Blood Group Antibodies and Their Significance in Transfusion Medicine

Joyce Poole and Geoff Daniels

The discovery of almost universally present naturally occurring antibodies in blood plasma led to the discovery of the ABO blood group system which remains, more than 100 years later, the most important and clinically significant of all blood groups. Blood group antibodies play an important role in transfusion medicine, both in relation to the practice of blood transfusion and in pregnancy, but not all are clinically significant. Clinically significant antibodies are capable of causing adverse events following transfusion, ranging from mild to severe, and of causing hemolytic disease of the fetus and newborn following placental transfer from mother to fetus. Assessing the clinical significance of antibodies relies heavily on mode of reactivity and historical data relating to specificity; functional assays are sometimes employed. The principals of methodology for blood typing and antibody identification have changed little over the years, relying mainly on serological methods involving red cell agglutination. The recent advent of blood typing using DNA technology, although still in relative infancy, will surely eventually supersede serology. However, deciding on the clinical significance of an antibody when compatible blood is not immediately available is likely to remain as one of the most common dilemmas facing transfusion practitioners.

The observation by Landsteiner in 1900 that blood plasma from some individuals will agglutinate red cells from some others led to the discovery of the ABO blood groups, which is probably the most important factor in making blood transfusion a safe clinical practice. Following the identification of the A and B blood group antigens, blood group serology blossomed throughout the 20th century, such that the International Society of Blood Transfusion now recognizes 302 blood group antigens, most of which belong to 1 of 29 genetically discrete blood group systems. Antibodies to many of these 302 antigens have the potential to be clinically significant; that is, they can facilitate accelerated destruction of red cells carrying the corresponding antigen.

The pathological effects of blood group antibodies can be summarized as follows:

- Destruction of allogeneic red cells. This usually manifests itself as a hemolytic transfusion reaction (HTR) and occurs as the result of a red cell transfusion. The severity of the reaction can vary from mild, with reduced efficacy of the transfusion therapy, to extremely severe causing rapid death of the recipient. Morbidity caused by antibody-induced red cell destruction can also result from red cells introduced with other blood components, such as platelets, fresh frozen plasma, or with a bone marrow allograft.
- Destruction of autologous red cells. Production of red cell autoantibodies can lead to autoimmune hemolytic anemia. The specificities of blood group autoantibodies are often more broadly reactive than those of alloantibodies. Autologous hemolysis can also occur following transplantation of an allograft as a consequence of the activity of donor lymphocytes producing antibodies to antigens on the red cells of the immunosuppressed recipient, but not on the red cells of the donor. These topics will not be covered further in this review.
- Destruction of fetal red cells. Maternal blood group antibodies may cross the placenta and facilitate immune destruction of fetal red cells or erythroid precursors to give rise to the condition broadly known as hemolytic disease of the fetus and newborn (HDFN).
- Damage of transplanted tissue. Many blood group antigens are present in tissues other than blood and blood group antibodies can facilitate immune damage to transplanted tissues, often leading to rejection of the allograft. This topic will not be covered in this review.
HEMOLYTIC TRANSFUSION REACTIONS

Intravascular HTRs

Intravascular HTRs are characterised by antibody-mediated hemolysis within the circulation and liberation of hemoglobin into the plasma. Hemolysis is rapid, with most of the cells being destroyed within 10 minutes. Intravascular HTRs are caused by immunoglobulin M (IgM) antibodies, which activate the complete classical complement pathway through to the formation of the membrane attack complex and the puncturing of the red cell membrane. The usual signs are chills, shock, hypotension, hemoglobinemia, and hemoglobinuria, with additional complications of disseminated intravascular coagulation and renal failure. The fatality rate associated with intravascular HTRs is generally considered to be approximately 10%.1 The antibodies that most commonly cause intravascular HTRs are those of the ABO system (anti-A, -B, -A,B), although anti-PP1Pk, -Vel, Lewis, and Kidd antibodies have been implicated on rare occasions.

Extravascular HTRs

Most HTRs that do not involve the ABO system are extravascular reactions. They generally involve immunoglobulin G (IgG) antibodies that do not bind complement or bind insufficient C3 to initiate the hemolytic pathway. Extravascular HTRs may be immediate (occurring within a few hours of transfusion) or delayed (occurring within a few days of transfusion). The main signs of extravascular HTRs are similar to those associated with intravascular reactions, but are generally less severe. Disseminated intravascular coagulation and renal failure are rare and the fatality rate is much lower. Although hemoglobinemia and hemoglobinuria may occur, presumably because of leakage of hemoglobin from red cells destroyed by macrophages in the liver and spleen, hyperbilirubinemia is a more common characteristic. Extravascular HTRs may be caused by IgG antibodies (IgG3 and IgG1), typically of the Rh system, that do not activate complement. Red cells coated with IgG become attached to the Fc receptors (FcγR) on macrophages, mostly in the spleen, and become phagocytosed. Alternatively, IgG antibodies such as those of the Kidd and Duffy systems, may cause C3 binding to the transfused red cells, but in insufficient quantity for significant extravascular hemolysis. Red cells coated with IgG and C3b will be mainly sequestered by macrophages in the liver, but splenic macrophages may also be involved. In some cases, an intravascular HTR and an immediate extravascular reaction may occur concurrently.

Immediate or acute extravascular HTRs occur either during the transfusion or within a few hours.1,2 In contrast to delayed reactions, immediate extravascular HTRs almost always occur when an antibody was serologically detectable in the plasma of the recipient before the transfusion and, therefore, are usually avoidable.

