The Rh system is complex, and certain aspects of its genetics, nomenclature, and antigenic interactions are not fully understood. This chapter avoids exhaustive theoretical considerations, and instead concentrates on commonly encountered observations, problems, and solutions.

The D Antigen and Its Historical Context

Rh Positive and Rh Negative

The unmodified descriptive terms Rh positive and Rh negative refer to the presence or absence of the red cell antigen D. An earlier name for D, Rh_o, is no longer used and remains of only historical interest. This chapter uses the CDE nomenclature originally proposed by Fisher and Race, which has been able to accommodate our present understanding of the genetics and biochemistry of this complex system. The Rh-Hr terminology of Wiener is presented only in its historical context, as molecular genetic evidence to date does not support Wiener’s one-locus theory.

Discovery of D

The first human example of the antibody against the D antigen was reported in 1939 by Levine and Stetson, who found it in the serum of a woman whose fetus had hemolytic disease of the newborn (HDN) and who experienced a hemolytic reaction after transfusion of her husband’s blood. In 1940, Landsteiner and Wiener described an antibody obtained by immunizing guinea pigs and rabbits with the red cells of Rhesus monkeys; it agglutinated the red cells of approximately 85% of humans tested, and they called the corresponding determinant the Rh factor. In the same year, Levine and Katzin found similar antibodies in the serum of several recently delivered women, and at least one of these sera gave reactions that paralleled those of the animal anti-Rhesus sera. Also in 1940, Wiener and Peters observed antibodies of the same specificity in the se-
rum of persons whose red cells lacked the determinant, who had received ABO-compatible transfusions in the past. Later evidence established that the antigen detected by animal anti-Rhesus and human anti-D were not identical, but by that time the Rh blood group system had already received its name.

Clinical Significance

Other than the A and B antigens, D is the most important red cell antigen in transfusion practice. In contrast to A and B, however, persons whose red cells lack the D antigen do not regularly have the corresponding antibody. Formation of anti-D almost always results from exposure, through transfusion or pregnancy, to red cells possessing the D antigen. A high proportion of D-negative persons who receive D-positive blood do produce anti-D.

The D antigen has greater immunogenicity than virtually all other red cell antigens; more than 80% of D-negative persons who receive a D-positive transfusion are expected to develop anti-D. To prevent this, the blood of all recipients and all donors is routinely tested for D to ensure that D-negative recipients are identified and given D-negative blood.

Soon after anti-D was discovered, family studies showed that the D antigen is genetically determined; transmission of the trait follows an autosomal dominant pattern. With only a few interesting exceptions, persons who have the gene for D will have D antigen detectable on their red cells.

Other Important Antigens

By the mid-1940s, four additional antigens, C, E, c, and e, had been recognized as belonging to what is now called the Rh system. Subsequent discoveries have brought the number of Rh-related antigens to over 50 (Table 13-1), many of which exhibit both qualitative and quantitative variations. The reader should be aware that these exist, but in most transfusion medicine settings, the five principal antigens (D, C, E, c, e) and their corresponding antibodies account for more than 99% of clinical issues involving the Rh system.

Genetic and Biochemical Considerations

Attempts to explain the genetic control of Rh antigen expressions have been fraught with controversy. Wiener proposed a single locus with multiple alleles determining surface molecules that embody numerous antigens. Fisher and Race inferred from the existence of antithetical antigens the existence of reciprocal alleles at three individual but closely linked loci. Tippett's prediction that two closely linked structural loci on chromosome 1 determine production of Rh antigens is presently considered to be correct.

Rh Genes

Two highly homologous genes on the short arm of chromosome 1 encode the nonglycosylated polypeptides that express Rh antigenic activity. One, designated RHD, determines the presence of a membrane-spanning protein that confers D activity on the red cell. D-positive individuals possess one or two examples of this gene. D-negative persons have no genetic material at this site; the absence of an antithetical allele explains why, after decades of searching, serologists have never found a d antigen.

At the other, adjacent locus, the gene RHCE determines the C, c, E, and e antigens; its alleles are RHCE, RHCE, RHcE, and RHce. Much effort has gone into determining if different proteins, resulting from alternative splicing of
mRNA, carry C/c and E/e or if a single polypeptide expresses both sites. Recent evidence derived from transfection studies suggest that both C/c and E/e reside on a single polypeptide product.

**Biochemical and Structural Observations**

The products of both *RHD* and *RHCE* are proteins of 416 amino acids that, modeled studies suggest, traverse the red cell membrane 12 times and display only short exofacial loops of amino acids on the exterior. The polypeptides are fatty-acid acylated and, unlike most blood group-associated proteins, carry no carbohydrate residues. Within the red cell membrane, the Rh polypeptides complex with glycoproteins that have partial homology with the Rh polypeptides but are encoded at a locus on chromosome 6.

<table>
<thead>
<tr>
<th>Numerical Designation</th>
<th>CDE</th>
<th>Rh-Hr</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh1</td>
<td>D</td>
<td>Rh₀</td>
<td></td>
</tr>
<tr>
<td>Rh2</td>
<td>C</td>
<td>rh’</td>
<td></td>
</tr>
<tr>
<td>Rh3</td>
<td>E</td>
<td>rh”</td>
<td></td>
</tr>
<tr>
<td>Rh4</td>
<td>c</td>
<td>hr’</td>
<td></td>
</tr>
<tr>
<td>Rh5</td>
<td>e</td>
<td>hr”</td>
<td></td>
</tr>
<tr>
<td>Rh6</td>
<td>ce,(f)</td>
<td>hr</td>
<td></td>
</tr>
<tr>
<td>Rh7</td>
<td>Ce</td>
<td>rh₁</td>
<td></td>
</tr>
<tr>
<td>Rh8</td>
<td>Cw</td>
<td>rhw₁</td>
<td></td>
</tr>
<tr>
<td>Rh9</td>
<td>Cx</td>
<td>rhₓ</td>
<td></td>
</tr>
<tr>
<td>Rh10</td>
<td>ce₅</td>
<td>hrᵥ</td>
<td>V</td>
</tr>
<tr>
<td>Rh11</td>
<td>Ew</td>
<td>rhw₂</td>
<td></td>
</tr>
<tr>
<td>Rh12</td>
<td>G</td>
<td>rh₆</td>
<td></td>
</tr>
<tr>
<td>Rh17</td>
<td>G</td>
<td>hr₀</td>
<td></td>
</tr>
<tr>
<td>Rh18</td>
<td>G</td>
<td>hr₅</td>
<td></td>
</tr>
<tr>
<td>Rh20</td>
<td>eₛ</td>
<td>VS</td>
<td></td>
</tr>
<tr>
<td>Rh21</td>
<td>C G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rh22</td>
<td>CE</td>
<td>rhᵧ</td>
<td>Jarvis</td>
</tr>
<tr>
<td>Rh23</td>
<td>Dw</td>
<td>Wiel</td>
<td></td>
</tr>
<tr>
<td>Rh26</td>
<td>“c-like”</td>
<td>Deal</td>
<td></td>
</tr>
<tr>
<td>Rh27</td>
<td>cE</td>
<td>rh</td>
<td></td>
</tr>
<tr>
<td>Rh28</td>
<td>hr₇</td>
<td>Hernandez</td>
<td>Hernandez</td>
</tr>
</tbody>
</table>

The table is compiled from the findings of the ISBT Working Party on Terminology for Red Cell Surface Antigens.

