

**CHAPTER**  
**6**

# Introduction to the Rh blood group system

**Learning objectives**

*After studying this chapter you should be able to:*

- Discuss the historical aspects of the Rh systems
- Outline the cellular and molecular bases of the Rh systems
- Describe the genetics of the Rh systems
- Describe the frequency of Rh types in different populations
- Discuss the contribution of molecular techniques to knowledge of the Rh blood group systems

## 6.1 INTRODUCTION TO THE RH SYSTEM (ISBT 004, SYMBOL RH)

The Rh system was discovered in 1940 by Landsteiner and Wiener. They injected rabbits and guinea pigs with the red cells from *Macacus rhesus* monkeys and the resulting antibody reacted with the red cells of 85% of New York blood donors. Those who reacted were said to have the Rhesus factor and were Rhesus positive, whilst those that did not react lacked the Rhesus factor and were Rhesus negative. The terms Rhesus positive, or Rh positive, and Rhesus negative, or Rh negative, are still used (incorrectly) today, especially by clinical staff, to describe what we now know as Rh D positive and Rh D negative.

In 1939, Levine and Stetson had described an antibody in a mother who had recently had a stillborn foetus. The antibody caused a haemolytic transfusion reaction when she was transfused with ABO-compatible blood from her husband. They suggested that the antibody had been produced in response to an antigen carried by the foetus, which had been inherited from the father. This antibody was subsequently shown to have the same reaction pattern as Landsteiner and Wiener's anti-Rh, and so Rh haemolytic disease of the newborn was described for the first time.

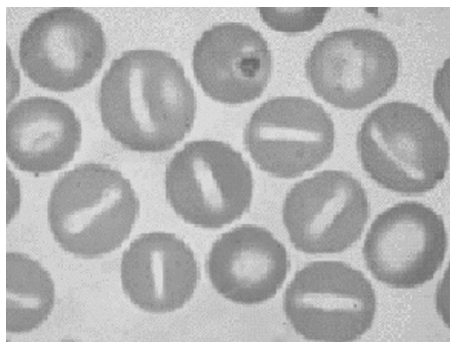
By 1945, the original Rh factor had been renamed D and four more Rh antigens discovered. These were the antithetical antigens C and c, and E and e (for further information about antithetical genes, see Chapter 4). There

**92 Chapter 6 Introduction to the Rh blood group system****Box 6.1 Numerical system for Rh**

In 1962, Rosenfield and colleagues proposed a numerical system for describing the Rh antigens. This system was free from the genetic implications of either Wiener's or Fisher's systems, as it merely recorded the observed serological reactions. The known Rh antigens were numbered from 1 (for D) to 24, in order of discovery. The numbering of Rh antigens has now reached 52, although, because of obsolete forms, there are now 45 antigens in the system.

are now 45 antigens (see *Box 6.1*) in the Rh system but D, C, c, E and e are the most commonly identified and the most significant in blood transfusion. The Rh antigens are expressed on polypeptides. The Rh polypeptides span the red cell membrane exposing six extracellular loops on which are expressed the Rh antigens. These polypeptides are associated in the membrane with an Rh glycoprotein to form tetramers (two Rh polypeptides and two Rh glycoproteins), which form the Rh core complex. The Rh glycoprotein is essential for the formation of this Rh core complex. Mutations in the genes controlling the expression of the Rh polypeptides or the Rh glycoproteins can result in the Rh<sub>null</sub> phenotype, in which no Rh antigens are expressed. Red cell defects seen in the Rh<sub>null</sub> phenotype include abnormal cation transport across the red cell membrane and red cell morphological abnormalities, in the form of stomatocytes (red blood cells that exhibit a slit or mouth-shaped pallor rather than a central pallor), which may cause a mild, compensated haemolytic anaemia (see *Fig. 6.1*). The function of the Rh polypeptide is not known for certain, but it seems likely that it is involved in cation transport across the red cell membrane. The Rh antigens are well developed before birth, being detectable in the 6-week-old foetus. They are fully expressed on cord red blood cells. Rh antigens have not been demonstrated on leucocytes and platelets, or found in saliva or amniotic fluid.

The Rh antigens are distributed with considerable variation in different populations. This is illustrated in *Table 6.1*, which shows examples of the variation in distribution of the D antigen.



