Complement receptors

samples of serum by hemolysis *in vitro* using the complement activity in the serum or they may be detected by examining the surface of erythrocytes for activated complement proteins. The presence of complement in serum diminishes with storage, therefore it is recommended that samples of sera should be stored at –20°C to retain activity if they are to be used for hemolysis determination. Further, some anticoagulants, such as EDTA, inhibit complement, which may be significant if plasma rather than serum is available. Other sera may have 'anticomplementary' activity due to the presence of a denatured form of complement known as complementoid.

Receptors for C3b are found on all the major types of phagocytic cells and also on erythrocytes themselves, so that even these cells have a role in the clearance of immune complexes from the blood. Antigen–antibody complexes coated in C3b bind to erythrocytes and are removed by macrophages in the spleen and liver.

In vivo, other complement proteins trigger inflammatory reactions. For example, C3a, C4a and C5a cause blood basophils and tissue mast cells to degranulate. The mediators released stimulate inflammation (*Chapter 4*), which may have consequences in a patient who has anti-erythrocyte antibodies. In addition, both C3a and C5a are chemotactic factors for neutrophils and promote a build up of these cells, which may itself lead to clinical problems.

The alternative pathway for complement activation is a positive feedback loop which is usually initiated by microorganisms such as bacteria and yeasts. However, feedback may utilize C3b, produced in the classical pathway, and amplify the amount of C3b produced. The positive feedback loop is controlled to prevent an overproduction of C3b. One regulatory step binds C3b to a plasma protein called Factor H and in the bound form is inactivated by Factor I, which converts it to C3bi, a form that can no longer enter the amplification loop. C3b may then be degraded into smaller fragments, C3dg and C3d, which may remain bound to the erythrocyte membrane. The presence of natural regulators means that many antibodies that are potentially able to lyze erythrocytes are unable to do so *in vitro* and the transfusion scientist may look for the presence of C3d on erythrocytes to determine whether antibodies to them are present.

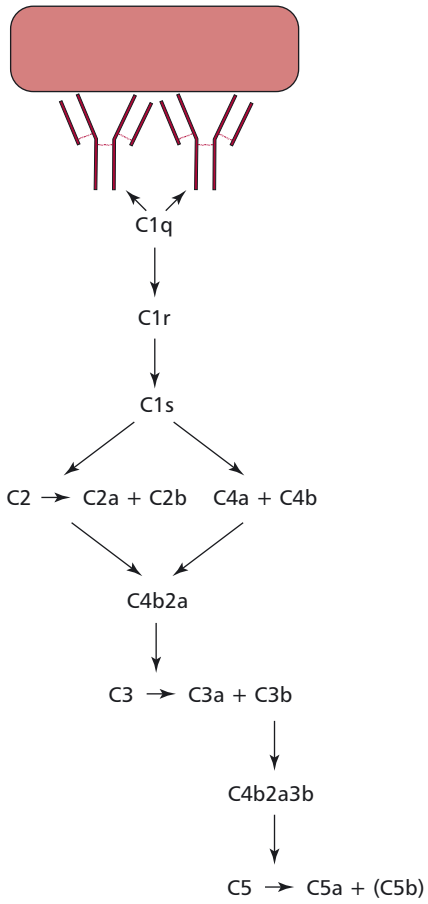


Figure 6.12 The classical pathway for complement activation. See main text for details.

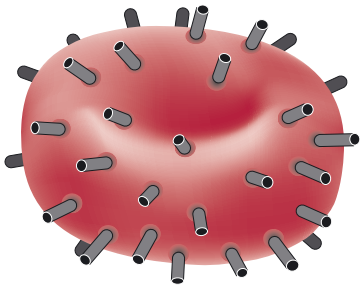


Figure 6.13 Schematic to show an erythrocyte covered with membrane attack complexes (MACs) inserted into the cell membrane.

6.9 HAZARDS OF TRANSFUSION

One hazard of transfusion is a hemolytic transfusion reaction (HTR) if preexisting antibodies are present in the recipient. This may result in acute intravascular hemolysis, as in ABO incompatibility, or in delayed extravascular hemolysis, as with several of the other blood group systems. Acute intravascular hemolysis has serious clinical consequences and, indeed, may be fatal. With delayed extravascular hemolysis the patient may suffer fever and general malaise as the donated erythrocytes are destroyed and the hemoglobin levels

Margin Note 6.2 The Serious Hazards of Transfusion group

In the UK, adverse reactions to transfusion are reported to the Serious Hazards of Transfusion (SHOT) group based at the Manchester Blood Centre. Here, the data are collected and an annual report is produced. This process enables trends to be recognized, and recommendations on safe practice in transfusion to be made.

fall. To prevent either occurrence, the recipient should be screened before the transfusion for the presence of anti-erythrocyte antibodies.

The transfusion of leukocytes may also lead to adverse reactions, such as **nonhemolytic febrile transfusion reactions**. Such patients exhibit flushing, fever, rigors and hypotension. These may be caused by the reaction between antibodies and leukocyte antigens in the recipient, resulting in the lysis of donated leukocytes and release of cytokines from them. In addition, activated complement proteins cause the release of histamine from basophils, triggering inflammatory reactions. As all blood is leukodepleted before transfusion, such reactions are rare.

TRANSFUSION RELATED ACUTE LUNG INJURY

Transfusion related acute lung injury (TRALI) is a life-threatening complication of transfusion. It presents as a rapidly progressing and severe respiratory failure with diffuse damage to alveolar cells and the filling of alveolar spaces with fluid. Histological examinations reveal an infiltration of the alveoli with neutrophils and monocytes, indicative of an acute inflammatory reaction.

The symptoms of TRALI include dyspnea, cyanosis, hypotension, fever and pulmonary edema, which usually occur within 6 h of transfusion. The condition is thought to arise from the interaction of leukocytes and specific antileukocyte antibodies, usually in the donor plasma, though occasionally in that of the recipient. The most likely antibodies are those to the Major Histocompatibility (MHC) antigens, which are commonest in women who have had multiple pregnancies (*Section 6.11*) and in males and females who have received multiple transfusions. In addition, antibodies to neutrophil antigens have also been implicated.