Delayed HTRs

Delayed HTRs usually occur in patients who have previously been immunized to the offending antigen, but in whom antibody levels have dropped to a level too low to cause significant intravascular or extravascular hemolysis and often to a level too low to be detected serologically. Consequently, transfusion of red cells expressing that antigen will initiate a secondary or anamnestic response which, after a period of several days, will initiate clearance of the transfused red cells. Typically the HTR occurs approximately 5 to 7 days after the transfusion, but in extreme cases the reaction may be as early as 3 days and as late as 14 to 23 days.1,2 Clinical features often involve fever, fall in hemoglobin level, jaundice, and hemoglobinuria; renal failure is rare.1 It is common for the presence of transfused red cells to be undetectable 2 weeks after transfusion. Delayed reactions that are only detectable by serological tests, especially a positive direct antiglobulin test, and cause no significant morbidity, may be referred to as delayed serological transfusion reactions. Antibodies most

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Total</th>
<th>Acute HTR</th>
<th>Delayed HTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death definitely attributed to transfusion</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Death probably attributed to transfusion</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Death possibly attributed to transfusion</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Death due to underlying condition</td>
<td>23</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Major morbidity</td>
<td>49</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Minor or no morbidity</td>
<td>373</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>457</td>
<td>39</td>
<td>25</td>
</tr>
</tbody>
</table>
frequently involved in delayed HTRs are those of the Rh, Kidd, Duffy, and Kell systems, though other blood group antibodies are occasionally implicated.\(^1\)

**Incidence of HTRs**

The Serious Hazards of Transfusion (SHOT) report in the United Kingdom showed that of 2087 reported adverse events between 1996 and 2003, 233 (11.2\%) were acute HTRs and 213 (10.2\%) were delayed HTRs (http://www.shot-uk.org). Table 1 shows the outcomes of 457 adverse events in 2003 and of 64 transfusion reactions in that year. For a 10-year period (1976-1985) in the United States, Sazama\(^3\) reported that 158 (44.5\%) of 355 transfusion-related fatalities were caused by acute hemolyis, of which 131 (82.9\%) were due to ABO incompatibility. In the same period, 26 fatal- ities were attributed to delayed hemolysis, but analysis of the antibodies deemed to be responsible suggests that this figure is not completely valid.

**HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN**

Hemolytic disease of the fetus and newborn is caused by blood group antibodies in pregnant women which cross the placenta into the fetus where they initiate immune destruction of fetal erythroid cells. It is restricted to IgG (mostly IgG1 and IgG3), as antibodies of other classes are not transported across the placental barrier. Immunoglobulin G–coated fetal and neonatal red cells are probably lysed by splenic macrophages, giving rise to hyperbilirubinemia and anemia.\(^4\) In blood group antigens, such as K (KEL1), that are expressed at an early stage of erythroid development, it is feasible that monocytes in the fetal liver may destroy early erythroid progenitors, in which case anemia rather than hyperbilirubinemia is the predominant symptom.\(^5,6\)

Hemolytic disease of the fetus and newborn at its most severe may cause fetal death at about the 17th week of pregnancy. If the infant is born alive, the disease can result in hydrops and jaundice, which can develop to kernicterus and infant death or permanent cerebral damage. At its mildest, neonatal jaundice, often treated with phototherapy, is the only clinical sign of HDFN. For the purposes of this review, an antibody is only deemed to have caused clinically significant HDFN if at least neonatal blood transfusion was considered neces-

**ALLOIMMUNIZATION TO BLOOD GROUP ANTIGENS**

Anti-A, -B, and the cross-reacting antibody anti-A,B are often referred to as naturally occurring. Almost without exception, adults lacking A or B or both make IgM antibodies to the corresponding antigens. A few other “naturally occurring” blood group antibodies exist regularly, eg, anti-H and -PP1P\(^\ast\) in rare Bombay (O\(_h\)) and p phenotype individuals, respectively. However, most other clinically significant antibodies to red cell surface antigens are IgG and produced in response to immunization by antigen-positive red cells: either donor red cells following transfusion, or cells of fetal origin, following fetomaternal hemorrhage during pregnancy or at parturition.

The D-negative phenotype of the Rh system results from an absence of the RhD protein rather than from the minor modifications of cell-surface protein that are usually associated with blood group polymorphism. Consequently, D is the most immunogenic and clinically important blood group antigen after the ABO antigens. In healthy volunteer studies, approximately 85\% of D-negative individuals infused with 200 mL or more of D-positive red cells made anti-D.\(^1\) A recent study reported that formation of anti-D following transfusion of D-positive blood to D-negative patients was lower than this and estimated to be 30.4\%.\(^7\) The prevalence of anti-D has been reduced dramatically over the last 3 decades by administration of anti-D immunoglobulin to pregnant D-negative women to prevent sensitization by D-positive fetal cells.

From an analysis of published reports, Issitt and Anstee\(^2\) estimate that approximately 1\% to 1.5\% of patients will present with 1 or more clinically significant blood group antibodies. The figure for healthy donors is much lower, at around 0.2\%. In a prospective study of the incidence of red cell alloimmunization following transfusion, 8.4\% (38/452) of patients developed antibodies within 24 weeks of transfusion.\(^8\) Specificity within the Rh system was present in 76\% of these. The higher incidence in this study was presumed to be due to the testing of serial posttransfusion samples compared to only one test in previous studies. The
most commonly encountered immune antibodies are D > K > E > Fya > Jka.1,2