**Figure 6.1**  
Stomatocytes in peripheral blood.

**Table 6.1 Examples of the frequency of the D antigen in various populations**

	Rh D	
	positive (%)	negative (%)
Europe	83	17
West Africa	97	3
India	90	10
Japan	99.7	0.3
China	93	7

## 6.2 INHERITANCE AND NOMENCLATURE OF THE RH SYSTEM

Two genetic systems were originally proposed to explain the relationships and inheritance of these five original Rh antigens. In the USA, Wiener proposed a system comprising a single locus producing factors he called agglutinogens, which could express multiple antigens. In the UK, Fisher and Race proposed a system of three closely linked loci for *D/d*, *C/c* and *E/e*, each gene coding for the production of a single antigen. Thus, the antigens *C* and *c* were thought to be the products of the co-dominant alleles *C* and *c*. Antigens *E* and *e* were thought to be the products of the co-dominant alleles *E* and *e*. The *D* antigen was the product of the *D* gene and the proposed allelic gene *d* was considered an amorph as no *d* antigen or anti-*d* antibody was ever discovered. Fisher also postulated that the order of the genes on a chromosome was *DCE* (see *Box 6.2*). It has become common practice to refer to them in this order.

### Box 6.2 Fisher's DCE theory

Race and Sanger showed their early Rh typing results to Fisher, in the Bun Shop (a Cambridge pub). His first outline of his *DCE* theory, on 22 June 1944, was described on a pub beer mat.

The clinical significance of the Rh system has led to considerable investigation of the system. As new Rh antigens and phenotypes were discovered, it became apparent that neither Wiener's nor Fisher's system could explain every new finding. However, Fisher's system of three closely linked loci was the most complete and allowed the deduction of phenotypes of offspring from different mating types. Fisher's shorthand notation was also very convenient for communicating information regarding phenotypes and genotypes.

It has now been shown by modern molecular biology techniques that neither of these earlier systems was completely correct and that in fact the

**Table 6.2 Fisher’s model of haplotypes and their frequency in the Caucasian population**

Haplotype	Shorthand notation	Approx. frequency in Caucasians (%)	Haplotype	Shorthand notation	Approx. frequency in Caucasians (%)
<i>DCe</i>	R <sub>1</sub>	41	<i>dCe</i>	r'	1
<i>DcE</i>	R <sub>2</sub>	14	<i>dcE</i>	r''	1
<i>Dce</i>	R <sub>0</sub>	3	<i>dce</i>	r	39
<i>DCE</i>	R <sub>Z</sub>	<1	<i>dCE</i>	r <sup>y</sup>	<1
Rh D positive			Rh D negative		

Rh system is controlled by two closely linked loci. One carries the gene for the Rh D polypeptide and is known as the *RHD* locus. The other carries the genes for the CcEe polypeptide and is known as the *RHCE* locus.

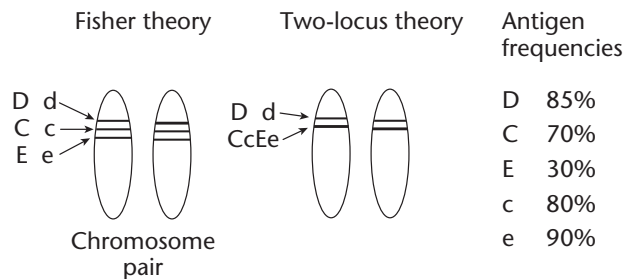
Despite current evidence concerning the genetics and biochemistry of the Rh system, Fisher’s model and shorthand notation continue to be a convenient way to explain and communicate Rh phenotypes and genotypes. For this reason, it is outlined below in more detail

### 6.3 FISHER’S DCE SYSTEM

The Fisher model proposed that three pairs of closely linked genes (now known to be two genes) allow for eight possible haplotype arrangements of Rh genes on a chromosome. This is shown in *Table 6.2* and *Fig. 6.2*, and the possible genotypes are shown in *Table 6.3*.

The three genes of each set were thought to be inherited together due to the close linkage of the loci. Each could then be paired with itself or any other arrangement, giving 36 possible genotypes. The eight most common ones are listed in *Table 6.2* with their frequencies.

It should be noted that the vast majority of Rh D-negative individuals are *dce/dce* (*rr*) and therefore capable of being immunized by exposure to C+ and E+ red cells.



**Figure 6.2** Suggested gene locations for the Fisher and two-locus theories.

**Table 6.3 Fisher's model of genotypes and their frequency in the Caucasian population**

Genotype	Shorthand notation	Approx. frequency (%)	Genotype	Shorthand notation	Approx. frequency (%)
<i>DCE/dce</i>	$R_1r$	33	<i>dce/dce</i>	$rr$	15
<i>DcE/dce</i>	$R_2r$	11			
<i>Dce/dce</i>	$R_0r$	2			
<i>DCE/DCE</i>	$R_1R_1$	18			
<i>DcE/DcE</i>	$R_2R_2$	2			
<i>DCE/DcE</i>	$R_1R_2$	14			
<i>DCE/Dce</i>	$R_1R_0$	2			
All other combinations		<1	All other combinations		<1
Rh D positive			Rh D negative		

Although current knowledge of the Rh genes has rendered the Fisher model obsolete, it is vital that students are familiar with the Fisher shorthand notations and their implied genotypes. It is with these shorthand expressions that Blood Transfusion Laboratory workers in the UK communicate information on the Rh system on a day-to-day basis.