TRALI has been reported to occur after transfusion of fresh frozen plasma, platelets, whole blood and concentrated erythrocytes. In the UK in 2003, 36 cases of suspected TRALI were reported to SHOT. Nine patients died, seven possibly, and one definitely due to transfusion. Plasma-rich components were implicated in 20/21 cases in which there was proven leukocyte incompatibility between the patients and the donor. In 2004, however, 23 suspected cases were reported in the UK. Of these, 13 were highly likely to be TRALI and were linked to fresh frozen plasma, in six cases, platelets in four, erythrocytes in two and whole blood in one. The incidence of TRALI has since decreased in the UK, due to the processing of fresh frozen plasma from untransfused male donors who have tested negative for antileukocyte antibodies.

IRON OVERLOAD

Patients who receive many transfusions over a long period of time may develop iron overload. The excess of iron, for which there is no excretory route, from transfused blood may cause tissue damage, especially to the liver, heart and endocrine glands. Signs of iron overload can be detected after 10–20 transfusions and the condition may be fatal if left untreated. Patients should be treated with a chelating agent such as deferrioxamine mesylate to remove unwanted iron (*Chapter 13*).

ALLOIMMUNIZATION

Patients who receive regular erythrocyte transfusions may become immunized to other blood group antigens present on the ABO compatible cells. This may have consequences for future transfusions. If patients receive whole blood, rather than leukodepleted blood, they may become immunized to the MHC antigens on the foreign leukocytes. This may become clinically significant if in the future they require an organ transplant (*Section 6.11*). However,

the increasing use of leukodepleted blood, in which the leukocytes have been removed prior to transfusion, prevents the immunization of recipient to these antigens.

GRAFT VERSUS HOST DISEASE

Graft versus host disease (GVHD) is a potentially fatal condition which is associated with the transfusion of whole blood or blood products, such as packed erythrocytes or platelets, which contain residual lymphocytes. It usually results from the transfusion of leukocytes into an immunodeficient patient or when blood is transfused into neonates or premature babies. The small lymphocytes present in the donated blood recognize the antigens of the recipient as foreign and mount an immune response against them. The donor lymphocytes proliferate in the patient and attack tissues, causing enlargement of the spleen and liver, diarrhea and an extensive skin rash. Acute GVHD may be fatal and for this reason it is recommended that products such as packed erythrocytes be irradiated prior to use to prevent residual small lymphocytes from reacting to the host antigens. Graft versus host reaction can also be a consequence of bone marrow transplantation (*Section 6.14*). Frozen plasma is safe in this respect, since freezing destroys leukocytes. Transfusion associated GVHD is not usually linked to AIDS.

INFECTIONS

One potential hazard of transfusion is infection with microorganisms present in the donor. In the past, blood transfusions have spread infections such as HIV (*Chapter 4*) and hepatitis C (*Chapter 11*) to the patient. For this reason there is now extensive screening of blood donors (*Section 6.10*).

6.10 SCREENING OF BLOOD DONORS

Transfusion scientists must be assured that the process of transfusing blood poses minimal risk to the patient and donor alike. Aside from the obvious need for blood to be carefully matched to avoid a possibly fatal transfusion reaction, it is essential that donors are carefully screened to avoid those who are ill or who may be harmed by giving blood, or whose blood poses a health risk because, for example, it is contaminated with certain viruses even though the donor shows no signs of ill health.

In the UK, blood is taken from healthy donors aged between 17 and 70 and is a voluntary and unpaid activity. Potential donors who are excluded from donation include individuals with HIV or hepatitis viral infections, as well as individuals who are at risk of becoming HIV and/or hepatitis virus positive, for example prostitutes, drug abusers who inject themselves with drugs, and individuals who have had sex with men or women living in Africa (*Table 6.10*). In addition, people with low hemoglobin levels (below 135 g dm^{-3} and 125 g dm^{-3} for men and women respectively), those who have had infectious diseases such as a cold or sore throat within the last seven days or viral infections such as measles, mumps, rubella, chickenpox, shingles or herpes simplex cold sores within the last three weeks. Other reasons for exclusion include the recent use of therapeutic drugs, for example aspirin, antibiotics, antihistamines and antidepressants.

All donated blood is screened for a variety of infectious agent as shown in *Table 6.11*. Some tests are mandatory while others are optional. Optional tests such as those for cytomegalovirus (CMV) are used when the blood is to be transfused into immunocompromized individuals.

Margin Note 6.3 Plasmapheresis and leukapheresis

Different components of the blood can be isolated as the donation is occurring by sending the blood through a cell separator. A catheter is first inserted into the vein of the donor. Blood entering the catheter is transferred to the cell separator where it is centrifuged at a speed necessary to collect the required component. The blood, minus the component that has been removed, is returned to the donor via a catheter inserted into a vein in the other arm. **Plasmapheresis** is the collection of plasma, with the return of the erythrocytes and leukocytes to the donor. **Leukapheresis** is the collection of leukocytes, with plasma and erythrocytes being returned. Similarly, **plateletpheresis** involves the collection of platelets, with all other components being returned. The term **apheresis** is a general term that covers the collection of specific blood components in this way.