Antibodies in Transfusion-Dependent Patients

Transfusion-dependent patients are those who require frequent and long-term transfusion support to sustain their life and include some patients with sickle cell disease (SCD), severe thalassemia syndromes, severe aplastic anemia, myelodysplastic syndromes, and other congenital or acquired chronic anemias. The frequency of alloimmunization in transfusion-dependent patients is higher than in other patient groups and is highest in SCD. Blood transfusion in SCD is used as a life-saving measure and for secondary prevention of strokes and other complications. Alloimmunization is reported with a varying incidence from 8% to 76% in multiply transfused patients, generally tending to increase with transfusion load and age. A comparison of the rates of alloimmunization of SCD patients in Manchester, UK, and Jamaica showed a significantly higher incidence in UK patients, 76% of those transfused compared to 2.6% in Jamaicans. Multiple antibody production occurred in 63% of UK patients and in none of those from Jamaica. The overall percentage of patients receiving transfusion did not differ between the communities. The higher incidence of alloimmunization among UK patients was considered to be due to the greater number of units of blood received by each patient in the UK and the disparity between donor (predominantly white) and SCD recipient (predominantly black) populations in the UK. The Dce S- K- Fy(a-b-) Jk(b-) phenotype is commonly found in blacks and is exceptionally rarely in whites, and antibodies to C, E, S, K, Fya, and Jkb are among those most frequently made, the highest incidence being C, E, and K. Several studies have reached the same conclusion that the high incidence of alloimmunization in SCD patients is due to racial differences between donor and recipient phenotypes and that the risk of alloimmunization can be reduced by performing extended red cell phenotyping prior to initiating a transfusion regimen. If the patient is already receiving transfusions, and autoantibodies or multiple alloantibodies are present, molecular genotyping can overcome the problem of phenotyping in the presence of transfused cells. Many centres, including the National Blood Service (NBS) in England, recommend the use of D, C, E, c, e, and K-matched blood for SCD patients. Transfusion in some SCD patients is further complicated by a delayed HTR causing acute life-threatening anemia termed hyperhemolysis syndrome by some investigators.10,11 Autologous as well as allogeneic red cells appear to be destroyed during the reaction.12 Continuation of blood transfusion can further exacerbate hemolysis even when phenotypically matched or cross-match compatible blood is used. It has been reported by several workers that provision of antigen-matched and cross-match compatible blood does not always prevent the development of serious delayed HTRs.13,14 A high incidence of autoantibody formation in SCD adds to the cross-matching and transfusion problems.

DETECTION AND IDENTIFICATION OF ANTIBODIES

Antibody detection and identification are fundamental to transfusion practice and provide information which aids in the selection of suitable blood for transfusion. Red cell antibodies are detected in serological tests and certain characteristics can be an indication of their clinical significance: antibody strength, mode of reactivity (notably the indirect antiglobulin test [IAT]), thermal range, specificity, immunoglobulin class, IgG subclass, and affinity and ability to bind complement. Perhaps the most important of these is thermal amplitude since, if the antibody does not react at 37°C, it should cause no significant in vivo red cell destruction and no immediate clinical effects due to an immune reaction. Other factors that can influence the pathogenicity of an antibody are the quantity and distribution of target antigen on the red cell membrane, the quantity of IgG and/or complement bound to the red cell, and the presence of target antigen in tissues and/or body fluids. Although it is often easy to predict clinical significance by evaluating the serology, these tests do not always distinguish between clinically significant and clinically benign antibodies. In some circumstances, antibody identification can be a difficult and time-consuming process and may cause a delay in patient care. There are guidelines for antibody detection and ABO and D typing,15 but very little guidance for the procedures necessary for antibody identification.

When a positive antibody screen is encountered, there are many pathways that can be followed and
each laboratory should have a policy outlining its procedures. It is important that a systematic approach is taken in assigning specificities and in excluding the possibility of the presence of additional antibodies. Although more sophisticated techniques may be necessary for some antibodies it is important that routine techniques are not oversensitive. The most likely situations that cause problems in the identification process are a complex mixture of antibodies within routine systems, ie, Rh, Kell, Kidd, Duffy, MNS, Le, Lu, P, or an antibody to a high-incidence antigen with or without underlying antibodies. In both of these cases, all, or at least the majority, of red cell samples matched against the serum are positive and compatible blood is difficult to find.

It is useful to know the ethnic background of a patient because some rare phenotypes are found almost exclusively in certain populations, eg, In(b-) in Asians and S-s-U-, Js(b-), hr²⁻ in blacks. A white patient is more likely to have an antibody to high-incidence antigens such as k, Kp, Yt, Vel, Co, and Lu. The clinical history of the patient and previous serological findings are useful information. Knowledge about the expected phases of reactivity for different antibodies will be a guide to certain specificities. Strength of reactivity is often a clue to what type of antibody is present and a very strongly reacting antibody is more likely to be clinically significant. When reactivity is generally weak the negative cells might not lack the antigen but have weaker expression of it, eg, Ch, Rg, Cs, Kn/McCa (complement receptor 1 [CR1]-related). Some antibodies react more weakly with cells carrying a single dose of antigen—notably M, N, S, s, Jk, and Jk. Hemolysis of test red cells, which may be partial or total, occurs with some antibodies, eg, Le, Jk, PP1P, Vel, H (made in O and must be noted as a positive reaction. These factors should be considered as clues and careful observation of them can aid in the antibody identification process.

**BIOASSAYS TO PREDICT THE CLINICAL SIGNIFICANCE OF ANTIBODIES**

The immunological mechanism of extracellular destruction of antibody-sensitised red cells is by phagocytosis and/or lysis by the mononuclear phagocytic cells of the spleen or the Kupffer cells of the liver. These cells have specific receptors for IgG1, IgG2, IgG3, and the C3 component of complement. Macrophages are capable of destroying red cells following attachment of the sensitized red cells to the IgG(Fc) and C3 (CR1 and CR3) receptors on the macrophage. In vitro cellular bioassays were developed to simulate these interactions and are carried out by mixing antibody-coated cells with peripheral blood Fc receptor-bearing cells, usually monocytes, incubating them and assessing different stages of the interaction. The interactions are as follows: (1) adherence as measured by the rosette assay and phagocytosis (and usually also adherence) by the monocyte monolayer assay (MMA) in which evaluation takes place on a stained slide; (2) extracellular lysis by the antibody-dependent cellular cytotoxicity (ADCC) assay, using either lymphocytes (L) or monocytes (M), in which red cells are labelled with ⁵¹Cr, the lysis being proportional to the amount of radiation recovered in the supernatant; (3) the metabolic response of monocytes during erythrophagocytosis in the chemiluminescence test (CLT) in which the amount of luminescence produced by luminol, in the presence of oxygen radicals, is measured.