*Rh32 is a low-incidence antigen that is a product of the predominately Black gene *Rh*, the other products of which include a reduced expression of C and e.

†Rh37 is the low-incidence antigen Evans, which occurs in association with the ·D· haplotype. This is similar to –D–, except for the presence of the Evans antigen and a lesser exaltation of D activity.
Considerable homology exists between the products of RHD and RHCE; the products of the different alleles of RHCE are even more similar. C and c differ from one another in only four amino acids, at positions 16, 60, 68, and 103, of which only the difference between serine and proline at 103 appears to be critical. The presence of proline or alanine at position 226 appears to be the sole characteristic that distinguishes E from e. The D polypeptide, by contrast, possesses 36 amino acids that will be perceived as foreign by D-negative individuals.

The study of Rh\textsubscript{null} red cells, which entirely lack Rh antigens, reveals that the Rh proteins are part of a large membrane complex, in which the presence of the Rh proteins appears to be essential for correct expression or presentation of other constituents. The glycoproteins that bear the LW, Duffy, and U antigens all seem to require the presence of Rh proteins for full expression. Rh\textsubscript{null} cells lack all the LW antigens, are negative for Fy5 of the Duffy system, and have weakened expression of the antigens carried on glycophorin B (S, s, and U).

### Rh Terminology

Three systems of nomenclature were developed before the recent advances in our understanding of the genetics of Rh. Each of these systems of nomenclature have been used to convey genetic and serologic information about the Rh system.

#### System Notations

The Rh-Hr terminology derives from the work of Wiener, who believed the immediate gene product to be a single entity he called an agglutinogen. Wiener’s concept was that each agglutinogen is characterized by numerous individual serologic specificities, called factors, identified by individual specific antibodies. Current biochemical and serologic data do not support this theory.

CDE terminology was introduced by British workers, Fisher and Race, who postulated three sets of closely linked genes (C and c, D and d, and E and e). Both gene and gene product have the same letter designation, with italics used for the name of the gene. Although this theory does not fully explain some of the observed Rh antigenic profiles, it does, however, provide the easiest way to communicate research and serologic findings at present.

Rosenfield and coworkers proposed a system of nomenclature based simply on serologic observations. Symbols were not intended to convey genetic information, merely to facilitate communication of phenotypic data. Each antigen is given a number, generally in order of its discovery or its assignment to the Rh system. The presence of an antigen on a red cell specimen is indicated by the appropriate number placed after the system designation, Rh followed by a colon; a minus sign before a number indicates that the antigen has been tested for and found to be absent. This system is cumbersome for verbal communication, but well-suited for written communication without genetic inference, and for computerized data entry. Table 13-1 lists the antigens currently included in the Rh system. Table 13-2 shows the most common combinations of antigens, expressed as haplotypes. Table 13-3 shows reaction patterns achieved by testing cells with antibodies to the five principal antigens, and the descriptive terms used for phenotypes in three systems of nomenclature.

#### Phenotypic and Genetic Notations

For informal designation of phenotype, particularly in conversation, many workers use a shorthand system based on Wie-
ner’s Rh-Hr notation. This does not fit into any system of nomenclature, but these shorthand symbols convey information in a convenient and efficient fashion.

**Haplotype Notations**

The phenotype notations convey haplotypes with the single letters R and r, in roman type, for the haplotypes that produce or do not produce D, respectively. Subscripts or, occasionally, superscripts, indicate the combinations of other antigens present. For example, R₁ indicates C, D, and e together; R₂ indicates c, D, and E; r indicates c and e; R₀ indicates c, D, and e; and so on. Phenotypes appearing to embody homozygous expression of a single haplotype have only one letter; others have two.

**Determining Phenotype**

In clinical practice, five blood typing reagents are readily available: anti-D, -C, -E, -c, and -e. Routine pretransfusion studies include only tests for D. Other reagents are used principally in the reso-

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### Table 13-2. The Principal Rh Genes (or Gene Complexes)

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Fisher-Race Terminology</th>
<th>Gene Combination</th>
<th>Antigenic Specificities</th>
</tr>
</thead>
<tbody>
<tr>
<td>R¹</td>
<td>cDe</td>
<td>C,D,e</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>ce</td>
<td>c,e</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>cDE</td>
<td>C,D,E</td>
<td></td>
</tr>
<tr>
<td>R₀</td>
<td>cDe</td>
<td>C,D,e</td>
<td></td>
</tr>
<tr>
<td>r’</td>
<td>Ce</td>
<td>C,e</td>
<td></td>
</tr>
<tr>
<td>r”</td>
<td>cE</td>
<td>C,E</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>CDE</td>
<td>C,D,E</td>
<td></td>
</tr>
<tr>
<td>r’</td>
<td>CE</td>
<td>C,E</td>
<td></td>
</tr>
</tbody>
</table>

*Shorthand terminology*

### Table 13-3. Determination of Some Rh Phenotypes from the Results of Tests with the Five Principal Rh Blood Typing Reagents

<table>
<thead>
<tr>
<th>Anti-D</th>
<th>Anti-C</th>
<th>Anti-E</th>
<th>Anti-c</th>
<th>Anti-e</th>
<th>Rh-Hr*</th>
<th>CDE</th>
<th>Numerical</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>R₁r</td>
<td>CcDe</td>
<td>Rh:1,2,–3,4,5</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>R₁</td>
<td>CDe</td>
<td>Rh:1,2,–3,–4,5</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>R₁R₂</td>
<td>CcDe</td>
<td>Rh:1,2,3,4,5</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>R₀</td>
<td>cDe</td>
<td>Rh:1,–2,–3,4,5</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>R₂r</td>
<td>CcDe</td>
<td>Rh:1,–2,3,4,5</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>R₂</td>
<td>cDe</td>
<td>Rh:1,–2,3,–4,5</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>R₂R₁</td>
<td>CcDe</td>
<td>Rh:1,2,3,–4,5</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>R₂R₂</td>
<td>CcDe</td>
<td>Rh:1,2,3,4,–5</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>R₂</td>
<td>CDE</td>
<td>Rh:1,2,3,4,–5</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>r</td>
<td>ce</td>
<td>Rh:–1,–2,–3,4,5</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>r”r</td>
<td>Cce</td>
<td>Rh:–1,–2,3,4,5</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>r”r”</td>
<td>CcEe</td>
<td>Rh:–1,2,3,4,5</td>
</tr>
</tbody>
</table>

*Shorthand terminology*
olution of antibody problems or in family studies. The assortment of antigens detected on a person’s red cells constitutes that person’s Rh phenotype.