Category	Examples
Recent history of clinical intervention	had a minor operation within the last month had a major operation within the last six months had a child or a miscarriage within the last 12 months had a local anesthetic for dental treatment within the last two days had a general anesthetic for dental treatment within the last month is currently receiving medical treatment or under medical investigation
Donor is currently unwell	has a hemoglobin level below 135 g dm^{-3} (male) or 125 g dm^{-3} (female) is currently taking aspirin or has taken antibiotics, antihistamines or antidepressants within the last seven days
Donor has recently been unwell/ is unwell	recently had chicken pox, shingles, measles, mumps, rubella, herpes simplex cold sores within the last three weeks had a sore throat, cold, cough in the last seven days is known to be infected with HIV or hepatitis viruses
Donor is in a high risk category for HIV and/or hepatitis viruses	has injected recreational drugs at any time since 1977 had sex with men or women living in Africa at any time since 1977 is a prostitute

Table 6.10 Reasons for exclusion from donating blood

Mandatory tests	Optional tests
Hepatitis B virus (HBV)	cytomegalovirus (CMV)
Hepatitis C virus (HCV)	malaria (*Chapter 3*)
Human immunodeficiency viruses (*Chapter 3*)	
Syphilis (*Chapter 3*)	

Table 6.11 Mandatory and optional tests for infectious agents in donated blood

BOX 6.2 Artificial blood

The potential risks arising from the transfusion of blood have led to research into the development of an artificial blood or blood substitute that would alleviate these risks. In addition, the supply of blood in some regions of the world is too small to make its availability reliable. For an artificial material to be a 'good' blood substitute it should contain a material that will carry appropriate amounts of oxygen around the body. In addition, the fluid carrier must be isotonic to prevent cell lysis due to osmotic differences. It must also be able to withstand sterilization to prevent the risk of infection. Two classes of artificial blood substitute have been investigated. One is based on the use of solutions of modified hemoglobin, the other has involved the use of products based on perfluorocarbons.

MODIFIED HEMOGLOBINS

Originally, these substitutes were based on free hemoglobin extracted from erythrocytes. However, it became apparent that free hemoglobin is associated with renal toxicity. Hemoglobin inside the erythrocyte forms tetrameric molecules whereas outside the erythrocyte it forms toxic dimers. Cross-linking the tetramers or processing them to polymers prevents dimer formation. However, success has been limited. The use of hemoglobin-based solutions has been hampered by severe side effects including hypertension and vasoconstriction. One cross-linked hemoglobin product was withdrawn from a phase II clinical trial in 1998 because of unacceptably high mortality compared with standard treatments. However, other attempts may prove to be more useful.

6.11 TRANSPLANTATION OF SOLID TISSUES AND ORGANS

The transplantation of solid tissues from one individual to another for therapeutic purposes is a fairly routine medical procedure. Interest in transplantation mainly arose during the Second World War during attempts to treat badly burned airmen using skin grafts from unrelated donors. Indeed, it was from experiments with skin grafting in rodents that the role of the immune system in the rejection of transplants was identified. Moreover, once this role was recognized, the search for drugs that could prevent rejection became more focused. The first successful kidney transplant was performed by Murray (1919–) in 1954 while the first human heart transplant, by Barnard (1922–2001), was performed in 1967.

Once technical problems associated with the transplantation of whole organs were overcome, the major problem associated with transplanting organs was, and remains, rejection. This is caused by the immune system of the recipient recognizing the cells of the donated organ as foreign and mounting an immune response against them. Unless steps are taken to prevent it, transplant rejection is inevitable, unless the donor and the recipient are identical twins. However, organ transplantation is now remarkably advanced compared with initial attempts. Indeed, a range of transplants are routinely performed (*Table 6.12*).

IMMUNOLOGICAL REJECTION OF TRANSPLANTS

The rejection of a transplant is due to the immune system of the recipient recognizing the donated cells as foreign, that is nonself. Thus, the greater the genetic disparity between the donor and recipient, the greater the chances of rejection. The commonest type of clinical transplant is called an **allograft**, that is, a graft between two genetically nonidentical people. However, **isografts**, in which pieces of tissue are transplanted from one site to another on the same individual are also routinely undertaken, for example in skin grafting to treat burns. Isografts are not rejected since the donor and recipient are, of course, genetically identical. Occasionally, the graft may come from another species, such as those occasions when a baboon heart has been transplanted into a human. Such grafts are known as **xenogeneic** transplants. Xenogeneic transplants are also subject to rejection,

In 2003, trials at the Karolinska hospital in Stockholm used an artificial blood to treat patients. The product was said to transport oxygen through the body 'better than real blood'. It is thought that the substitute was based on hemoglobin solutions. In October 2005, the American Food and Drug Administration (FDA) was reported to be conducting a trial with patients in Kansas. Patients traveling by emergency ambulance to hospitals in four counties in the state were being given an artificial blood substitute called PolyHeme, rather than saline, to treat severe bleeding.

PERFLUOROCARBON-BASED SOLUTIONS

Perfluorocarbons (PCFs) are compounds in which fluoride and bromide atoms are attached to an inert carbon chain. They are able to dissolve large quantities of oxygen and have been shown to improve the oxygenation of tissues, even at low doses. However, PCFs are immiscible with water and must be administered as emulsions. They have been investigated over a considerable period. In 1966 mice were found to survive a 10-min immersion in an oxygen saturated PCF liquid and were able to breathe atmospheric oxygen when removed from the trial. Several products based on PCFs have been the subjects of clinical trials, although patients also have to breathe 70–100% oxygen and flu-like symptoms have been reported. The use of PFCs to enhance artificially the performance of athletes has also been reported.

Tissue organ transplanted	Examples of clinical conditions
Bone marrow	immune deficiency; in treatment of leukemia
Cornea	types of blindness
Heart	heart failure
Heart and lungs	cystic fibrosis
Kidney	end-stage renal failure
Liver	cirrhosis
Pancreas	type 1 diabetes mellitus
Skin	treatment of burns

Table 6.12 Tissue and organ transplants

owing to the increased genetic disparity between the donor and the recipient. Other more common examples of xenogeneic grafts involve the use of porcine and bovine heart valves to replace diseased human ones. In the latter case the valves are treated with glutaraldehyde to stiffen them and to mask their antigenic determinants, and these are often referred to as bioprosthetic valves. There is much debate about proposals to breed genetically engineered **transgenic** pigs to carry some human antigens on their cells to provide transplants for humans. Apart from the ethics of breeding animals purely to supply organs for humans, there is also the risk of the transfer of an unknown virus from pigs to humans, with possible disastrous consequences.