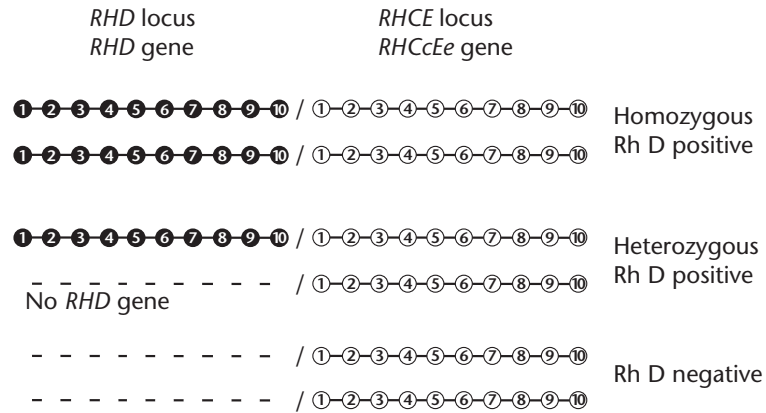
## 6.4 THE TWO-LOCUS MODEL

In this model, suggested by Tippett in 1986, the Rh system is controlled by two closely linked loci, *RHD* and *RHCE*. The *RHD* locus carries the gene for the RHD polypeptide, which expresses all the D antigen epitopes. The *RHCE* locus carries the genes for the RHCE polypeptide, which expresses both the C/c and E/e antigens. The genes which encode the C/c and E/e antigens are co-dominant alleles. *RHCE* exists in four allelic forms and each allele determines the expression of two antigens in Ce, ce, cE or CE combination (*RHCE* is the collective name of the four alleles). The Rh genes are located on chromosome 1. Figs 6.3 and 6.4 are diagrammatic representations of the *RHD* and *RHCE* genes and Rh D-positive and Rh D-negative gene arrangements. Fig. 6.5 shows the relationship between the two-locus haplotypes and the corresponding Fisher DCE notations.

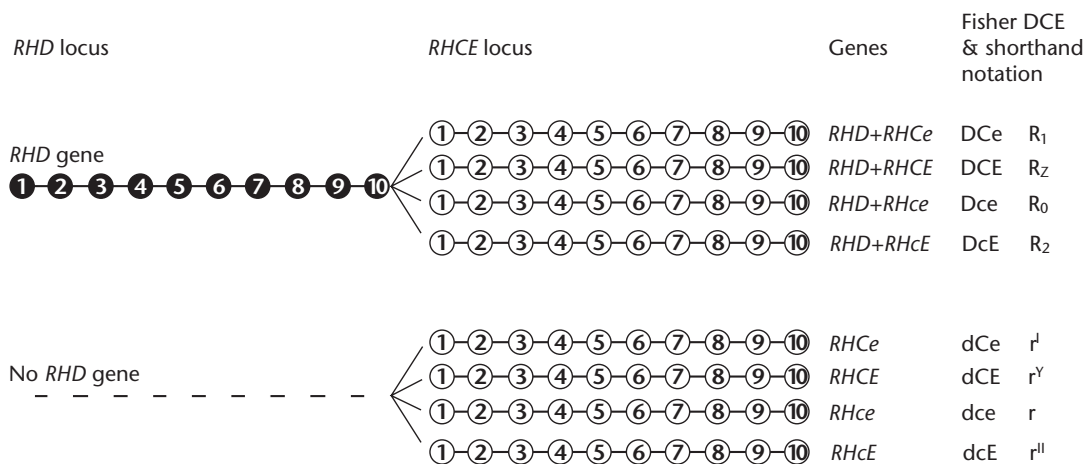
**Figure 6.3**

Representation of the *RHD* and *RHCE* genes. Each gene contains ten exons, which are shown numbered 1 to 10.

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**Figure 6.4**  
Representation of Rh D-positive and Rh D-negative gene arrangements.