**Inferring Genotype**

Identifying antigens does not always allow confident deduction of genotype. Presumptions regarding the most probable genotype rest on the frequencies with which particular antigenic combinations derive from an individual genome complex. For simplicity, the remainder of this chapter uses CDE terminology to express haplotypes, eg, CDE rather than RHD, RHCe.

Inferences about genotype are useful in population studies and in the investigation of disputed parentage. Such analyses are also used to predict whether the sexual partner of a woman with Rh antibodies is likely to transmit the genes that will result in offspring negative or positive for the particular antigen.

**Serologic Testing for Rh Antigen Expression**

To determine whether a person has genes that encode C, c, E, and e, the red cells are tested with antibody to each of these antigens. If the red cells express both C and c or both E and e, it can be assumed that the corresponding genes are present in the individual. If the red cells carry only C or c, or only E or e, the person is assumed to be homozygous for the particular allele. Titration studies can sometimes document this assumption because the amount or dose of antigen on the red cells from homozygotes often is greater than when the genome includes only a single copy. Tests for the D antigen indicate only its presence or absence; titration results to demonstrate dosage have not given reliable information.

**Expression of D**

D-negative persons either lack RHD, which encodes for the D antigen, or, much more rarely, have a nonfunctional D gene. Most D-negative persons are homozygous for RHce, the gene encoding c and e; less often they may have RHCe or RHcE, which encode C and e or c and E, respectively. The RHCE gene that produces both C and E is quite rare.

The genotype of a D-positive person cannot be determined serologically; dosage studies are not effective in showing whether an individual is homozygous or heterozygous for RHD. An individual’s D genotype can be assigned only by inference from the antigens associated with the presence of D. Recent techniques for cloning the Rh genes may eventually allow highly accurate determination of D genotype.

Interaction between genes results in so-called “position effect.” If the interaction is between genes, or the product of genes, on the same chromosome it is called a cis effect. If a gene or its product interacts with one on the opposite chromosome, it is called a trans effect. Examples of both effects were first reported in 1950 by Lawler and Race, who noted as a cis effect that the E antigen produced by cDE is quantitatively weaker than E produced by cE. They noted as trans effects that both C and E are weaker when they result from the genotype CDe/cDE than when the genotypes are CDe/ce or cDE/ce, respectively.

**Effect of Race**

A person whose red cells are of the phenotype CDe most likely has the genotype CDe/CDe, and will transmit a gene encoding D to all offspring. A less likely alternative genotype would be CDe/Ce. Racial origin influences deductions about genotype because the frequencies of Rh genes differ by race. For example,
a White person with the phenotype cDe would probably be cDe/cE, but a Black person of the same phenotype, cDe/cDe and cDe/cE are almost equally likely.

**Effect of Gene Frequency**

The phenotype CcDEe (line 3 of Table 13-3) can arise from any of several genotypes. In any population, the most probable genotype is CDe/cDE. Both these haplotypes encode D; a person with this phenotype will very likely be homozygous for the D gene, although heterozygous for the actual combination of genes present on the two chromosomes. Some less likely alternative genotypes could render the person heterozygous at the D locus, for example, CDe/cE, cDE/Ce, or CDE/cE, but these are uncommon in all populations. An even less likely possibility is cDe/CE. Table 13-4 gives the frequencies of the more common genotypes in D-positive persons. The figures given are for Whites and Blacks. In other racial groups, the likelihood of being heterozygous for D is reduced because absence of RHD is so uncommon.

**Weak Expression of D**

Different D-positive red cell specimens may have differing reactivity with anti-D reagents. Most D-positive red cells show

### Table 13-4. Frequencies of the More Common Genotypes in D-Positive Individuals

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Genotype Frequency (%)</th>
<th>Frequency (%)</th>
<th>Likelihood of Zygosity for D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Whites</td>
<td>Blacks</td>
</tr>
<tr>
<td>CDE</td>
<td>CDE</td>
<td>Rh-hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CcDe</td>
<td>CDe/cE</td>
<td>R(^1)r</td>
<td>31.1</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>CDe/cDe</td>
<td>R(^1)R(^0)</td>
<td>3.4</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Ce/cDe</td>
<td>r(^R)(^0)</td>
<td>0.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Cde</td>
<td>CDe/CDe</td>
<td>R(^1)R(^1)</td>
<td>17.6</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>CDe/cDe</td>
<td>R(^1)r</td>
<td>1.7</td>
<td>0.7</td>
</tr>
<tr>
<td>CDee</td>
<td>cDE/cE</td>
<td>R(^2)r</td>
<td>10.4</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>cDE/cDe</td>
<td>R(^2)R(^0)</td>
<td>1.1</td>
<td>9.7</td>
</tr>
<tr>
<td>cDE</td>
<td>cDE/cDE</td>
<td>R(^2)R(^2)</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>cDE/cE</td>
<td>R(^2)r</td>
<td>0.3</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>CDe/cDE</td>
<td>R(^1)R(^2)</td>
<td>11.8</td>
<td>3.7</td>
</tr>
<tr>
<td>CcDEe</td>
<td>CDe/cE</td>
<td>R(^1)r</td>
<td>0.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>Ce/cDE</td>
<td>r(^R)(^2)</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>cDe</td>
<td>cDe/cE</td>
<td>R(^0)r</td>
<td>3.0</td>
<td>22.9</td>
</tr>
<tr>
<td></td>
<td>cDe/cDe</td>
<td>R(^0)R(^0)</td>
<td>0.2</td>
<td>19.4</td>
</tr>
</tbody>
</table>

For the rare phenotypes and genotypes not shown in this table consult the reference books listed at the end of this chapter.
clear-cut macroscopic agglutination after centrifugation with reagent serum and can readily be classified as D-positive. Red cells that are not immediately or directly agglutinated cannot as easily be classified. For some D-positive red cells, demonstration of the D antigen requires prolonged incubation with the anti-D reagent or addition of antiglobulin serum after incubation with anti-D. These cells are considered D-positive, even if there has been need for an additional step in testing.