CAUSES OF GRAFT REJECTION

The transplanted cells of an allograft carry histocompatibility antigens on their membranes, which are recognized as foreign by the cells of the immune system. These histocompatibility antigens are found on proteins that are encoded by the **Major Histocompatibility Complex** (MHC) which, in humans, is also known as the Human Leukocyte Antigen (HLA) complex (*Chapter 4*). The MHC complex is a genetic region that encodes several classes of proteins, some of which are membrane proteins. Class I MHC proteins (MHC I) are found on the membranes of all nucleated cells and are involved in the recognition of virus-infected cells by the precursors of cytotoxic T lymphocytes (T_C cells). Class II MHC proteins (MHC II) are found on the membranes of antigen presenting cells such as macrophages and are involved in the recognition by T_H lymphocytes of foreign proteins on the surface of antigen presenting cells. It is relatively minor differences between the amino acid sequences of the MHC molecules of the donor and of the recipient that lead to the rejection of the transplanted tissue.

The rejection of an allograft usually takes place a few weeks after the transplant unless immunosuppressive treatments are given. Small lymphocytes recognize the transplanted cells as foreign. An **acute rejection** is caused by T lymphocytes that infiltrate the graft. The presence of T lymphocytes and monocytes in the infiltrate is a strong indicator of cell-mediated immunity. Both T_H and T_C cells are involved in graft rejection. The T_C cells develop into cytotoxic T lymphocytes directed against the foreign histocompatibility antigens of the grafted cells and are able to destroy cells of the grafts directly, or indirectly by producing cytokines that attract monocytes and macrophages. The T_H lymphocytes respond by producing cytokines that activate a variety of nonspecific cells to attack the graft.

Sometimes rejection may take place within hours or minutes of transplantation, once the tissue has become revascularized. This is known as **hyperacute rejection** and is due to antibodies against graft antigens being already present in the plasma of the recipient. These antibodies bind to the graft cells and activate complement leading to the rapid destruction of donor cells. Hyperacute rejection can also be brought about if the recipient already has antibody to MHC antigens present on the graft. These antibodies may be present for a number of reasons. For example, women who have had a number of pregnancies often have antibodies to the MHC antigens on the fetus, which were inherited from the father. Secondly, patients who have had a number of blood transfusions may become immunized to the MHC antigens on residual leukocytes present in the transfusion. Thirdly, patients who have had a previous transplant and rejected it will almost certainly have antibodies to any foreign MHC antigens that were present on that graft. Finally, antibodies to blood group antigens can also cause hyperacute rejection if they are already present in the recipient. For example, the blood group A, B and H antigens are present on the endothelial cells lining blood vessels. If a recipient of blood group A is given a transplant, for example a kidney from a person of blood group B, the anti-B antibodies in the plasma of the recipient will attack the endothelial cells of the graft and activate complement causing destruction of the graft. For this reason, transplants are no longer carried out against a major blood group barrier. Given that pre-existing antibodies can cause a rapid rejection of a graft it is essential to know which potential recipients have such antibodies. Hence, a cross match is performed in which serum from the recipient is incubated with cells from the donor. If the donor cells are killed in the presence of recipient serum and complement, the transplant will not be undertaken and another potential recipient will be sought.

A **chronic rejection** takes place months or years after transplantation and is brought about by a combination of cell-mediated and humoral mechanisms.

6.12 THE HLA SYSTEM

The HLA system of genes is found on the short arm of chromosome 6. This region encodes MHC proteins. The structures of MHC I and II were discussed in *Chapter 4* in the context of their roles in the immune response. Here their involvement in triggering rejection will be emphasized. Molecules of MHC I consist of a single polypeptide encoded by the MHC that is always associated with a smaller protein, β_2M, encoded outside the MHC. However, MHC II proteins consist of two polypeptides, α and β, both of which are encoded by the MHC region.

Figure 6.14 illustrates the structure of the HLA-region although it has been greatly simplified to aid understanding. The HLA complex contains a number of genetic loci, including those that encode different types of Class I proteins. Thus, the HLA-A, B and C regions contain genes that encode HLA-A, B and C proteins respectively. These are all found on nucleated cells and are distinct types of proteins and not allelic forms of each other. However, there *are* allelic forms of each of the *HLA-A*, *B* and *C* genes, and these encode HLA proteins, each of which have small differences in their amino acid sequences. Each of the Class I genes is **polyallelic**, which means that many different alleles exist although, of course, each individual only expresses a maximum of two alleles at each locus, given chromosomes occur in pairs (*Chapter 15*). Moreover, the alleles are codominant so that each nucleated cell expresses two different alleles of the *HLA-A* gene, as well as two different forms of the *HLA-B* and of the *HLA-C* gene. A large number of allelic forms of each gene exist (*Table 6.13*).

The HLA complex in humans is one of the most highly polymorphic systems known. Given that each individual has two of each of these alleles, and that the allelic forms are codominant, it can be seen that the chances of two unrelated

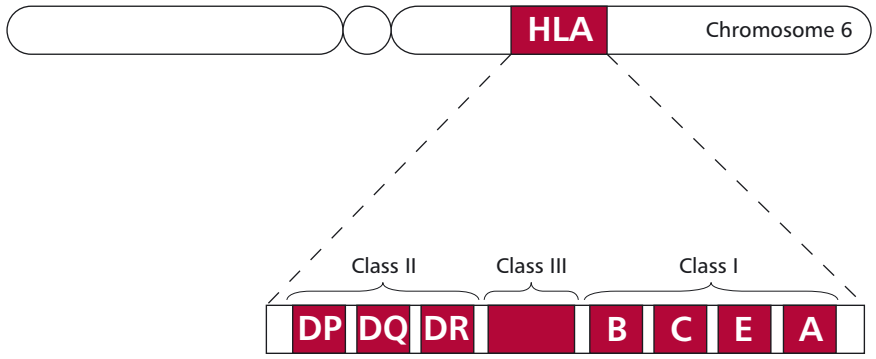


Figure 6.14 Schematic to show the structure of the HLA system on chromosome six.

individuals having the same 'set' of HLA genes is very small, making the possibility of finding 'good' transplant matches, other than identical twins, low.