Distinguishing between potentially clinically significant and nonclinically significant antibodies is important in alloimmunized women to predict the severity of HDFN and in patients with alloantibodies who require blood transfusion when compatible donors are not available. Although some specificities are usually clinically significant, eg, those to antigens in the Rh, Kell, Kidd, and Duffy systems, others have variable clinical significance, eg, anti-Yt, -Ge, and -Lu. Blood lacking Yt and Ge antigens, and to a lesser extent Lu, is not readily available, and it is important that it is not used inappropriately. It is therefore important to try to establish which patients require antigen-negative blood, and bioassays are employed to this end. Other applications are for patients with suspected autoimmune hemolytic anemia and investigating in vitro mechanisms of red cell interactions with FcR-bearing cells.

**Clinical Bioassays for Predicting the Severity of HDFN**

The clinical management of potential HDFN relies on the measurement of antibody levels in the maternal circulation. Antibody quantitation by autoanaylsers is generally accepted to have a greater predictive value for the severity of HDFN than
serological titrations. If antibody quantification levels are high (10-15 IU/mL), amniocentesis and percutaneous umbilical cord blood sampling may be performed to assess the extent of fetal hemolysis more accurately. However, these are invasive procedures which have associated risks including transplacental haemorrhage leading to stimulation of maternal antibody levels and a worsening of fetal anemia. Although inconvenient to use on a routine basis, cellular bioassays can be a better predictor of severity of outcome than antibody quantitation. The ADCC, MMA, and CLT have all been used for predicting the severity of HDFN. Monocyte monolayer assays have been shown to distinguish between mildly and severely affected infants\(^{16,17}\) and to be more reliable than amniotic fluid measurement in predicting the need for treatment.\(^{18}\) Despite these observations, several studies have failed to establish a correlation between MMA results and HDFN severity or show advantage over antibody titration. Garner et al\(^{19}\) in a study of 44 D-negative women with D-positive infants which included roughly equal numbers of unaffected, moderately affected, severely, and very severely affected infants found the ADCC (M) correctly predicted severity in 39 cases, the autoanalyser in 35, and the MMA in 32 cases. The better predictive value of ADCC compared to MMA was considered to be due to measurement of bound IgG3 to the target antigen by the MMA rather than IgG1 by the ADCC. In another study the ADCC (M) predicted severe disease in 75% of cases compared to only 15% by IAT titre.\(^{20}\) In a small series using the ADCC (L),\(^{21}\) of 10 women, all of whom had anti-D concentrations of 20 IU/mL or more, 3 had low assay values and their infants were the only ones who did not require exchange transfusion.

Several studies have shown that the results of the CLT have good predictions of severity of HDFN. In one study,\(^{22}\) the CLT was shown to be a better predictor of elevated bilirubin levels in amniotic fluid than the autoanalyser (\(P < .01\)). Results of >30% were always associated with elevated bilirubin levels, indicative of significant fetal hemolysis. The authors concluded that the CLT might be used to prompt the direct evaluation of fetal hemolysis in patients with borderline levels of anti-D (5-15 IU/mL), but the ability of the test to predict normal bilirubin levels in amniotic fluid was less clear.

**Clinical Bioassays in Patients Requiring Transfusion**

An in vivo test for establishing the survival of transfused incompatible red cells, when all normal donor red cells are incompatible, was published by the International Committee for Standardization in Hematology\(^{23}\) in 1971 and updated in 1980. The test involves labelling a small aliquot (eg, 0.5 mL) of incompatible red cells with \(^{51}\)Cr in vitro and injecting it into the patient. Samples are taken at intervals up to 60 minutes and the radioactivity measured in the plasma and on the red cells. When the amount of radioactivity in the plasma does not correspond to more than 3% of the radioactivity injected and the red cell survival at 60 minutes is not less than 70% it was deduced that the concentration of antibody is low and that destruction of a large volume of incompatible red cells will be either negligible or take place only slowly. Despite being the standard test for many years this test is not easy to procure and is not commonly used. In vitro assays, although not in themselves widely used, are done in preference.

Although the ADCC, MMA, and CLT have been used for predicting the severity of HDFN the MMA has been most extensively used for predicting the outcome of transfusion of incompatible red cells; the CLT has been used more recently by other workers. In the original validation report on the MMA\(^{24}\) there was a 100% correlation between the MMA and \(^{51}\)Cr survival studies or clinical outcome when incompatible blood was transfused to 12 patients with antibodies to high-incidence antigens. Results indicating no clinical significance (<3% reactivity) were never associated with clinical ill effects and/or abnormal 1- or 24-hour \(^{51}\)Cr results. A recent retrospective analysis of the MMA was carried out by Arndt and Garratty\(^{25}\) after approximately 20 years of use instead of the 1-hour \(^{51}\)Cr survival to aid in the decision to transfuse incompatible red cells to patients with antibodies to high-incidence antigens. The authors concluded that a negative result (<5%) indicated that incompatible blood could be given without risk of an overt HTR, but normal long-term survival of the cells could not be guaranteed. It was also predicted that most unusual antibodies would cause shortened survival, but transfusion of incompatible blood may not result in clinical or laboratory signs of an HTR. The authors concluded that the approach was safe and
Table 2. Clinical Significance of Blood Group Antibodies