In the past, red cells that required additional steps for demonstration of D were classified as D\textsuperscript{u}. The term D\textsuperscript{u} is no longer used; red cells that carry weak forms of D are classified as D-positive, and may be described as “weak D.” Monoclonal anti-D reagents may cause direct agglutination of some D-positive cells that would have been considered weak D after use of polyclonal reagents. Additionally, they may detect hitherto unrecognized epitopes of D or, occasionally, fail to react with variant configurations of the D antigen.

**Weak D Due to Transmissible Genes**

Weak D phenotypes can result from several different genetic circumstances. Some examples of RHD appear to encode weak expression of the D antigen. This quantitative characteristic follows a regular pattern of Mendelian dominant inheritance. Weak D expression of this type is fairly common in Blacks, often occurring as part of a cDe haplotype. Transmissible genes for weak D expression are considerably less common in Whites, but may be seen as part of an unusual CDe or cDE haplotype.

On testing with most anti-D reagents, most red cell samples with weak D antigens due to a transmissible gene either fail to react or react very weakly in direct agglutination tests, but react strongly when antiglobulin serum is added to the system.

**Weak D as Position Effect**

Perhaps the best-known example of position effect is weakening of the D antigen by C gene trans to the D gene. Red cells from some persons of the genotype CDe/Ce have weakened expression of D, a suppressive effect exerted by C in the trans position to an unremarkable D on the opposite chromosome. Similar depression of D can be seen with other D-positive haplotypes accompanied by Ce. For example, the CDe/c and CDe/Ce genotypes encode for the same antigens, but D expression is often perceptibly weaker on cells from the latter individual. Many weak D phenotypes reported in the early literature would, with currently available reagents, appear as normal D. Weak D antigens that have qualitative differences will be discussed later in this chapter.

**Significance of Weak D in Blood Donors**

Transfusion to D-negative recipients of blood with weak expression of the D antigen has long been proscribed, based on the possibility that such red cells could elicit an immune response to D. This possibility may be more apparent than real, as weak forms of the D antigen seem to be substantially less immunogenic than normal D-positive blood. Transfusion of a total of 68 units of blood with weak D to 45 D-negative recipients failed to stimulate production of a single example of anti-D.\textsuperscript{21} Although 15 of the 45 recipients were receiving the level of immunosuppression available in the late 1950s, one person in the series made anti-E and a second made anti-K. A report\textsuperscript{22} of anti-CD produced in response to CD\textsuperscript{u}e red cells can be better explained as anti-G than as anti-C and an anti-D elicited by the cells described as D\textsuperscript{u}.
More important than the potential immunogenicity of red cells with weakened expression of D is the possibility of such cells experiencing accelerated destruction if transfused to a recipient with circulating anti-D. Hemolytic transfusion reactions were reported in the early literature, but it is probable that the responsible cells would, with currently available reagents, have been considered straightforwardly D-positive. Hemolytic disease of the newborn has also been reported, but not recently.

Significance of Weak D in Recipients

The transfusion recipient with weak D is sometimes a topic of debate. Most such patients can receive D-positive blood without risk of immunization, but if the weak D expression reflects lack of one or more D epitopes, the possibility exists that transfusion of D-positive blood could elicit alloanti-D. The same possibility exists, however, for epitope-deficient persons whose red cells react strongly with anti-D reagents. AABB Standards for Blood Banks and Transfusion Services requires donor blood specimens to be tested for weak expression of D and be labeled as D-positive if the test is positive, but recipients’ specimens need not be tested with anti-D by any procedure other than direct agglutination. Currently available, licensed anti-D reagents are sufficiently potent that most patients with weak D are found to be D-positive. The few patients classified as D-negative, whose D-positive status would have been detected by antiglobulin testing, can receive D-negative blood without problems. Some workers consider this practice wasteful of D-negative blood and prefer to test potential recipients for weak D, and then issue D-positive blood.

If D-positive blood is given to recipients of the weak-D phenotype, it is important to safeguard against careless or incorrect interpretation of tests. D-negative recipients erroneously classified as D-positive, possibly because of a positive direct antiglobulin test (DAT), run the risk of immunization to D if given D-positive blood. Individuals whose weakly expressed D antigen is detectable only in the antiglobulin test will ordinarily be classified as D-negative recipients. If they give blood, however, they will be classified as D-positive at the time of blood donation. Personnel in blood centers and transfusion services should be prepared to answer questions from puzzled donors or their physicians. This can present special problems in autologous donations, when the D-negative patient’s own blood is labeled as D-positive. In this case, confirmation of the patient’s D status by antiglobulin testing resolves the apparent discrepancy between recipient and donor types.

Partial D

The concept that the D antigen consists of multiple, individually determined constituents arose from observations that some people with D-positive red cells produced alloanti-D that was nonreactive with their own cells. Most D-positive persons who produce alloanti-D have red cells that react strongly when tested with anti-D, although some have test results that suggest weak D. Red cells lacking parts of the D antigen complex have been referred to in the past as “D mosaic” or “D variant.” Current terminology indicates such red cells, deficient in components of D, are more appropriately described as expressing “partial D.”

Tests of various monoclonal anti-D reagents with red cells of various D categories confirm that the D antigen comprises many epitopes. Numbered D cate-
gories and other partial-D phenotypes can now be defined in terms of their D epitopes. Lomas, McColl, and Tippett established nine D epitopes, expanding on the seven epitopes demonstrated in 1989. Complete exposition of the D antigen, its sum and its parts, has not yet been achieved.

**Quantitative vs Qualitative Differences**

Red cells lacking part of the D antigen may appear phenotypically similar to those that reflect inheritance of a gene for weak D expression unless tested with selected anti-D. The D antigen of the epitope-deficient types is qualitatively different from normal; red cells of a person with a gene that encodes weak expression of D simply have fewer examples of the normal D polypeptide. The distinction can seldom be made serologically, unless the epitope-deficient person makes alloanti-D. Red cells of many partial-D phenotypes, although epitope-deficient, react as strongly with most anti-D reagents as do normal cells. In such cases, the qualitative abnormality becomes apparent only if the person makes alloanti-D. Such alloanti-D is indistinguishable from the anti-D made by D-negative individuals except that it is nonreactive with D-positive red cells that lack the same epitope.