In the HLA complex, the Class II region is located nearest to the centromere, which contains the DP, DQ and DR loci. Within each of these loci there are genes encoding the α and β chains of the class II molecules. The situation is rather more complex than for Class I because each locus may contain more than one gene that encodes the α and β chains. For example, the HLA-DR region contains three or four genes for the HLA-β chain. All of the β gene products may be expressed in a single cell, making the degree of variation much higher than that of class I proteins. Like Class I, the Class II region also displays a high degree of polymorphism (*Table 6.14*).

HLA TYPING

Human Leukocyte Antigen typing is the process whereby the HLA antigens present on the cells of the recipient and the potential donor are determined. By determining the HLA types of potential transplant recipients, and storing these details on computer databases it is possible to match the donor organ, when one becomes available, to the most appropriate recipient. The degree

Gene	Number of alleles	Number of proteins
HLA-A	372	348
HLA-B	661	580
HLA-C	190	153

Table 6.13 Genes and alleles of Class I genes of the HLA complex

Gene	Codes for	Number of alleles
HLA-DRB	β chain of HLA-DR	249
HLA-DQA1	α chain of HLA-DQ	20
HLA-DQB1	β chain of HLA-DQ	36
HLA-DPA1	α chain of HLA-DP	13
HLA–DPB1	β chain of HLA-DP	82

Table 6.14 Alleles of the Class II HLA genes

of match required depends on the organ being transplanted, and is much more stringent for bone marrow transplants than for solid organ transplants. The transplantation of bone marrow is used to treat a number of conditions, including immunodeficiency and some kinds of cancer (*Chapter 17*). Transplantation of bone marrow presents particular difficulties because bone marrow contains immunocompetent cells that can lead to fatal GVHD.

The traditional method for HLA typing is a serological technique that uses antibodies to known HLA antigens. This method is still used but is being superseded by molecular biological techniques. The serological method is the lymphocytotoxicity assay. In this assay, the cells to be typed are peripheral blood mononuclear cells (PBMC) that are readily obtained from whole blood. For typing Class II HLA antigens it is necessary to use purified B lymphocytes, since resting T lymphocytes do not express Class II molecules. Aliquots of the cells to be typed are pipetted into the wells of 96-well trays, known as Teresaki plates, after the inventor of this test. Antibodies to individual HLA antigens are added to each well and will bind to the lymphocytes if they express the appropriate antigen. The addition of complement to all the wells results in the death of cells with bound antibody. Viability stains, such as the fluorescent acridine orange and ethidium bromide are then used to reveal wells containing dead cells. Acridine orange enters living cells and stains the nuclei green, while ethidium bromide enters dead cells and stains the nuclei red (*Figure 6.15*). The anti-HLA antibodies used in these tests may be obtained from people already sensitized to HLA antigens or they may be monoclonal antibodies with specificities for known HLA antigens. Rabbit serum is used as a source of complement. A test is scored as strongly positive when more than 50% of the cells in a well are killed.

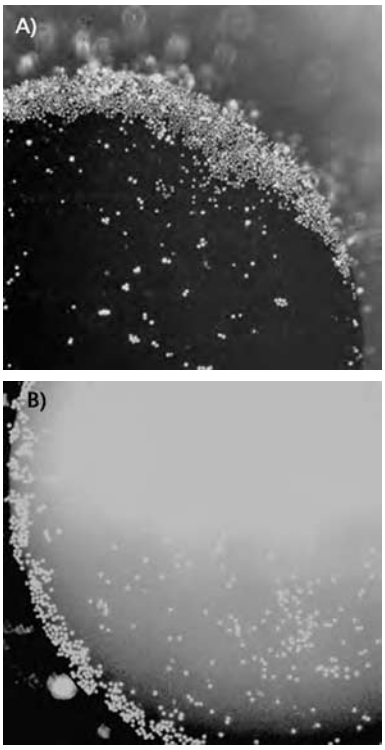


Figure 6.15 Serological test to determine HLA type. (A) A negative result, that is, no cell death and (B) a positive result in which cells have been killed as indicated by different flourescent colors. Courtesy of the Transplantation Laboratory, Manchester Royal Infirmary, UK.

Molecular biology techniques

The main molecular biological technique used in histocompatibility testing is the polymerase chain reaction (PCR; *Chapter 3*) in which samples of DNA from the individual being typed is amplified many times over. Methods that use PCR require very little DNA and do not require living cells. For example, they can be carried out on whole blood that has been stored frozen. In the sequence specific primer (SSP) assay the primers used in the PCR are those specific for individual HLA alleles. The DNA will only be amplified in those mixtures that contain a probe complementary to that of the DNA being typed (*Figure 6.16*). Another method, called a sequence specific oligonucleotide probe (SSOP) assay, amplifies all the DNA by PCR and then uses sequence specific oligonucleotide probes to identify the products. In some laboratories the oligonucleotide probes are attached to microspheres. Incubation of the microspheres with the PCR product allows the latter to bind to any complementary HLA sequence. The microspheres, with bound DNA, are then analyzed in a flow cytometer to identify target HLA sequences.

The serological cross match

A serological cross match is carried out in order to detect the presence of antibodies to graft antigens in the serum of a potential recipient. Serum from the potential recipient is added to PBMC from the donor. Complement is added and the viability of the cells tested as previously. If donor cells are killed then

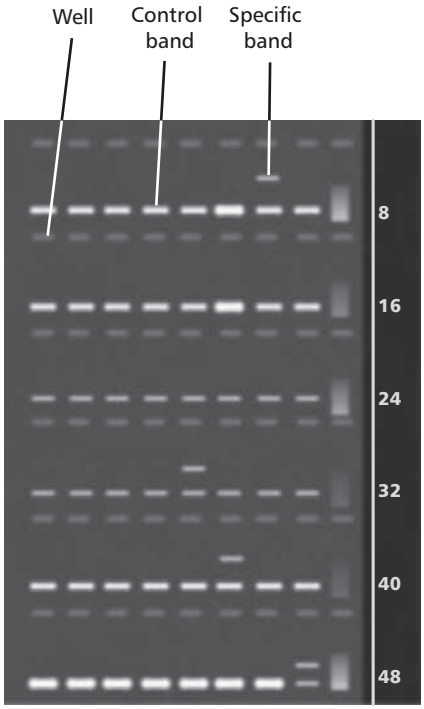


Figure 6.16 Sequence Specific Primer Assay to determine HLA type. The specific band shows that the individual being typed is positive for that specific HLA gene. The controls ensure that the individual's DNA has been amplified correctly. The numbers down the right-hand side are reference values to indicate the appropriate sizes of the bands. Courtesy of the Transplantation Laboratory, Manchester Royal Infirmary, UK.

it is probable that the recipient already has antibodies against graft antigens and the donor is unlikely to be a suitable match.