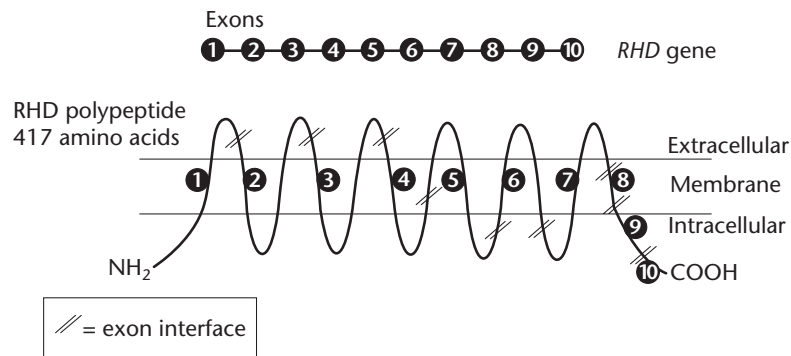
**Figure 6.5**  
Relationship between two-locus haplotypes and Fisher DCE notation.

The RHD and RHCE loci are very similar, each comprising ten exons. The corresponding polypeptides are therefore very similar, differing only at 36 of the 417 amino acid residues in each polypeptide. The C/c antigen polymorphism appears to be associated with four amino acid substitutions, whereas the E/e polymorphism is associated with a single amino acid substitution (see Table 6.4). Transfer of exons between RHD and RHCE loci, and vice versa, is known to occur. This causes variations in epitope expression and hence antigen expression. The location of the RHD and RHCE polypeptide chains in the red cell membrane are depicted in Figs 6.6 and 6.7. It can be seen that they are transmembrane proteins.

**Table 6.4 Amino acids (AA) involved in the C/c and E/e polymorphisms**

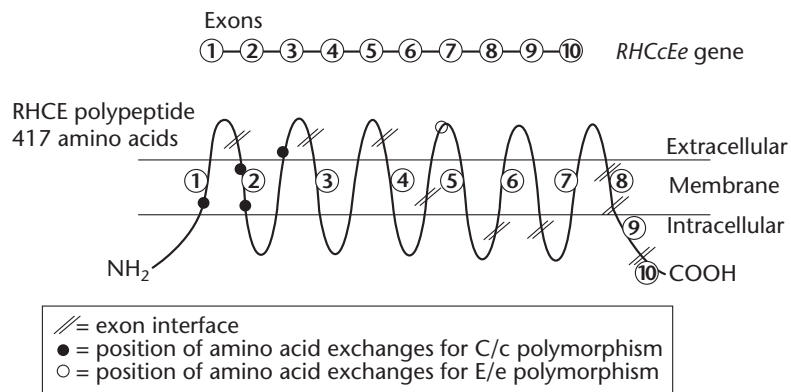
Antigen expressed		AA position	Exon
C	c		
cysteine	tryptophan	16	1
isoleucine	leucine	60	2
serine	asparagine	68	2
serine	proline	103*	2
E	e		
proline	alanine	226	5

\*This amino acid is located on the second external loop and considered crucial for C/c expression.



**Figure 6.6**

RHD polypeptide chain within the red cell membrane. The RHD polypeptide chain is a transmembrane protein which spans the red cell membrane and frequently protrudes on both intra- and extracellular surfaces. Both the NH<sub>2</sub> and COOH termini are located intracellularly. The interface between each of the ten exons and the amino acid point is shown.



**Figure 6.7**

RHCE polypeptide.

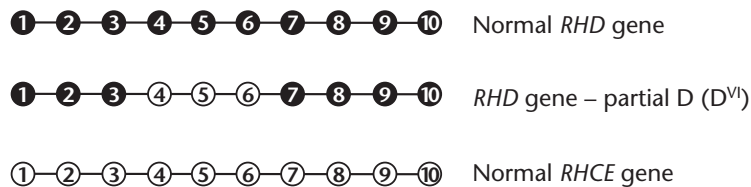
Expression of Rh epitopes is dependent on the sequence and resulting conformation of the amino acids within the polypeptide chain. Changes in amino acid sequences or exchange of exons can not only lead to the expression of new epitopes, but also cause conformational changes which may affect the expression of other epitopes.

Epitopes are the sites that are recognized by antibodies and thus each antibody has a specific epitope to which it can bind (see Chapter 2). Polyclonal antibodies produced in humans are usually a mixture of antibodies to several related epitopes or groups of epitopes. Monoclonal antibodies, which are used in laboratory testing for Rh status, are more usually specific for a single epitope or group of epitopes (see Chapter 2). The RHD and RHCE polypeptides may be thought of as expressing many different epitopes which together express the antigens of the Rh system.