**Spurious Alloanti-D**

Not all persons who are D-positive and produce what appear to be anti-D should be assumed to have epitope-deficient red cells. Weakly reactive anti-LW\textsuperscript{ab} or anti-LW\textsuperscript{a} may react with D-positive cells but not with D-negative cells. A D-positive person whose antibody is a weakly reactive anti-LW\textsuperscript{a} may be indistinguishable on initial serologic testing from an individual with a partial-D antigen who has made anti-D to missing epitopes. (See section on LW in Chapter 14.) Anti-LW can easily be differentiated from anti-D in tests with sulfhydryl-treated red cells; the LW antigen is destroyed by sulfhydryl reagents and D is not.

**Other Rh Antigens**

Numbers up to 51 have been assigned to red cell antigens (see Table 13-1); some of the numbers have been rescinded or the antigens reassigned, but of the currently included 44, most beyond D, C, c, E, and e (Rh:1-5) are rarely encountered in routine blood transfusion therapy.

**Cis Product Antigens**

The membrane components that display Rh activity have numerous possible antigenic subdivisions. Each gene or gene complex determines a series of interrelated surface structures, of which some portions are more likely than others to elicit an immune response. The polypeptides determined by the genes in the haplotype CDe express determinants additional to those defined as D, C, and e. These include Ce, a cis product that almost always accompanies C and e when they are encoded by the same haplotype. The Ce antigen is absent from red cells on which the C and e were encoded by different haplotypes, for example, in a person of the genotype CDe/ce. Similar cis product antigens exist for c and e determined by the same haplotype (the antigen called ce or f), for c and E (cE), and for C and E (CE).

Although antibodies directed at cis product antigens are encountered infrequently, it would not be correct to consider them rare. Such antibodies may be present, unnoticed in serum containing antibodies of the more obvious Rh specificities; only adsorption with red cells of selected phenotypes would demonstrate their presence. Anti-f (ce) may be present, for example, as a component of
some anti-c and anti-e sera, but its presence would have little practical significance. The additional antibody should not confuse the reaction patterns given by anti-c and anti-e, since virtually any cell that reacts with anti-f would express both c and e.

Anti-Ce is frequently the true specificity of the apparent anti-C that a cDE/cDE person produces after receiving D-positive blood. This knowledge can be helpful in establishing an individual’s Rh genotype. If anti-Ce is the predominant specificity in a reagent anti-C, however, the individual whose C antigen resulted from a CDE haplotype may be mistyped unless test methods and control red cells are chosen carefully.

Deletions

Rare genes exist that either fail to encode Rh material or encode Rh material lacking activity at the CcEe sites. Some portions of the surface configuration are not detectable, leaving D (or C/c and D) as the only remaining site(s). Red cells that lack C/c and/or E/e antigens may show exceptionally strong D activity, an observation by which such red cells have sometimes been recognized during routine testing with anti-D. The −D− phenotype may be identified in the course of studies to investigate an unexpected antibody. Such persons may have alloantibody of complex specificity, because the person’s red cells lack all the epitopes expressed on the CcEe polypeptide. A single antibody with anti-HR (anti-Rh17) specificity is often made by persons of this rare phenotype, although some such sera have been reported to contain apparently separable specificities, such as anti-e.

The •D• phenotype is similar in most respects to −D−, except that the D antigen is not exalted to the same degree. •D• red cells may be agglutinated weakly by some examples of serum from immunized Rh-deletion persons if the serum contains anti-Rh47 in addition to anti-Rh17. The •D• gene encodes Rh47. A distinguishing characteristic of •D• red cells is that they possess a low-incidence antigen known as Rh37 (Evans).

The Antigen G and Cross-Reactions

The G antigen is almost invariably present on red cells possessing either C or D. Antibodies against G appear superficially to be anti-C+D, but the anti-G activity cannot be separated into anti-C and anti-D. The fact that G appears to exist as an entity common to C and D explains the fact that D-negative persons immunized by C−D+ red cells sometimes appear to have made anti-C as well as anti-D. It may also explain why D-negative persons who are exposed to C+D− red cells may develop antibodies appearing to contain an anti-D component. Rare red cells have been described that possess G but lack D altogether and show greatly diminished, altered, or absent expression of the C antigen C(−−eG). The D−G+ phenotype occurs in Blacks but evidently is not quite the same as when it occurs in Whites. Red cells also exist that express at least a part of D but lack G entirely.

Variant Antigens

There must be innumerable subtle differences in composition among various Rh gene products. Although red cells from most people give straightforward reactions with common antibodies, some cells give atypical reactions and others stimulate the production of antibodies that do not react with red cells of common Rh phenotypes. It has been convenient to consider C and c, and E and e, as antithetical antigens at specific surface sites. This scheme can be expanded to include variant antigens that seem to occupy the same surface site but
have been determined by genes, possibly conversion or misalignment products, that encode proteins that differ from the common Rh determinants. For example, several variant forms of the e antigen have been identified, described as hr^\text{S} or hr^B (Rh19 and Rh31). Most people have red cells with all epitopes of the Rh antigens, but some lack some of the epitopes of certain Rh antigens, such as D, C, V, VS, Hr, and Hr_o.

Red cells lacking one or both of the epitopes of e are sometimes found in Blacks. Diminished C and markedly diminished e activity characterize the products of the \( R^w \) gene complex, which also encodes a low-incidence antigen, Rh32. Genes that encode weakly expressed antigens may be designated by parentheses, i.e., \((C)D(e)\). Among Whites, a weakened e antigen is among the products of genes that encode the low-incidence Rh33 and the Rh36 (Berrens, Be^e) antigens. Antigens that behave as if they had an antithetical relationship to C/c or E/e have been found, mainly in Whites. The most common is C^w, found in 2% or more of some White populations. Table 13-1 lists the antigens that belong to the Rh system and contribute to the advancing complexity of the system and its nomenclature.

**Rh\textsubscript{null} Syndrome and Rh\textsubscript{mod}**

**Genetic Determination**

The literature reports at least 43 persons in 14 families whose red cells appear to have no Rh antigens; others are known but have not been reported. The phenotype, described as Rh\textsubscript{null}, may be produced by at least two different genetic mechanisms. In the more common regulator type of Rh\textsubscript{null}, the absence of a very common regulator gene, \( X^r \), was thought to prevent expression of the person’s perfectly normal genes at the Rh locus on chromosome 1. Such persons appear to transmit normal Rh genes to their offspring, in a manner roughly analogous to that in which the A or B transferases are transmitted by people of the Bombay phenotype. These Rh\textsubscript{null} persons were considered to be homozygous for X^o,r, a rare allele of X^1,r, that segregates independently of genes of the Rh system. In some cases, parents or offspring of people with the regulator type of Rh\textsubscript{null} show overall depression of their Rh antigens.