Matching donor and recipient

Individuals inherit a set of HLA genes from each parent. Thus, siblings are more likely to have a closer match than unrelated donors and, occasionally, people have donated a healthy kidney to a sibling with renal failure. Other sources of kidneys for transplantation include cadavers, often people who have been killed in accidents. The HLA types of potential recipients are stored in computer databases so that when a kidney donor becomes available, they can be HLA typed and the kidneys given to those recipients whose HLA antigens match as closely as possible.

Retrospective studies on the outcome of kidney transplantation have shown that matching the donor to the recipient improves graft survival. Thus, in the most favorable match, that is no mismatches of the HLA-DR, HLA-A and HLA-B alleles graft survival is superior to those with no mismatch of HLA-DR and only one mismatch of HLA-A and/or HLA-B alleles. These also gave significantly better graft survival than any other mismatched graft.

6.13 IMMUNOSUPPRESSION

All patients who receive an allograft are liable to reject the transplant, even if the recipient and host are closely matched for histocompatibility antigens. This is because relatively few of the HLA antigens are tested for, and complete matches are rare. Thus, all patients who receive an allograft have to take immunosuppressive drugs to prevent rejection resulting from an immune response. Immunosuppressive treatments fall into a number of categories. The first generation of immunosuppressive drugs were used to prevent lymphocytes from proliferating (*Table 6.15*). As these drugs act by inhibiting cell division, they are also used in the treatment of cancer. Their actions are described in more detail in *Chapter 17*. Corticosteroids, such as cortisol, are also immunosuppressive agents but act principally by suppressing inflammation. They are still used, often in combination with other drugs such as methotrexate. All the first generation drugs produce a 'blanket' immunosuppression and prevent all immune responses. This makes the patient more susceptible to infections of all kinds, but especially to opportunistic infections caused by such organisms as *Candida albicans*. Immunosuppressed patients are also more susceptible to the types of cancers associated with viruses, including lymphoma, associated with the Epstein-Barr virus (EBV) and Kaposi's sarcoma, associated with the Kaposi's sarcoma associated herpes virus (KSV). First generation immunosuppressive treatments also have significant toxicity, because they affect all dividing cells, including those of the bone marrow and of the GIT. Some, such as methotrexate, also show liver toxicity.

Type of treatment	Mechanisms of action	Examples
Purine analogs	incorporated into DNA during the process of DNA synthesis; prevent further DNA elongation	azathioprine; mercaptopurine
Folic acid antagonists	prevent the action of dehydrofolate reductase, an enzyme required for the synthesis of purines and pyrimidines	methotrexate; aminopterin
Alkylating agents	become incorporated into developing DNA and cross-links DNA strands, preventing further replication	cyclophosphamide

Table 6.15 First generation immunosuppressive drugs

The second generation of immunosuppressive treatments target T lymphocytes rather than B cells. These included the use of antilymphocyte globulin, an antibody raised against human T lymphocytes. The third generation of immunosuppressive treatments are much more selective in their action and target only those T cells which have been activated by an antigen. Cyclosporin A, a cyclic peptide (*Figure 6.17*) derived from the soil fungus *Tolypocladium inflatum gams*, is most commonly used. The peptide shows considerable immunosuppressive action, without the bone marrow toxicity demonstrated by other drugs. Tacrolimus, a macrolide antibiotic (*Figure 6.18*) derived from *Streptomyces tsukubaensis*, is similar to cyclosporin A in action. It is more powerful than cyclosporin A but also has more side effects. Mycophenolate mofetil is an example of a prodrug, which is converted in the body to mycophenolic acid, another powerful immunosuppressive agent.

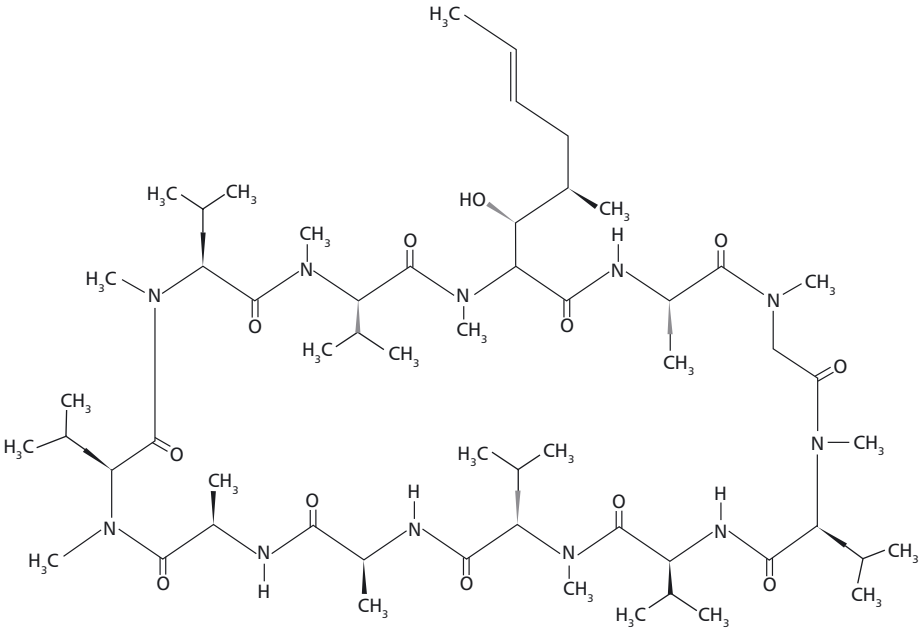


Figure 6.17 Cyclosporin A.