## 6.5 QUALITATIVE DIFFERENCES IN RH ANTIGENS

The Rh D antigen is now considered to contain at least 30 different epitopes, as revealed by tests using monoclonal antibodies. Rare individuals who lack certain epitopes are known as **partial D** and may be stimulated to produce antibodies to the missing epitopes by transfusion or pregnancy. The partial D type known as category D<sup>VI</sup> is the clinically most important partial D (see Box 6.3 for an explanation of the development of the nomenclature systems for partial D types). Severe cases of haemolytic disease of the newborn have occurred in Rh D-positive babies born to category D<sup>VI</sup> mothers with anti-D antibody. Category D<sup>VI</sup> is the most common partial D occurring in 6–10% of weak D samples and 0.02–0.05% of all Caucasian samples. The majority of Rh D-positive individuals with allo-anti-D are category D<sup>VI</sup>.

This partial D state may arise from the replacement of an exon segment from *RHD* by an equivalent segment from *RHCE*, creating a hybrid *RHD-CE-D* gene (see Fig. 6.8) or as a result of point mutation within *RHD*. The replacement of several exon segments from *RHD* with the equivalent segments from *RHCE* can destroy the ability to produce D antigen altogether. Thus, the individual only expresses the C/c and E/e antigens and serologically appears as Rh D negative although they do possess an *RHD* gene.



**Figure 6.8**

This shows how exon exchange gives rise to a partial D gene. Exons 4, 5 and 6 of the *RHD* gene have been replaced with copies of exons 4, 5 and 6 from the *RHCE* gene. This results in the production of an RHD polypeptide which does not express all the normal D epitopes, that is, a partial D.

**Box 6.3 The D antigen mosaic**

In 1953, Wiener proposed that the Rh D antigen was a mosaic of four parts, which he termed Rh<sup>A</sup>, Rh<sup>B</sup>, Rh<sup>C</sup> and Rh<sup>P</sup>. Rare individuals who lack part of this mosaic (partial D) may produce immune anti-D antibody specific for the epitopes they lack. In the 1960s, Tippett identified seven D categories by cross-testing the cells and sera of Rh D-positive individuals with anti-D in their serum. These D categories were designated using superscript Roman numerals I to VII, e.g. category D<sup>III</sup>, category D<sup>VI</sup>. Some of the categories were also subdivided. Further partial D types have been identified by their reaction patterns with monoclonal anti-D. There has been no clear systematic approach to naming these partial D types. By 1993, there was a nine-epitope model for Rh D and by 1995 a 30-epitope model. It is now most usual to see partial D types written as their D category (or name if not in a category) together with the epitopes they express and/or lack in either the nine- or 30-epitope models or both. This can make for difficult reading for students, and for even more difficult conversation.

**The G antigen**

The G antigen is usually only detected on red cells expressing D antigen or C antigen or both. Its expression appears to be dependent on amino acid sequences derived from exon 2 of the *RHD* gene. Anti-G has been implicated in haemolytic disease of the newborn. The presence of the G antigen

**Box 6.4 Apparent inconsistencies in blood grouping and antibody identification due to the presence of the G antigen**

In her first pregnancy, JF (a 24-year-old female) had been grouped as O Rh D negative rr (dce/dce) with anti-D antibody in her plasma at a level of 0.67 IU ml<sup>-1</sup>. Fortunately, her baby had not been affected by her low level of antibody. Unfortunately, the blood group of the father had not been checked during this pregnancy. Twelve months later, JF was again pregnant and attended the antenatal booking clinic on 20 August. She was confirmed as O Rh D negative (rr) with an anti-D level of 1.57 IU ml<sup>-1</sup>. This time a blood sample was obtained from her husband and he tested as O Rh D positive R<sub>2</sub>r (DcE/dce).

On 29 October, a routine check indicated that JF had now developed anti-C in addition to anti-D (which was now at a level of 1.95 IU ml<sup>-1</sup>). Again, on 26 November, anti-C+D was confirmed and by now the anti-D level had risen dramatically to 12.9 IU ml<sup>-1</sup>. Presumably, the anti-C (and the rising level of anti-D) were due to the baby being both C+ and D+. This was disturbing news for the family in two ways: the rising level of antibody indicated a potential risk to the baby and as the husband was grouped as R<sub>2</sub>r, and therefore C negative, it appeared that he was not the father of the baby.

JF's anti-D level eventually rose to 20.3 IU ml<sup>-1</sup> and the baby was born by caesarean section, 6 weeks early in January. The baby was grouped as O Rh D positive R<sub>2</sub>r (DcE/dce) with a strongly positive direct antiglobulin test (see Chapter 10). Fortunately, the baby responded well to phototherapy and mother and baby both made full recoveries. In addition, and of most importance, the baby's Rh type was consistent with the husband being the father.