<table>
<thead>
<tr>
<th>System</th>
<th>No. of antigens</th>
<th>HTR</th>
<th>HDFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
<td>4</td>
<td>Anti-A, -B, -A,B cause severe and often fatal intravascular HTRs.</td>
<td>Rare and usually mild</td>
</tr>
<tr>
<td>MNS</td>
<td>46</td>
<td>Rare examples of anti-M and -N active at 37°C cause I and D HTRs. Anti-S, -s, and some other antibodies cause severe HDFN.</td>
<td>Anti-M, rarely.</td>
</tr>
<tr>
<td>P1</td>
<td>1</td>
<td>Only very rare examples active at 37°C cause I and D HTRs.</td>
<td>No</td>
</tr>
<tr>
<td>Rh</td>
<td>49</td>
<td>Cause severe I and D HTRs</td>
<td>Cause severe HDFN</td>
</tr>
<tr>
<td>Lutheran</td>
<td>19</td>
<td>Anti-Lu* and -Luβ have caused mild D HTRs</td>
<td>No</td>
</tr>
<tr>
<td>Lewis</td>
<td>6</td>
<td>Not generally considered clinically significant</td>
<td>No</td>
</tr>
<tr>
<td>Kell</td>
<td>28</td>
<td>Cause severe I and D HTRs</td>
<td>Cause severe HDFN</td>
</tr>
<tr>
<td>Duffy</td>
<td>6</td>
<td>Anti-Fy^A^, -Fy^B^, and -Fy^3^ cause I and D HTRs; anti-Fy^5^, D HTR. Anti-Fy^A^ and -Fy^B^ cause HDFN.</td>
<td>Not usually</td>
</tr>
<tr>
<td>Kidd</td>
<td>3</td>
<td>Common cause of D HTRs. Anti-Jk^a^ and -Jk3 also cause I HTRs.</td>
<td>No</td>
</tr>
<tr>
<td>Diego</td>
<td>21</td>
<td>Anti-Di^a^ and -Di^b^, no evidence. Anti-Wr^A^ has caused HTRs.</td>
<td>Anti-Di^a^, -Di^b^, -Wr^A^, and -Wr^B^, plus some others have caused severe HDFN.</td>
</tr>
<tr>
<td>Yt</td>
<td>2</td>
<td>Anti-Yt^a^ very rarely caused HTR.</td>
<td>No</td>
</tr>
<tr>
<td>Xg</td>
<td>2</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Scanna</td>
<td>7</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Dombrock</td>
<td>5</td>
<td>Anti-Do^a^ and -Do^b^ cause I and D HTRs.</td>
<td>No</td>
</tr>
<tr>
<td>Colton</td>
<td>3</td>
<td>Anti-Co^a^ causes I and D HTRs. Anti-Co^b^ has caused severe and anti-Co^3^ mild HDFN.</td>
<td>No</td>
</tr>
<tr>
<td>LW</td>
<td>3</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ch/Rg</td>
<td>9</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>Anti-H in Bombay phenotype can cause severe intravascular HTRs. Anti-H in para-Bombay not usually clinically significant.</td>
<td>Anti-H in Bombay phenotype has potential to cause severe HDFN.</td>
</tr>
<tr>
<td>Kx</td>
<td>1</td>
<td>Anti-Kx + -Km in McLeod syndrome has caused severe HTRs.</td>
<td>Antibodies only found in males</td>
</tr>
<tr>
<td>Gerbich</td>
<td>8</td>
<td>No</td>
<td>One example of anti-Ge3 causing HDFN</td>
</tr>
<tr>
<td>Cromer</td>
<td>15</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Knops + COST</td>
<td>9 + 2</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Indian</td>
<td>4</td>
<td>One example of anti-In^b^ causing an HTR</td>
<td>No</td>
</tr>
<tr>
<td>Ok</td>
<td>1</td>
<td>Very rare and no HTR reported</td>
<td>No</td>
</tr>
<tr>
<td>JMH</td>
<td>5</td>
<td>One example of anti-JMH reported to have caused IHTR</td>
<td>No</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>Anti-I in adult i phenotype has caused increased destruction of I+ red cells.</td>
<td>No</td>
</tr>
<tr>
<td>GLOB + PP1P^θ and LKE</td>
<td>1 + 2</td>
<td>Anti-P and -PP1P^θ cause intravascular HTRs. LKE not clinically significant.</td>
<td>No, but high rate of spontaneous abortion with anti-P and -PP1P^θ</td>
</tr>
<tr>
<td>GIL</td>
<td>1</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Er</td>
<td>2</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Vel</td>
<td>2</td>
<td>Anti-Vel causes severe intravascular HTRs. Little is known about anti-ABTI.</td>
<td>Generally no, but 1 case reported</td>
</tr>
<tr>
<td>LFAs (700 series)</td>
<td>19</td>
<td>Little evidence as compatible red cells readily available</td>
<td>Anti-JFV, -Kg, -JONES, -HJK, and -REIT have caused HDFN.</td>
</tr>
<tr>
<td>HFAs (901 series)</td>
<td>9</td>
<td>Anti-Lan: 1 example caused IHTR. Anti-Ar^a^: reported to have caused HTR. Anti-Jr^a^-: 1 example caused IHTR. Anti-AnWi: severe HTRs. Anti-Pel: -MAM: very rare, but could be clinically significant Anti-Emm, -Sd^a^: no</td>
<td>Anti-Ar^a^: 1 report of mild HDFN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anti-MAM: caused severe HDFN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anti-Lan, -Jr^a^, -Emm, -AnWi, -PEL, -Sd^a^: no</td>
</tr>
</tbody>
</table>
preferable to the more conservative approach of searching for antigen-negative units for all patients with antibodies to high-incidence antigens. If the MMA or equivalent assay (e.g., CLT or ⁵¹Cr survival) were not available or time did not allow they would support the UK guidelines,²⁶ based on predicting clinical significance from the specificity of the antibody, as a compromise approach. In a study to compare the CLT with the MMA²⁷ using sera containing well-characterised IgG antibodies to high-incidence antigens, approximately 50% of which the clinical significance was already known, overall results showed good agreement between the 2 methods. Both MMA and CLT results were in concordance with clinical significance, where known, in >80% of cases. Both assays gave false-positive reactions with a Kna (complement receptor 1 [CR1])–related antibody, which was known not to have caused adverse effect in a patient. This appeared to be due to the ability of anti-Kna to cross-link CR1 on red cells to the CR1 present on monocytes; negative results were obtained using autologous monocytes. The data highlighted the need for caution when interpreting CLT or MMA results on antibodies of unidentified specificity, in which the presence of the corresponding antigen on monocytes is not known.