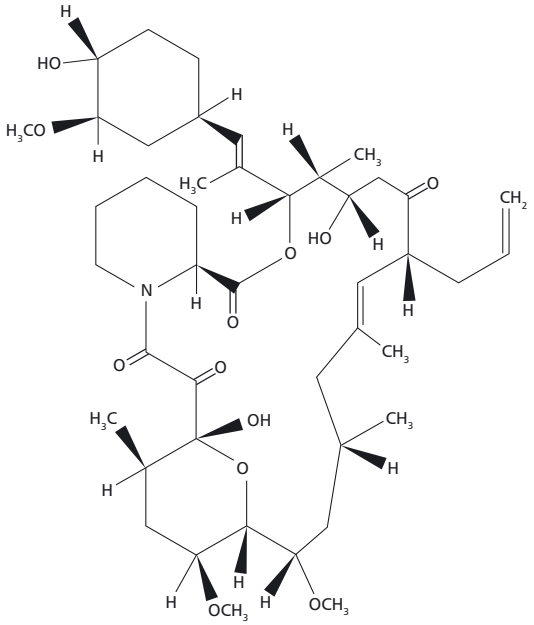


Figure 6.18 Tacrolimus.

6.14 HEMOPOIETIC STEM CELL TRANSPLANTATION

The transplantation of hemopoietic stem cells is used to correct some immunodeficiency diseases (*Chapter 5*) and during the treatment for some types of cancer, such as leukemias and lymphomas (*Chapter 17*). Patients with total bone marrow failure, such as in aplastic anemia, or partial failure, as seen in some of the hemoglobinopathies, may also benefit from a stem cell transplant (SCT).

Hemopoietic stem cells for transplantation may be derived from bone marrow, from peripheral blood, and from umbilical cord blood and SCTs may be autologous**, syngeneic** or **allogeneic**. In an **autologous** transplant the patient's own stem cells are harvested prior to a treatment in which their own stem cells are destroyed, as, for example, in patients who receive radiation treatment or high dose chemotherapy to treat leukemia. The stem cells are stored in liquid nitrogen until the transplant can take place, when they are thawed and reinfused.

A SCT between identical twins is called a syngeneic transplant. In contrast, an allogeneic SCT involves donors and recipients who are genetically nonidentical. The donor could be a sibling or a potential donor identified in a bone marrow registry (*Margin Note 6.4*). Donors who are related to the recipient are more likely to be compatible. There is a much greater need for close HLA matching of the donor and recipient involved in bone marrow transplants (BMTs), as GVHD can be a major hazard of this type of transplantation and can occur between seven and 30 days post-transplantation. In the acute form of GVHD epithelial cells in the skin and those lining the intestine are attacked by the sensitized T cells originating in the graft. Patients present with a severe maculopapular skin rash and sloughing of the intestinal epithelium may lead to diarrhea. Splenomegaly and hepatomegaly occur, as these organs become attacked by T lymphocytes and the patient may become jaundiced. Acute GVHD is frequently fatal. A chronic form with similar symptoms may occur over a longer time frame. The patients may suffer frequent secondary infections.

One method of avoiding GVHD is to remove T lymphocytes from SCTs prior to giving the graft. This process, known as T cell depletion involves the use of an antibody to T cells. Once GVHD develops it must be treated with immunosuppressive drugs.

Margin Note 6.4 Bone marrow databases

Potential bone marrow donors are always required to increase the number of HLA types available. The National Marrow Donor Program in the USA maintains an international registry of stem cell donors. Similarly, in the UK the two main bone marrow registries are the British Bone Marrow registry and the Anthony Nolan Bone Marrow registry.

BONE MARROW TRANSPLANTATION

The first successful bone marrow transplant (BMT) was achieved in 1968. Bone marrow contains the hemopoietic stem cells that give rise to all the formed elements of the blood (*Chapter 13*). In bone marrow transplantation (BMT), the donor receives a general or local anesthetic and the marrow is harvested using a needle inserted through the skin over the pelvic bone and into the bone cavity. The process takes approximately one hour to harvest sufficient quantity; usually a minimum of 3×10^8 nucleated bone marrow cells containing approximately 2×10^6 stem cells per kilogram of recipient body weight is required. At this stage the marrow may be infused into the recipient or it may require further processing. The marrow is treated to remove blood and plasma, especially if there is a disparity between the ABO blood group of the donor and the recipient. Fragments of bone are removed and the marrow may also be depleted of T cells to reduce the risk of GVHD prior to intravenous transfusion into the recipient. The recipient may be given antibiotics because, until the marrow is engrafted and starts to produce blood cells, the patient is at risk of infection. They may also be given platelet and erythrocyte transfusions to prevent bleeding and anemia. A patient receiving a bone marrow transplant may show adverse side effects, such as nausea, fatigue, hair loss and loss of appetite.

PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

Peripheral blood stem cell transplantation (PBSCT) is the most common form of SCT. Collection of stem cells from peripheral blood is easier both for the collector and the donor. In addition, engraftment of PBSCT is often more rapid than with bone marrow. The donor is treated with granulocyte colony stimulating factor (G-CSF) to increase the number of stem cells in the blood. The stem cells are then obtained from the donor by leukapheresis (*Margin Note 6.3*). Leukapheresis may take several hours to complete and more than one session may be needed, with stem cells being stored frozen between donations.

UMBILICAL CORD STEM CELL TRANSPLANTATION

Stem cells may also be obtained from umbilical cord blood, of course with the permission of the family involved. Following birth, blood derived from the baby is obtained from the umbilical cord and the placenta. Since only a small amount of blood is retrieved in this way, the collected stem cells are typically used to treat children.

IDENTIFICATION OF HEMOPOIETIC STEM CELLS

Hemopoietic stem cells have a marker protein called CD34 that may be used in their identification. Thus, the numbers of CD34 positive cells in a preparation can be assessed if a sample is stained with a fluorescent antibody to CD34. The cells can be estimated either by using a fluorescence microscope or by flow cytometry (*Box 6.1* and *Figure 6.19*). Both peripheral and cord blood may be further processed to obtain the stem cells. For example, an anti-CD34 antibody linked to magnetic particles will bind to CD34+ cells, which can then be purified using a magnet.