The other form of Rh\textsubscript{null} was thought to arise through homozygosity for an amorphic gene at the Rh locus itself, \( \bar{r} \), which appears to have no products detectable with Rh testing reagents. Recent studies, however, suggest Rh\textsubscript{null} gene complexes could direct low levels of Rh polypeptides to be produced. This type of Rh\textsubscript{null} phenotype is considerably rarer than the regulator type. Parents and offspring of this type of Rh\textsubscript{null} are obligate heterozygotes for the amorph.

**Red Blood Cell Abnormalities**

Whatever the genetic origin, red cells lacking Rh antigens have membrane abnormalities that shorten their survival. The severity of hemolysis and resulting anemia varies among affected persons, but stomatocytosis, shortened red cell survival, and variably altered activity of other blood group antigens, especially S, s, and U, have been consistent features.

**Serologic Observations**

A few Rh\textsubscript{null} probands were recognized because their serum contained Rh antibodies. Some came to light, however, when routine Rh phenotyping of their red cells revealed the absence of any Rh antigens, and in three cases, the discovery resulted from deliberate testing for Rh antigens in patients with morphologically abnormal red cells and
hemolytic anemia. Immunized Rhnull people have produced antibodies varying in specificity from apparently straightforward anti-e or anti-C to several examples that reacted with all red cells tested except those from other Rhnull people. This antibody, considered to be “anti-total Rh,” has been given the numerical designation anti-Rh29.

**Rhmod**

The Rhmod phenotype represents less complete suppression of Rh gene expression. As for the regulator type of Rhnull, an unlinked recessive modifier gene is thought to be responsible; it has been named Xq. Unlike Rhnull red cells, those classified as Rhmod do not completely lack Rh and LW antigens. Rhmod red cells show much reduced and sometimes varied activity, depending on the Rh system genes the proband possesses and on the potency and specificity of the antisera used in testing. Sometimes the Rh antigens have sufficiently weakened expression that only adsorption-elution techniques will demonstrate their presence. As in Rhnull, hemolytic anemia is a feature of the Rhmod condition. It may be appropriate to think of the two abnormalities as being essentially similar, differing only in degree.

**Rh Antibodies in a Patient’s Serum**

Except for some non-red-cell-stimulated examples of anti-E, anti-Cw, and antibodies to rare low-incidence antigens, most Rh antibodies result from exposure to human red cells through pregnancy or transfusion. D is the most potent immunogen, followed by c and E. Although a few examples of Rh antibodies behave as saline agglutinins, most react best in high-protein, antiglobulin, or enzyme test systems. Even sera containing potent saline-reactive anti-D are usually reactive at higher dilutions in antiglobulin testing. Some workers find enzyme techniques especially useful for detecting weak or developing Rh antibodies.

Detectable antibody usually persists for many years. If serum antibody levels fall below detectable thresholds, subsequent exposure to the antigen characteristically produces a rapid secondary immune response. With exceedingly rare exceptions, Rh antibodies do not bind complement when they combine with their antigens, at least to the extent recognizable by techniques currently used.

**Dosage Effect**

Anti-D seldom shows any difference in reactivity between red cells from individuals homozygous or heterozygous for RHD, but D expression seems to vary somewhat with the accompanying alleles of the genotype. For example, red cells from a cDE/cDE individual carry more D antigen sites than red cells from a CDe/CDe person, and may show higher titration scores with anti-D. Dosage effects can sometimes be demonstrated with some antibodies directed at the E, c and e antigens and occasionally, at the C antigen. Quantifying the antigen sites requires specialized techniques such as radioisotope labeling, flow cytometry, immunoferritin localization, or automated procedures.

**Concomitant Antibodies**

Some Rh antibodies tend to occur in concert. For example, the CDe/CDe person manifesting immune anti-E has almost certainly been exposed to c as well as E. Anti-c may be present in addition to anti-E, although substantially weaker and possibly undetectable at the time the anti-E is found, and transfusion of
seemingly compatible E–c+ blood may elicit an immediate or somewhat delayed hemolytic reaction. Generally, it is not a sound practice to select donor blood negative for all or most of the antigens absent from the recipient’s cells, but some workers feel that the CDe/CDe recipient with detectable anti-E is a case that merits special consideration. Since anti-c occurs frequently with anti-E in immunized people whose red cells are E-negative and c-negative, some select blood of the patient’s own Rh phenotype for transfusion even when the presence of anti-c cannot be demonstrated by test procedures routinely used. Anti-E less consistently accompanies anti-c, as the patient can easily have been exposed to c without being exposed to E. There is little clinical value in pursuing anti-E in a serum known to contain anti-c, as the vast majority of c-negative donor blood will be negative for the E antigen.

**Rh Typing Tests**

Routine Rh typing for donors and patients involves only the D antigen, and techniques to demonstrate weak D are required only for donor blood. Tests for the other Rh antigens are performed only for defined purposes, such as identifying unexpected Rh antibodies, obtaining compatible blood for a patient with an Rh antibody, investigating disputed parentage or other family studies, selecting a panel of phenotyped cells for antibody identification, or evaluating whether a person is likely to be homozygous or heterozygous for RHD.

In finding compatible blood for a recipient with a comparatively weak Rh antibody, tests with potent blood grouping reagents more reliably confirm the absence of antigen than mere demonstration of a compatible crossmatch. Testing the patient’s phenotype may provide confirmation of the antibody specificity and suggest which other Rh antigens could also be present.

**Prudent Use of Resources**

Routine testing for Rh antigens other than D is not recommended. Besides the expenditure of time and money involved, excessive testing wastes scarce resources. Reagent anti-e sera, in particular, should be used sparingly as raw material suitable for manufacture is often scarce. In most cases, red cells negative for the E antigen can be considered e-positive without actual testing. This practice preserves precious supplies of anti-e for cases with specific indications, such as evaluating E-positive blood specimens or investigating a suspected deletion at the E/e sublocus.

**Routine Testing for D**

Until recently, high-protein anti-D reagents of human, polyclonal origin and suitable for slide, tube, or microplate tests were used for most routine testing. More recently, monoclonal anti-D reagents have become widely available. Tests may employ red cells suspended in saline, in serum, or in plasma, but permissible test conditions should be confirmed by reading the manufacturer’s directions before use. Recommended test procedures may vary somewhat among manufacturers.