The explanation for the apparent inconsistencies in blood grouping and antibody identification can be explained by the presence of the G antigen. The cell panels used to identify the mother's antibodies had given positive reactions with all C+ and D+ cells, hence the conclusion that the antibody was anti-C+D and the implication that the husband was not the father (as he was C negative). However, all C+ and D+ cells also carry the G antigen. The husband was R<sub>2</sub>r (DcE/dce) and, although he was C negative, he was G positive. The baby was also R<sub>2</sub>r and had therefore inherited the father's G antigen. The antibody produced by the mother was in fact anti-G+D.

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explains the not uncommon observation that some non-transfused pregnant women apparently produce anti-C+D antibody even though the father of their foetus is found to be C negative (see *Box 6.4*). In such cases, the father has been shown to have passed an  $R_2$  chromosome (DcE) to the foetus. The G antigen would also be expressed by this gene arrangement. Thus, the mother has actually been immunized to produce anti-D and anti-G rather than anti-D and anti-C.

### Compound antigens

Not unexpectedly, given that the C/c and E/e antigens are produced by the same gene, antibodies have been described which only react with **compound antigens** that are produced by the same gene. For example, the antibody produced in response to the ce compound antigen is anti-ce (it is also known as anti-f). This antibody will only react with cells expressing both c and e antigens derived from the same gene. This means that anti-ce will react with dce (r) or Dce ( $R_0$ ) red cells but not with DCe/DcE ( $R_1R_2$ ) cells where the c and e antigens have been produced by different genes. Other examples of compound antigens with corresponding antibodies are cE, CE and Ce.

## 6.6 QUANTITATIVE DIFFERENCES IN RH ANTIGENS

### Antigen dosage

Dosage is the property displayed when antibodies give stronger reactions in laboratory tests with red cells showing homozygous expressions of the corresponding antigen than with heterozygotes. The dosage effect when the D antigen is reacted with anti-D is not very noticeable as there is considerable overlap in the number of Rh D among the various genotypes. *Table 6.5* gives typical examples of D antigen site numbers for various genotypes and clearly indicates the overlapping of D antigen expression among genotypes.

The replacement of several exon segments from the *RHCE* gene with the equivalent segments from the *RHD* gene can destroy the ability to produce C/c and E/e antigens. Such individuals may only express the D antigen so their phenotype is written as D--. These individuals express much more D antigen on their red cells than those who are normal Rh D positive. A stronger dosage effect is seen with anti-E and anti-c than anti-D, and the most marked effect is seen in antibodies to compound Rh antigens.

### Influence of other Rh antigens

The expression of low-frequency antigens often affects the expression of other more common antigens. Low-frequency Rh antigens, for example  $C^w$  (RH8) and  $C^x$  (RH9), will not be discussed here in detail. However, they are

**Table 6.5 The distribution of antigens on red cells**

Group	No of antigen sites per cell
DCe/dce ( $R_1r$ )	D sites: 9900–14 600
Dce/dce ( $R_0r$ )	D sites: 12 000–20 000
DcE/dce ( $R_2r$ )	D sites: 14 000–16 600
DCe/dCe ( $R_1R_1$ )	D sites: 14 500–19 300
DCe/DcE $R_1R_2$	D sites: 23 000–31 000
DcE/DcE ( $R_2R_2$ )	D sites: 15 800–33 300
D <sup>u</sup> cE/dce ( $R_2^ur$ )	D sites: 340–470
D <sup>u</sup> Ce/D <sup>u</sup> cE ( $R_1^uR_2^u$ )	D sites: 540
D--D--	D sites: 110 000–202 000
cc	c sites: 70 000–85 000
cC	c sites: 37 000–53 000
ee	e sites: 18 200–24 000
eE	e sites: 13 400–14 500

usually associated with abnormal expression of one or more of the polymorphic Rh antigens.

In addition, students should be aware of two further effects, commonly known as the *cis* and the *trans* effect.

**Cis effect:** this term is used to describe the observation that when the gene for the D antigen is on the **same** chromosome as a gene for the C or E antigens, the expression of C and E antigens may be depressed. Thus, there is usually more E antigen produced on the red cells of  $r''$  (dcE) than  $R_2$  (DcE) type individuals, and more C antigen on the red cells  $r'$  (dCe) than an  $R_1$  (DCe) type individuals.