ANTIBODIES THAT CAUSE HEMOLYTIC TRANSFUSION REACTIONS

For references to many of the antibodies described in the following sections, see Daniels,²⁸ and for a précis of the information, see Table 2.

Antibodies Likely to Cause Severe HTRs

Antibodies of the ABO system are clinically the most important in transfusion medicine for 2 main reasons: (1) almost all adults have anti-A or -B or both when the corresponding antigen is absent and (2) ABO antibodies cause acute intravascular HTRs. The severity of these HTRs is highly variable, but it has been estimated that approximately 10% of ABO-incompatible blood transfusions lead directly to the death of the patient.¹ Transfusion of ABO-incompatible red cells is never an acceptable transfusion practice. However, anti-A₁ is not generally considered clinically significant (see below). Anti-H in individuals with the very rare O₅ phenotype also has the potential to cause severe intravascular HTRs, but finding the necessary compatible blood is a far greater problem.

Rh antibodies often have the capacity to cause severe immediate or delayed extravascular HTRs and all Rh antibodies should be considered to be potentially clinically significant. Whereas anti-D typically causes immediate HTRs, other Rh antibodies are more likely to cause delayed reactions. Rh antibodies are mostly IgG, and predominantly IgG₁, although IgG₂, IgG₃, and IgG₄ have all been detected.²⁹ Rh antibodies are not generally naturally occurring, but approximately 85% of D-negative healthy individuals make anti-D following infusion of 200 mL or more of D-positive red cells.¹

In the Kell system, anti-K, -k, and -Ku (the antibody made by individuals with the rare Kell-null phenotype, K₀) have caused severe immediate HTRs. All other Kell antibodies should be considered to have the potential to cause HTRs, although these are likely to be delayed.

Anti-Fya, -Fyb, and -Fy₃ (the rare antibody made by Fy(a-b-) individuals of non-African origin) have caused severe immediate and delayed HTRs, and anti-Fy₅ has been incriminated in delayed reactions.

Kidd antibodies, anti-Jka, -Jkb, and -Jk₃, have caused severe and fatal immediate and delayed HTRs. Kidd antibodies have a tendency to fall rapidly to very low levels in the plasma and so are a common cause of delayed HTRs, as they are often not detected in pretransfusion testing. It has been estimated that over one third of delayed HTRs are caused by anti-Jka.³⁰

The antibodies of the MNS system to glycoporphin B, anti-S, -s, and -U can all cause immediate and delayed HTRs, and anti-U, the antibody made by individuals lacking glycoporphin B (GPB), is a particularly dangerous antibody. Anti-M and -N, on the other hand, rarely cause HTRs and are usually inactive at 37°C. Antibodies to GP.Mur phenotype red cells (anti-Mur, -Mia) have been responsible for immediate and delayed HTRs. Although rare elsewhere, both GP.Mur and anti-Mur are relatively common in Southeast Asia, where it is important that GP.Mur red cells are included in antibody screening panels. Antibodies to the low-frequency antigens Vw (MNS9) and Far (MNS22) are reported to have caused severe HTRs. Anti-En², a rare antibody to high-frequency determinants on glycoporphin A, has caused a fatal HTR.

Dombrock antibodies, anti-Doᵃ and -Doᵇ, have been responsible for immediate and delayed HTRs and are often difficult antibodies to identify. Apart
Table 3. Recommendations for Red Cells to be Selected for Transfusion

<table>
<thead>
<tr>
<th>Antigen-negative red cells</th>
<th>Red cells compatible by IAT at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>eg, ABO, Rh, Kell, Duffy Kidd antibodies, anti-Coa, -Vel</td>
<td>eg, Lewis antibodies, anti-A_1, -P_1, -Lu^a, -Do^a, -Do^b, -Co^a</td>
</tr>
<tr>
<td>Least incompatible red cells, but antigen-negative red cells</td>
<td>for strong examples of the antibody</td>
</tr>
<tr>
<td>eg, Cromer antibodies, -Yt^a, -Gy^a, -Hy, -Jo^a, -Lan, -At^a, -Jr^a</td>
<td>Least incompatible red cells</td>
</tr>
<tr>
<td>eg, Gerbich, Knops antibodies, anti-LW^a, -LW^ab -JMH, -Emm, -PEL, -ABTI</td>
<td>Ideally antigen-negative red cells, but, due to their extreme rarity, least incompatible red cells should be used with extreme caution.</td>
</tr>
<tr>
<td>eg, Anti-Sc3, -Co3 -Ok^a, -MAM</td>
<td></td>
</tr>
</tbody>
</table>

from one report of an HTR caused by anti-Hy, other Dombrock antibodies have not been implicated, although they are rare.

Anti-P and -PP1P^a are naturally occurring antibodies invariably present in individuals with the very rare P^a and p phenotypes, respectively, in which the red cells lack globoseries glycolipids. They are IgM and often also IgG, are usually reactive at 37°C, and can cause severe HTRs.

Rare antibodies to the high-frequency antigen Vel are often IgM and complement-activating, and can cause severe intravascular HTRs. There is no information on the related antibody, anti-ABTI. Anti-AnW^j, a rare antibody to an antigen of very high frequency that appears to be associated with CD44 glycoprotein, has caused severe HTRs.

Anti-Wr^a of the Diego system, a relatively common antibody to an antigen of very low frequency, has caused severe HTRs. Anti-Co^a, an antibody to a high-frequency antigen, has caused delayed HTRs, whereas anti-Co^b and -Co3, the other antibodies of the Colton system, have only been associated with mild reactions.

Anti-Kx is a rare antibody found in immunized individuals with McLeod syndrome, usually together with anti-Km. McLeod syndrome often occurs together with chronic granulomatous disease and anti-Kx + -Km have caused HTRs in boys with this disease.

Antibodies Unlikely to Cause Severe HTRs

Anti-A_1 of the ABO system, Lewis antibodies, anti-P_1, -LKE, and -I in adult i individuals are examples of antibodies to carbohydrate determinants that are mostly IgM, rarely active above 25°C, and unlikely to cause an HTR. There are exceptions, however. Anti-P_1 has caused severe immediate and delayed HTRs, and a few HTRs have been attributed to anti-A_1, -Le^a, and possibly -Le^b.