Slide tests produce optimal results only when a high concentration of red cells and protein are combined at a temperature of 37 C. Because the slide test must be read within 2 minutes, the red cells and serum on the glass slide must reach 37 C quickly. The viewing surface used to perform slide tests should be kept lighted at all times to maintain a temperature of 45-50 C, permitting rapid warming of the test mixture. The slide test has the serious disadvantage that drying of the reaction mixture can
cause the red cells to aggregate, which may be misinterpreted as agglutination. Another disadvantage is the risk that specimens will spill during manipulation. Procedures for microplate tests are similar to those for tube tests but very light suspensions of red cells are used. Representative procedures for tube, slide, and microplate tests are given in Methods 2.6, 2.7, and 2.8. If there is an indication to test for weak D, an antiglobulin test should be performed.

A reliable test for weak D expression cannot be performed on a slide. Red cells with weak D, if they are agglutinated at all on a slide test, will invariably exhibit weaker agglutination than that seen with normal D-positive cells. This distinction may not be recognized, however, especially if the reagent has high potency and avidity, unless normal D-positive cells and weak D cells are tested in parallel and results compared.

**High-Protein Reagents**

Some anti-D reagents designated for use in slide, rapid tube, or microplate tests contain high concentrations of protein (20-24%) and other macromolecular additives. Such reagents are nearly always prepared from pools of human sera and give rapid, reliable results when used in accordance with manufacturers’ directions.

Because the macromolecular medium may cause red cells coated with immunoglobulin to aggregate spontaneously, antisera with these additives may produce false-positive reactions. A false-positive result due to spontaneous aggregation could cause a D-negative patient to receive D-positive blood and become immunized. To detect this kind of spurious results when the reagent has high-protein formulation, the red cells must be simultaneously tested with an immunologically inert reagent, identical in formulation to the anti-D in use but lacking the antibody component. If red cells exhibit aggregation in the control test, the results of the anti-D test cannot be considered valid. In most cases the presence or absence of D can be determined, with other reagents, as detailed later in this chapter.

**The Control for High-Protein Reagents**

High-protein reagents may give false-positive results in several other circumstances. Factors in the patient’s serum may affect the test if the test is performed on unwashed red cells suspended in the patient’s own serum or plasma. Strong autoagglutinins, abnormal serum proteins that promote rouleaux formation, or antibodies directed at an additive in the reagent may cause aggregation that can be mistaken for agglutination by anti-D. The best way to detect possibly invalid reactions is to use, as the immunologically inert control, the diluent used to manufacture the particular anti-D reagent. This material will contain all the additives present in the reagent except for the active antibody. It should, therefore, potentiate spontaneous aggregation to the same degree as the active anti-D.

Manufacturers offer their individual diluent formulations for use as control reagents; if patients’ red cells are typed with high-protein reagents, the tests must be controlled with this material. The nature and concentration of additives differ significantly among reagents from different manufacturers. The control reagent from one manufacturer may not produce the same pattern of false-positive reactions as that of another; tests on patients’ cells must use a control suitable for the testing procedure. Using 22% or 30% bovine albumin detects even fewer false positives, because reagent solutions of bovine albumin lack the other high-molecular-weight potentiators that manufacturers use in high-protein reagents.
Misleading Results with High-Protein Reagents

False Positives. False-positive test results with high-protein reagents include:
1. Cellular aggregation resulting from immunoglobulin coating of the patient’s red cells or serum factors that induce rouleaux will give positive results in both the active and the control tubes. Serum factors can be eliminated by thoroughly washing the red cells (with warm saline if cold agglutinins are present or suspected) and retesting the washed red cells. The original high-protein agent may be used, concurrently with a new control tube, if the manufacturer’s directions state that the product is suitable for use with saline-suspended cells. If the cells in the control test remain unagglutinated and the anti-D test gives a positive result, the red cells are D-positive. If agglutination still occurs in the control tube, the most likely explanation is immunoglobulin coating of the red cells, which may then be tested with a saline-reactive reagent.
2. Rouleaux, simulating agglutination, may occur if red cells and anti-D are incubated together for too long before the test is read. This may occur rapidly, particularly on a warm slide on which evaporation causes drying, which further increases the protein concentration of the reaction mixture. It is important to follow the manufacturer’s recommendation to interpret the test within a limited period, usually no more than 2 minutes.

False Negatives. False-negative test results with high-protein reagents include:
1. Too heavy a red cell suspension in the tube test or too weak a suspension in the slide test may weaken agglutination. To achieve the 40-50% cell suspension required for slide testing, it may be necessary to centrifuge blood from a severely anemic patient and remove some of the plasma before testing the red cells.
2. Saline-suspended red cells may react poorly with some Rh reagents, and must not be used for slide testing.
3. Red cells possessing weakly expressed D antigen may not react well within the 2-minute limit of the slide test or upon immediate centrifugation in the tube test.

Low-Protein Reagents

The low-protein, saline-reactive Rh reagents in current use are formulated predominantly with monoclonal antibodies. Immunoglobulin-coated red cells can usually be successfully typed with low-protein Rh reagents that contain saline-agglutinating antibodies and no additives that promote spontaneous cellular aggregation. If such additives are present, the fact should be stated in the “Reagent Description” section of the package insert.

Monoclonal Source Anti-D

Monoclonal anti-D reagents are made predominantly from human IgM antibodies, which require no potentiators and agglutinate most D-positive red cells from adults and infants in a saline system. Monoclonal anti-D reagents usually promote reactions stronger than those with polyclonal IgG reagents, but they may fail to agglutinate red cells of some partial-D categories. Adding small amounts of polyclonal anti-D to the monoclonal antibodies provides a reagent that will react with partial-D red cells in antiglobulin tests. Just the right
amount of polyclonal material must be added. If the reagent contains too much polyclonal material, IgG molecules may attach to the D sites and block the reactivity of the monoclonal IgM component. If there is too little polyclonal anti-D, the reagent may fail to detect partial D.

Licensed monoclonal/polyclonal blends can be used in slide, tube, or microplate tests, and are as satisfactory as high-protein or chemically modified reagents in antiglobulin tests for weak D. False-negative findings can result, however, if tests using monoclonal reagents are incubated in excess of a manufacturer’s product directions. These reagents, prepared in a low-protein medium, can be used to test red cells with a positive DAT, provided those tests are not subjected to antiglobulin testing.

