Recent improvements in gene chip technology now permit rapid identification of common partial D and weak D genotypes.

It is important to understand the consequences of weak D for blood collection and transfusion services. As noted in Chapter 3, all blood donors who initially type as Rh negative by direct agglutination are screened for weak D expression by the indirect antiglobulin test (IAT). Blood donors who are positive for D antigen, regardless of the strength of D expression, are considered Rh positive for red cell donation. In contrast, patients who initially type as Rh negative by direct agglutination are not tested for weak D and are considered Rh negative for blood transfusion.

In female patients, there are additional precautions because of the potential risk of hemolytic disease of the fetus and newborn (HDFN) if these patients express partial D and thus are at risk for making anti-D. Some centers treat women with weak D, even in direct agglutination testing, as Rh negative for transfusion and pregnancy. To identify at-risk women and avoid overutilization of Rh Immune Globulin (RhIG), a recent joint task force has recommended RHD genotyping in all obstetric patients with weak D expression. Women with weak D types 1, 2, and 3 can be managed as Rh positive.

Women with partial D should be considered Rh negative for transfusion and given RhIG prophylaxis to prevent HDFN due to allo-anti-D. Women with weak D types 1, 2, and 3 can be managed as Rh positive.

C/c, E/e, and Compound Antigens

The C/c and E/e antigens are the result of single amino-acid polymorphisms on the RhCE protein. The C/c antigen resides at amino acid 103 on the second extracellular loop, where C is Ser103 and c is Pro103. The E/e antigen resides at amino acid 226 on the fourth extracellular loop, where E is Pro226 and e is Ala226. Because C/c and E/e antigens reside on the same protein, they can stimulate alloantibodies that require the presence of both C/c and E/e antigens on the same protein for antibody recognition. Four "compound antigen" specificities are recognized: ce, or f (RH6); Ce (RH7); cE (RH27); and CE (RH22). As shown in Table 5-4, those antigens are associated with specific Rh haplotypes. Anti-f is the most common compound alloantibody encountered clinically, particularly among R_1R_2 individuals (*DCe/DcE*).

Table 5-4. Compound Rh Antigens

Compound Antigen	Name	Rh Haplotypes		Prevalence (%) in Whites
		Fisher-Race	Wiener	
ce	f or RH6	ce + Dce	$r + R_0$	41
Ce	RH7	Ce + DCe	$r' + R_1$	44
cE	RH27	cE + DcE	$r'' + R_2$	15
CE	RH22	CE + DCE	$r^y + R_Z$	0.2

G Antigen

G is an Rh antigen present on D-positive cells and C-positive cells. The G antigen epitope is a C-active antigen (Ser103) shared by both RhD and RhCE proteins. After transfusion of C-positive blood, Rh-negative patients can make an anti-G that reacts like a mixture of anti-C and anti-D in laboratory testing. In fact, many examples of anti-G are accompanied by anti-C. Like other Rh antibodies, anti-G is clinically significant because it is capable of causing hemolytic transfusion reactions (HTRs) and HDFN. For transfusion purposes, it is not necessary to discern whether a patient has an anti-C with anti-D, or an anti-G. In both instances, the patient should receive Rh-negative blood (rr, or *ce*/*ce*), which essentially lacks D, C, and G antigens.

Rh-negative women with anti-G (without anti-D) are still at risk for making anti-D and should receive RhIG prophylaxis during pregnancy.

In prenatal samples, however, it is important to investigate and distinguish between an anti-G and an anti-D with anti-C. A patient with an anti-G or an anti-G and anti-C mixture may still become immunized to D antigen. To prevent sensitization to D antigen, patients with anti-G (without anti-D) should receive Rh immunoprophylaxis. In contrast, patients with a true anti-D should not receive RhIG because they are already sensitized to D (see Chapter 11). One clue to help discriminate between an anti-G vs an anti-D with anti-C is the antibody titer against D-positive and C-positive red cells. Anti-G has a stronger reaction with C-positive red cells in antibody titration studies, with anti-C titers at least four times higher than anti-D titers. That reaction is the opposite of an anti-D with anti-C, where anti-D titers should be significantly higher than anti-C. However, definite distinction between anti-G and anti C + anti-D requires serial

adsorptions and elution testing, which may be available only at reference laboratories.