Some Rh antibodies only react with red cells that have been protease treated. These are mainly anti-E, but some have had D, C, c, e, and ce specificity. These “enzyme-only” antibodies are generally considered to be clinically insignificant, although a few examples including an anti-C that bound C3 are reported to have caused HTRs.

Lutheran antibodies are not generally considered to be clinically significant, although anti-Lu^a, -Lu^b, and some other Lutheran-system antibodies have caused mild delayed HTRs. Although anti-Wr^a does cause severe HTRs, there is no firm evidence that any other Diego system antibody has caused an HTR. They do have hemolytic potential though, as demonstrated by their ability to cause severe HDFN.

Other antibodies that are unlikely to initiate an HTR are those of the Yt, Scianna, LW, Chido/Rodgers, Gerbich, Cromer, Knops, and Indian systems, Er and COST antibodies, and anti-Xg^a, -MER2, -JMH, -GIL, -LKE, -Sd^a, -Lan, -At^a, -Jr^a, and -Emm. There are, however, a few reports of the following causing severe HTRs: anti-Yt^a, -Te^a (CROM2), -In^b, -Lan, -At^a, and -Jr^a. In addition, there is one report of a low-titre anti-JMH associated with acute intravascular HTR, but evidence that the anti-JMH is responsible for the reaction is limited.31

Anti-Ok^a, -GIL, -PEL, -MAM, and some antibodies to low-frequency antigens are extremely rare and, although there is no evidence that they have caused HTRs, they are likely to have the potential should the opportunity arise.

HLA antigens are occasionally expressed on red cells and HLA antibodies have been implicated in HTRs, although proof of their culpability is incomplete.32-34 In one case, multiple HLA antibodies were blamed for a severe delayed HTR and, following further transfusion, a severe intravascular HTR.33

SELECTING SUITABLE RED CELLS FOR TRANSFUSION TO PATIENTS WITH BLOOD GROUP ANTIBODIES

It is often difficult, expensive, and unnecessary to provide antigen-negative red cells for transfusion to
patients with blood group antibodies. Consequently, in 2002, Daniels et al.26 devised a policy for the NBS in England for selecting suitable blood, based on information about the clinical significance of the antibodies and availability of compatible red cells. The policy is summarised in Table 3. In the majority of cases, suitable blood can be provided from local sources, but occasionally this may be difficult. These cases include patients with complex mixtures of antibodies and/or antibodies to high-incidence antigens. In vitro clinical bioassays may be employed when an unusual or rare blood group antibody of undetermined clinical significance is present and compatible blood is not readily available. If it is deemed necessary to obtain compatible blood for these patients, judged from the assessment of previously described criteria, there are options to consider for availability: national lists of donors of rare phenotype which exist in many countries around the world and are often supported by frozen blood banks; the World Health Organization international panel of donors of rare blood type,35 compiled and maintained at the International Blood Group Reference Laboratory (Bristol, UK), currently includes data from 60 blood centres in 26 countries. Autologous donation may also sometimes be used prior to surgery.

Little information is available with regard to transfusion support and outcome for patients with antibodies to high-frequency antigens. A recent collaborative study from reference laboratories in Switzerland, Germany, and Austria36 assessed the quality of transfusion support to such patients. A total of 52 patients with antibodies to high-frequency antigens were studied over a 20-month period. Four antibody specificities were present in two thirds of the patients: anti-Kp\(^b\), -Vel, -Lu\(^b\), and -Yt\(^l\). Twenty-two were transfused with antigen-negative blood, of which 20% was supplied internationally. Non-antigen-matched blood was used in 8 cases resulting in 5 delayed HTRs. The authors concluded that a lack of sufficient blood supply resulted in a deviation from standard transfusion policy in 23 of the cases, which was unsatisfactory and due to inadequate donor typing resulting in lack of readily available blood of rare phenotype. The NBS South London Centre has had an active screening programme for over 30 years and lists a substantial number of donors of rare blood type which are available for patients around the world. They are currently screening with anti-Vel, -I, -Lu\(^b\), -Kp\(^b\), and Lan (A Gray, personal communication).

According to the NBS guidelines in England, if incompatible blood is to be transfused, where possible, serologically “least incompatible” units should be selected. Many antibodies to high-incidence antigens are sufficiently rare to have provided little or no evidence of their clinical significance, but absence of evidence does not mean that transfusion of incompatible blood will be uneventful. Where appropriate, strategies for ameliorating the immune response, eg, the use of intravenous immunoglobulin and high-dose steroids, should be considered. Transfusion should be given at the slowest rate consistent with clinical condition and the patient observed closely throughout.26

**ANTIBODIES THAT CAUSE HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN**

*Antibodies that Most Commonly Cause Severe HDFN*

Despite the anti-D immunoglobulin prophylaxis programme for preventing D-negative women making anti-D after or during a D-positive pregnancy, anti-D remains the most common cause of HDFN. The prevalence of HDFN is now approximately 1 in 21 000 births. In England and Wales, about 500 fetuses develop HDFN each year, of which 25 to 30 babies die, and at least 20 pregnancies per year are lost to spontaneous abortion before 24 weeks’ gestation.37