**Trans or Ceppellini effect:** this describes the depressed expression of the D antigen which is due to the presence of the gene for the C antigen on the **opposite** chromosome. For example, red cells from an individual who is type  $R_1r$  (DCe/dce) will express more D than red cells of the  $R_0r'$  (Dce/dCe) type. In some cases, the D antigen may be so depressed as to appear as a weak D ( $D^u$ ).

### Weaker forms of Rh antigens

Amino acid substitutions or exon exchanges to produce *RHD/RHCE* hybrid genes can give rise to gene products and conformational changes which result in altered or weakened expression of common antigens. In addition, some individuals express fewer antigens than normal for no apparent reason. Weak expression of the D antigen arises from the expression of a

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reduced number of D antigen sites on red cells. The D antigen expressed is the same as that expressed by a normal D-positive person but there is less of it (see *Table 6.5*). This weakened form of D, historically designated  $D^U$ , is capable of stimulating the production of anti-D in Rh D-negative individuals. Failure to detect the weakened D antigen in determining the Rh group of blood samples in the transfusion laboratory may result in mis-typing blood donors or neonates as Rh D negative. This could result in an Rh D-negative patient receiving a transfusion of Rh D-positive ( $D^U$ ) blood or the failure to give anti-D prophylaxis to an Rh D-negative mother with an Rh D-positive ( $D^U$ ) infant.

### 6.7 LABORATORY ASPECTS OF RH BLOOD GROUP TYPING

#### Rh D typing

When undertaking Rh D typing of patients, and when selecting blood donors, consideration must be given to the qualitative and quantitative variations in the expression of the Rh D antigen.

During D typing of patients, it is important to detect all but the very weakest forms in order that the patient is not unnecessarily transfused with Rh D-negative blood, which would be wasteful of a scarce resource. Also, when D typing babies of Rh D-negative women, it is important that the women receive anti-D prophylaxis if the baby has a weak form of D (see also *Box 6.5*).

When D typing donors, it is essential to detect weak forms in order to avoid the chance of transfusing Rh D (weak)-positive donor blood to Rh D-negative patients. This could induce the production of anti-D antibody in the patient.

The detection of partial D types (particularly  $D^{VI}$ ) is not necessary when typing patients. It is safer to treat them as Rh D negative for transfusion purposes. Patients with a partial D type may produce anti-D antibodies when exposed to normal Rh D-positive donor blood (see *Section 6.5*).

The partial D expressed on a baby's cells is poorly immunogenic and it is therefore not necessary to offer anti-D prophylaxis to the Rh D-negative mothers of such infants. It is a different approach when D typing blood donors where it is necessary to detect partial D types in order to avoid mistakenly transfusing Rh D (partial)-positive to Rh D-negative patients, as this may result in the induction of anti-D antibody.

#### **Box 6.5 Summary of practical considerations for weak and partial D types**

Weak D patients should be treated as Rh D positive.

Partial D patients should be treated as Rh D negative.

Weak D blood donors should be treated as Rh D positive.

Partial D blood donors should be treated as Rh D positive.

### Phenotyping and genotyping

It is often necessary to make an assessment of the phenotype and genotype of an individual when selecting blood for transfusion to patients with Rh antibodies, assessing the likely effect on the foetus of a woman's Rh antibodies or when performing family studies. It is usually possible to derive the genotype from information about the phenotype in Rh D-negative individuals. However, it is usually impossible to tell whether Rh D-positive individuals are homozygous or heterozygous for D and therefore their genotype has to be assumed from the statistically most likely arrangement for their ethnic group. The presence of Rh antigens on red cells is most usually detected using the antisera anti-D, anti-C, anti-E, anti-c and anti-e. The phenotype of an individual may be established from the reactions of their red cells when added to these reagents.

Table 6.6 shows an example of the results obtained using Rh antisera and an unknown sample of red cells. Using the example shown in the table, the observed phenotype is DCce. The first assumption is that, as no E antigen was detected, then e antigen must be present on both chromosomes. The second assumption is that, as both C and c antigens were detected, they must be located on opposite chromosomes. Thus, the blood sample is phenotypically  $\text{? C e / ? c e}$ , where  $\text{?} = \text{D}$  or **non-D (d)** and / divides the two chromosomes.

**Table 6.6 Example of the possible genotype of a blood sample using (Caucasian) population frequency statistics**

Antisera					Phenotype	Genotype		
-D	-C	-E	-c	-e		Most likely	Less likely	Least likely
+	+	-	+	+	DCce	<i>DCe/dce</i> $R_1r$	<i>DCe/Dce</i> $R_1R_0$	<i>Dce/dCe</i> $R_0r'$

+ indicates a positive reaction, - indicates a negative reaction.