Control for Low-Protein Reagents

Most monoclonal/polyclonal blended reagents have a total protein concentration approximating that of human serum. False-positive reactions due to spontaneous aggregation of immunoglobulin-coated red cells occur no more often with this kind of reagent than with other saline-reactive reagents. False-positive reactions may occur in any saline-reactive test system if the serum contains cold autoagglutinins or a protein imbalance causing rouleaux and the red cells are tested unwashed. It is seldom necessary to perform a separate control test, however. Absence of spontaneous aggregation can usually be demonstrated by observing absence of agglutination by anti-A and/or anti-B in the cell tests for ABO. For red cell specimens that show agglutination in all tubes (ie, give the reactions of group AB, D-positive), a concurrent control must be performed on patients’ cells; this is not required when donors’ cells are tested. A suitable control is to centrifuge a suspension of the patient’s red cells with autologous serum or with 6-8% bovine albumin at the same time the anti-D test is centrifuged. If the test is one of several requiring incubation before centrifugation, any negative result on a test performed concurrently serves as an adequate control. A separate control tube would be required only for a red cell specimen that gives positive reactions with all the Rh reagents, ie, is typed as D+C+E+c+e+.

Control for Medium-Protein Reagents

Some manufacturers offer reagents containing chemically modified IgG in a diluent with a protein concentration between that of human serum and that of high-protein reagents. This type of reagent should not be confused with those formulated in a low-protein diluent, because at least some immunoglobulin-coated cells may experience spontaneous aggregation in the higher protein concentration. The incidence of false-positive reactions is not as high as with high-protein reagents, but for patients’ specimens, a suitable parallel control is needed. The need for a control will be stated in the manufacturer’s instructions.

Testing for D in Hemolytic Disease of the Newborn

Because red cells from an infant suffering from HDN are coated with immunoglobulin, a saline-reactive reagent is usually necessary for Rh testing. Occasionally, the infant’s red cells may be so heavily coated with antibody that all antigen sites are occupied, leaving none available to react with a saline-reactive antibody of appropriate specificity. This “blocking” phenomenon should be suspected if the infant’s cells have a strongly positive DAT, and are not agglutinated by
a saline-reactive reagent of the same specificity as the maternal antibody.

Anti-D is the specificity responsible for nearly all cases of blocking by maternal antibody. It is usually possible to obtain correct typing results with a saline anti-D after 45°C elution of the maternal antibody from the cord red cells. (See Method 2.14.) Elution liberates enough antigen sites to permit red cell typing, but must be performed cautiously because overexposure to heat may denature or destroy Rh receptors.

Tests for Antigens Other Than D

Reagents are readily available to test for the other principal Rh antigens: C, E, c, and e. These are formulated as either low-protein (chemically modified or monoclonal) or high-protein reagents. High-protein reagents of any specificity have the same problems with false-positive results as high-protein anti-D and require a comparable control test. Observation of a negative result in the control test for anti-D does not determine the tests for other Rh antigens, because results with anti-D are usually obtained after immediate centrifugation and tests for the other Rh antigens are generally incubated at 37°C before centrifugation. A valid control procedure must be performed concurrently with the test, using the same duration and conditions of incubation, and be interpreted simultaneously with the actual test.

Rh reagents may give weak or negative reactions with red cells possessing variant antigens. This is especially likely to happen if anti-e is used to test the red cells from Blacks, among whom variants of e are relatively common. It is impossible to obtain anti-e reagents that react strongly and consistently with the various qualitative and quantitative variants of e. Variable reactivity with anti-C reagents may occur if the CDE or CE haplotypes are responsible for the expression of C on red cells. Variant E and c antigens have been reported but are considerably less common.

Whatever reagents are used, the manufacturer's directions must be carefully followed. The indirect antiglobulin technique must not be used unless the manufacturer's instructions state explicitly that the reagent is suitable for this use. The pools of human sera used to prepare reagents for the other Rh antigens have a significant risk of containing antiglobulin-reactive, contaminating specificities. Positive and negative controls should be tested in parallel with the red cells under study. Red cells selected for the positive control should be known to have a single dose of the antigen concerned or be known to show weak reactivity with the reagent. If these reagents are used regularly, they should be included in the routine quality assurance program.

Additional Considerations in Rh Testing

The following limitations are common to all Rh typing procedures, including those performed with high-protein reagents.

False-Positive Reactions

The following circumstances can produce false-positive red cell typing results.

1. The wrong reagent was inadvertently used.
2. An unsuspected antibody of another specificity was present in the reagent. Antibodies for antigens having an incidence of less than 1% in the population may occasionally be present and cause false-positive reactions, even when the manufacturer's directions are followed. Though the antigens occur infrequently, antibodies to them are com-
paratively common, even in persons with no history of pregnancy or transfusions.

For crucial determinations, many workers routinely perform replicate tests, using reagents from different sources. This reduces the likelihood of a false classification, because discrepant reactions would alert the serologist to the need for further testing. Replicate testing is not an absolute safeguard, however, because reagents from different manufacturers may not necessarily derive from different sources. The scarcity of donors or of clones producing acceptable titers of such relatively uncommon specificities as anti-C and anti-e, in particular, may cause the same source of raw material to be used by several manufacturers. Different manufacturers' reagents prepared from the same polyclonal source material may, in fact, contain the same contaminating antibody.

3. Polyagglutinable red cells may be agglutinated by any reagent containing human serum. Although antibodies that agglutinate these surface-altered red cells are present in most adult human sera, polyagglutinins very rarely cause problems with reagents. Aging, dilution, and various steps in the manufacturing process tend to eliminate these predominantly IgM antibodies.

4. Autoagglutinins and abnormal proteins in the patient’s serum may cause false-positive reactions when unwashed red cells are tested.

5. Reagent vials may become contaminated with bacteria, with foreign substances, or with reagent from another vial. This can be prevented by the use of careful technique and the periodic inspection of the vials’ contents. Bacterial contamination may not, however, cause recognizable turbidity because the refractive index of bacteria is similar to that of high-protein reagents.

False-Negative Reactions

The following circumstances can produce false-negative red cell typing results.

1. The wrong reagent was inadvertently used.

2. The reagent was not added to the tube, either because of oversight or because the drops of fluid ran down the outside of the tube. It is good practice to add serum to all the tubes before adding the red cells and any enhancement media.

3. A specific reagent failed to react with a variant form of the antigen.

4. A reagent that contains antibody directed predominantly at a cis-product Rh antigen failed to give a reliably detectable reaction with red cells carrying the individual antigens as separate gene products. This occurs most often with anti-C sera.

5. The reagent was used incorrectly because the manufacturer’s directions were not followed.

6. The red cell button was shaken so roughly during resuspension that small agglutinates were dispersed.

7. Contamination, improper storage, or outdated cause antibody activity to deteriorate. Chemically modified IgG antibody appears to be particularly susceptible to destruction by proteolytic enzymes produced by certain bacteria.

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