Storage of transplant material

Harvested stem cells may be stored for 2–3 days in a refrigerator at 4°C. This may be required, for example, if more than one harvesting procedure is needed or if a patient has to undergo radiation or chemotherapy for cancer treatment (*Chapter 17*) prior to receiving the graft. If longer term storage is required, the stem cells may be stored in liquid nitrogen vapour at –176°C. Prior to storage, the cryopreservative dimethylsulfoxide (DMSO) is added to prevent ice crystal formation, which would destroy the cells. A programmed freezer allows the cells to be cooled at the optimum rate for cell survival, which is normally approximately 1°C per min.

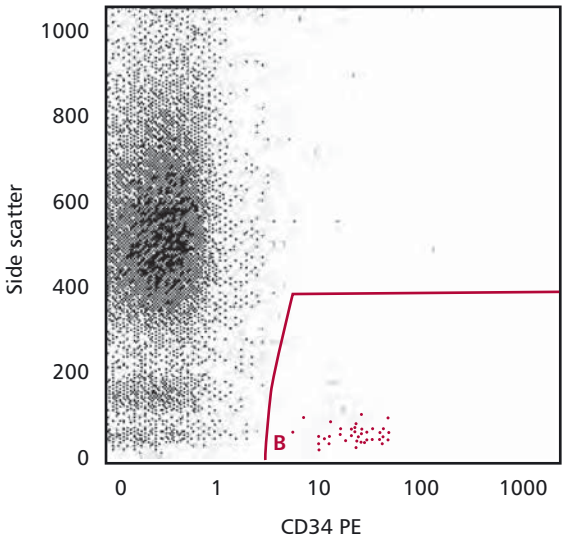


Figure 6.19 Flow cytometric characterization of CD34+ stem cells, shown in the gated Section B, in cord blood. Courtesy of Dr T.F. Carr, Royal Manchester Children's Hospital, UK.

Post-transplant support

Patients who have just undergone BMT or PBSCT transplantation are highly susceptible to infections. If transfusions of blood products are required, for example for anemia, these products should be free of CMV, a virus which is a latent infection in some individuals. In an immunosuppressed individual, CMV can cause serious illness.

CASE STUDY 6.1

Marie is a 31-year-old florist who is blood group O RhD negative and is seven months pregnant. The father of her baby is blood group A RhD positive. Marie is worried about the pregnancy, as she had several miscarriages, each of which occurred before three months of gestation. Despite these miscarriages, Marie has never become sensitized to the D and has no anti-D antibodies in her circulation.

Questions

(a) What are the chances of her baby developing hemolytic disease of the newborn?

(b) Is Marie likely to become sensitized to the D antigen after the birth of this child?

CASE STUDY 6.2

John is a 50-year-old school teacher who has recently been treated for acute myeloid leukemia. His consultant has recommended that he receives aggressive chemotherapy followed by a stem cell transplant. John is likely to die without this treatment. He has no living siblings who could donate bone marrow and both his parents are dead.

Questions

(a) What would be the best approach to treating John?

(b) Is a bone marrow transplant feasible?

CASE STUDY 6.3

Michael is a patient who was taken to the accident and emergency unit of his local hospital following a stabbing incident. Michael had lost a lot of blood and required an immediate transfusion. He was given compatible leukodepleted blood and fresh frozen plasma. However, he suffered acute respiratory distress approximately 4 h after the transfusion. He had hypotension and cyanosis and a temperature of 39.5°C. Examination of the chest showed signs of fluid in his lungs.

Question

What is the likely cause of Michael's respiratory problems?

6.15 SUMMARY

Transfusion of blood and blood products is a routine and safe clinical procedure, which rarely causes harm. Transfusion has been greatly facilitated by knowledge of the range of blood group antigens and the conditions under which antibodies to blood group antigens can cause problems. The screening of donors, for example for HIV or hepatitis viruses, has also increased the safety of the procedure. The transfusion of incompatible blood can cause the death of a recipient but this is a rare occurrence. Transfusion laboratories are involved in ensuring that compatible blood is given to patients and that all aspects of this procedure are safe. In addition, laboratories monitor the

presence of antibodies in pregnant women that might cause problems for a developing fetus.

Transplantation of solid tissue has progressed considerably since the first successful kidney transplant and a wide range of tissues and organs are now transplanted routinely. The rejection of transplants is minimized by a careful matching of donor and recipient and by the administration of immunosuppressive drugs. Bone marrow and other forms of stem cell transplant carry the risk of GVHD, although this is decreased if good HLA matches are achieved and if the patients are carefully monitored post-transplant.

Questions

1. Which of the following combinations of plasma and erythrocytes would result in agglutination?
   a) Group A erythrocytes and group B plasma;
   b) Group O erythrocytes and group O plasma;
   c) Group B erythrocytes and group O plasma;
   d) Group B erythrocytes and group B plasma;
   e) Group AB erythrocytes and group A plasma.

2. Which of the following statement(s) is (are) **TRUE**?
   a) MHC II proteins are found on all nucleated cells.
   b) MHC I proteins are found on antigen presenting cells.
   c) Complement is a protein complex which lyses bacteria.
   d) The classical pathway for complement activation is initiated by IgA.
   e) An ABO incompatible transfusion may be fatal.

3. Which of the following conditions would mean that a blood donor should be removed from the register of donors?
   a) previous infection with hepatitis virus;
   b) current infection with HIV;
   c) pregnancy;
   d) donor aged 78 years;
   e) intravenous drug abuse.

4. List the possible causes of a potential transplant patient having anti-HLA antibodies in their plasma.

5. Give one reason why PBSCT is preferable to bone marrow transplantation.

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Useful web sites:

http://www.anthonynolan.com

http://www.cyto.purdue.edu/ (an excellent website with all you need to know about flow cytometry)

http://www.shot-uk.org

http://www.transfusionguidelines.org.