The gene for D is either present on both chromosomes, making the phenotype  $\text{D C e / D c e}$  ( $R_1R_0 = 2\%$  in Caucasians), or on only one chromosome. In the latter case the second chromosome may either carry the gene for C, resulting in the arrangement  $\text{D C e / d c e}$  (i.e. type  $R_1r$ , found in 33% of Caucasians) or that for c, giving  $\text{D c e / d C e}$  (i.e. type  $R_0r'$ , which has a frequency of <1% in Caucasians).

The most likely genotype is *DCe/dce* ( $R_1r$ ), heterozygous with respect to D, although this is not necessarily the correct one. However, it is important to note that if these results were obtained from an individual of the black population, then the genotype *DCe/Dce* ( $R_1R_0$ ), homozygous with respect to D, would be the most probable (see Box 6.6).

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### Box 6.6 *Dce* distribution

The gene arrangement *Dce* ( $R_0$ ) is much more prevalent in black populations than any other population. In fact, it occurs with a frequency of 44% compared with 2% in Caucasian, oriental and Native American populations.

### Nature of Rh antibodies

Rh antibodies are usually immune in nature although naturally occurring forms of anti-D, anti-C, anti-E and anti-C<sup>w</sup> have been reported. The antibodies may be found in the IgM or IgG form. Anti-D production has even been reported following transfusion of fresh frozen plasma, presumably containing small amounts of red cell membrane. This is due to the highly immunogenic nature of the D antigen (see Box 6.7). Antibodies with specificities within the Rh system are often found in cases of autoimmune haemolytic anaemia, e.g. anti-e, anti-C+e, anti-c, anti-c+E (see Chapter 8).

### Box 6.7 Immunogenicity of Rh D antigen

The Rh D antigen is the most immunogenic of all the protein antigens. It has been reported that as many as 80% of Rh D-negative individuals receiving a transfusion of Rh D-positive red cells will produce anti-D antibody. Also, up to 16% of Rh D-negative mothers exposed to a foeto-maternal haemorrhage from an Rh D-positive foetus will produce anti-D antibody. Despite the success of anti-D prophylaxis, anti-D is still the most common cause of clinically significant haemolytic disease of the newborn.

Anti-D is the most common of all immune blood group antibodies in Rh D-negative individuals. It is sometimes found in association with anti-C (anti-C+D) or anti-E (anti-D+E), and rarely with both (anti-C+D+E).

In Rh D-positive patients, anti-E is often found in  $R_1r$  (*DCe/dce*) and  $R_1R_1$  (*DCe/DCe*) genotypes. Anti-c and anti-c+E are quite common in  $R_1R_1$  patients. Anti-C and anti-e are much less common.

Antibodies to compound Rh antigens (anti-ce, anti-Ce, etc.) may be present in sera containing other Rh antibodies but are difficult to differentiate by routine laboratory tests. Rarely, they may be the sole Rh antibody present in a serum.

### Laboratory detection

Immune Rh antibodies often have a wide thermal range. The majority react optimally at 37°C and are best detected using the indirect antiglobulin test or enzyme-treated red cells (see Chapter 10). The naturally occurring forms

usually react optimally at lower temperatures. Rh antibodies are not considered to be complement-fixing antibodies but exert their effects by opsonization and antibody-dependent cellular cytotoxicity (ADCC) (see Chapters 2 and 8).

### Clinical significance

Rh antibodies may cause immediate or delayed haemolytic transfusion reactions. In addition, anti-D is still the most common cause of severe haemolytic disease of the newborn despite anti-D prophylaxis programmes. Anti-c is also recognized as the cause of a significant number of severe cases of haemolytic disease of the newborn. Rh antibodies are found in many cases of autoimmune haemolytic anaemia. Their deleterious effects cause these antibodies to be of importance in the clinical environment, i.e. they are clinically significant. Antibodies in the Rh system are the most clinically significant of all blood group antibodies apart from the ABO system. The Rh antigens D and c, in particular, are highly immunogenic.

### SUGGESTED FURTHER READING

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**SELF-ASSESSMENT QUESTIONS**

1. Describe the *cis* and *trans* effects in relation to the expression of Rh antigens.
2. Name the genes and their products for the 'two-locus model' for the Rh genes.
3. List the five major antigens of the Rh system.
4. What is the thermal range and mode of detection for immune Rh antibodies?
5. Why is it not necessary to detect partial D types when performing routine Rh blood grouping on patients?
6. If a patient has *RHD* and *RHCe* genes on one chromosome and *RHD* and *RHcE* genes on the opposite chromosome, how would you write the patient's genotype using the Fisher notation?