Rh_{null}

Rh_{null} is a very rare (<1:6,000,000) autosomal recessive phenotype characterized by the complete absence of RhD and RhCE proteins on red cells. The absence of Rh proteins is accompanied by an absence of or decrease in other antigens (Fy5, LW, S/s/U). Rh_{null} red cells are abnormal; they possess a stomatocyte red cell morphology, increased osmotic fragility, and shortened red cell survival. As a result, Rh_{null} individuals have a chronic, mild hemolytic anemia (Rh-null syndrome). Rh_{null} individuals can make alloantibodies to several high-prevalence antigens on RhD and RhCE proteins, and these are reactive with all red cells except Rh_{null}.

The Rh_{null} phenotype arises from two genetic backgrounds—regulator or amorph (see Table 5-1). The Rh_{null} amorph type occurs in Rh-negative (D-negative) individuals who have inherited a nonfunctional RHCE gene. In the Rh_{null} amorph phenotype, RhAG protein is still present, although decreased relative to normal red cells. In contrast, Rh_{null} regulator is the result of mutations in the RHAG gene, thereby leading to a loss of RhD, RhCE, and RhAG proteins on red cells. Rh_{mod} is related to Rh_{null} regulator and is the result of nonlethal missense mutations in RhAG protein. Rh_{mod} red cells have markedly decreased RhD, RhCE, and RhAG proteins, as well as a mild, chronic hemolytic anemia.

Rh Antibodies

Alloantibodies against Rh antigens are of immunoglobulin G (IgG) isotype and are always clinically significant. They are capable of causing HTRs and severe HDFN. Rh antibodies are usually associated with extravascular hemolysis, although there are cases of acute intravascular hemolysis, especially during initial sensitization. Antibodies against Rh antigens are almost always the result of immune stimulation by transfusion or pregnancy; exceptions are anti-E and anti-C^w, which can sometimes be naturally occurring. For transfusion, antigen-negative, crossmatch-compatible red cells should be provided to patients alloimmunized to Rh-family antigens.

Rh antibodies react at 37 C and IAT, especially by gel method. Antibody reactivity is enhanced with ficin-treated cells and double-dose (homozygous) cells.

In laboratory testing, Rh antibodies are reactive at 37 C and in an IAT (see Chapter 3). Rh antibodies can also

demonstrate dosage (the dosage effect) by reacting more strongly with double-dose, or homozygous, cells. Antibody reactivity is enhanced by the protease digestion of red cells, which unmasks the Rh antigens (Table 3-4). In addition to alloantibodies, autoantibodies with apparent Rh specificity are not uncommon, particularly autoanti-e.

Clinically, D is the most immunogenic Rh antigen, followed by c, E, C, and e. Because D is so immunogenic, standard practice is to provide Rh-negative RBCs to all Rh-negative patients whenever possible (except in bleeding emergencies; see Chapter 12). This is particularly important for females of childbearing potential to avoid the risk of Rh-associated HDFN. Rh-negative women can also become sensitized during pregnancy or an event that may involve fetomaternal hemorrhage from exposure to Rh-positive fetal red cells. To prevent anti-D formation, in addition to provision after events that may involve fetomaternal hemorrhage, Rh-negative women are given Rh Immune Globulin prophylaxis during midpregnancy and immediately after delivery or a predisposing event (see Chapter 11). As discussed earlier in the section on G antigen, Rh Immune Globulin is not given to females who have already formed anti-D.

Kell and Kx Blood Group Systems

Kell is specific to red cells and is highly immunogenic. Kell antigens are destroyed by treating red cells with DTT, 2-ME, or ZZAP, which destroy disulfide bonds.

Kell is a large single-pass glycoprotein on red cells and erythroid progenitors. It is covalently linked to another red cell protein, XK, or to Kx antigen, which may help stabilize the Kell protein. Kell antigens are highly immunogenic and are second only to RhD in their ability to stimulate alloantibodies. The Kell system (KEL) has 36 antigens, including seven sets of antithetical antigens and 23 high-prevalence antigens. The most significant antigen pair is K (Kell, or K1) and k (cellano, or K2). Both are highly immunogenic. K is present in 10% of the population, whereas k is a high-prevalence antigen on 99% of donor red cells. $Kp^a/Kp^b/Kp^c$ and Js^a/Js^b are two additional antithetical antigen sets included on antibody identification panels (see Table 5-5). It is important to note that Js^a is significantly more prevalent in people of African ethnicity (20%) than in those of European ethnicity (<1%). Kell antigens can be targets for autoantibodies with suppression of Kell expression.