All antibodies to Rh-system antigens should be considered capable of causing severe HDFN, but the only Rh antibody other than anti-D that regularly causes severe HDFN is anti-c. In 3 series of studies, between 14% and 21% of c-positive babies born to women with anti-c required exchange transfusion.38 In England and Wales over the period from 1977 to 1987, 26 babies, representing 1 in 250 000 births, died from anti-c HDFN. Anti-C, -E, -e, and -G have all caused HDFN, but the occurrence is rare and the outcome seldom severe. One enzyme-only anti-E became reactive with untreated red cells during pregnancy and caused HDFN requiring exchange transfusion.39 Other Rh-system antibodies that have been reported to have caused serious HDFN are anti-ce (-I), -Ce, -C\(^w\), -E\(^w\), -Hr\(^\alpha\), -Hr, -Rh29, -Go\(^a\), -Rh32, -Be\(^a\), -Evans, -Tar, -Sec, -JAL, and -MAR-like.
Since the decline of HDFN caused by anti-D, much more attention has been focussed on HDFN due to anti-K of the Kell system. In one series of tests, maternal anti-K was detected in 127 (0.1%) of 127 076 pregnancies. Thirteen of these pregnancies with maternal anti-K resulted in a K-positive baby, 5 (38%) of whom were very severely affected with HDFN. Most anti-K appear to be induced by blood transfusion and it is becoming common practice for girls and women of childbearing age to be transfused only with K-negative red cells, though anti-K stimulated by transfusion seems to cause a less severe disease than anti-K stimulated by a previous pregnancy.

The pathogenesis of HDFN caused by anti-K differs from that due to anti-D. The severity of the anti-K disease is harder to predict than the anti-D disease. This is because there is very little correlation between anti-K titre and severity of disease and because anti-K HDFN is associated with lower concentrations of amniotic fluid bilirubin than in anti-D HDFN. Postnatal hyperbilirubinemia is not prominent in babies with anemia caused by anti-K. There is also reduced reticulocytosis and erythroblastosis in the anti-K disease compared with anti-D HDFN. These characteristics suggest that there is less hemolysis in HDFN caused by anti-K compared with HDFN of comparable severity due to anti-D. This has led to speculation that fetal anemia in anti-K HDFN results predominantly from a suppression of erythropoiesis. Kell glycoprotein is one of the first erythroid-specific antigens to appear on erythroid progenitors during erythropoiesis, whereas the Rh proteins appear much later, and it is likely that anti-K suppresses erythropoiesis through the immune destruction of early erythroid progenitors in the fetal liver, before they become hemoglobinised.

Antibodies that Occasionally Cause HDFN

ABO antibodies rarely cause HDFN and, when they do, it is generally mild. ABO HDFN is restricted almost exclusively to the fetuses of group O mothers and it is generally considered that IgG anti-A,B is culpable. Approximately 15% of pregnancies in women of European origin involve a group O mother with a group A or B fetus, yet ABO HDFN requiring clinical intervention is rare, although minor symptoms involving a small degree of red cell destruction may be relatively common. Hydrops fetalis due to ABO HDFN is exceedingly rare, but very occasionally exchange transfusion for the prevention of kernicterus is indicated. A variety of factors are responsible for the low prevalence of clinically significant ABO HDFN: (1) ABO antibodies are mostly IgM, and so cannot cross the placenta; (2) ABO antigens are present in many tissues and any IgG antibody crossing the placenta is likely to become bound to placental tissue; (3) IgG ABO antibodies are usually IgG2, which does not initiate red cell destruction; and (4) ABO red cell antigens are not fully developed in the fetus or at birth. Like ABO antibodies, anti-H in individuals with the Ob phenotype is unlikely to cause severe HDFN.

In addition to anti-K described above, it is likely that antibodies of all Kell-system specificities have the potential to cause HDFN. This is usually, but not invariably, mild. HDFN requiring at least fetal transfusion has been reported for anti-k, -Kpa, -Kpb, -Jsa, -Jsb, -Ula, -Ku, and -K22.

In the MNS system, severe HDFN has been attributed to anti-S, -s, and -U. Other MNS antibodies that are reported to have caused HDFN are antibodies to the high-frequency antigen Ena and antibodies to the low-frequency antigens Mi, Vw, Mur, Hut, Hil, M, M', Far, s, Or, and MUT. Most anti-M and -N are not active at 37°C and are usually benign, but severe HDFN due to anti-M, though not anti-N, has occurred. The MNS red cell phenotype GP.Mur is rare in most parts of the world, but is relatively common in Southeast Asia with frequencies between 2% and 10%. Antibodies to GP.Mur (anti-Mur, -Mi) have caused severe HDFN.

In a survey of 68 pregnancies in which anti-Fya was detected, 10 resulted in indications of mild HDFN and 3 resulted in severe HDFN, 2 requiring intrauterine transfusion. Only 1 case of HDFN due to anti-Fyb has been reported. Despite being a common cause of delayed HTRs, antibodies to Jka and Jkb do not usually cause HDFN, although 1 case of HDFN due to anti-Jka led to kernicterus. Antibodies to the high-frequency antigens Co and Co3 have both caused severe HDFN. In the Scianna system, the first 5 anti-Rd identified were reported to have caused mild or moderate HDFN, but only 1 baby required exchange transfusion.

In the Diego system, anti-Di can cause severe HDFN and, although this is rare in most populations, many examples have been described. Three cases of HDFN due to anti-Di requiring exchange
transfusion are reported. Anti-Wr⁺, -ELO, and -BOW, antibodies to 3 low-frequency antigens of the Diego system, have caused severe HDFN.

Gerbich antibodies are not generally considered to cause severe HDFN, but 2 babies from the same mother with anti-Ge3 required transfusion for HDFN that was not apparent or severe until 2 to 4 weeks after birth. Glycophorin C, which carries the Ge3 antigen, is one of the first blood group proteins to appear on erythroid cells during erythropoiesis and the HDFN caused by the anti-Ge3 had characteristics of suppressed erythropoiesis seen in Kell HDFN.

Only a few of the antibodies to antigens that have not been shown to belong to a blood group system have caused HDFN serious enough to merit transfusion therapy. Of the antibodies to low-frequency antigens, these are anti-HJK, -Kg, -REIT, -JFV, and -JONES. With the exception of anti-MAM, antibodies to high-frequency antigens have not caused severe HDFN.

**Antibodies Unlikely to Cause HDFN**

Antibodies to the glycolipid antigens P1PPk and LKE have not been implicated in severe HDFN. Red cells of the rare p phenotype lack all these antigens and an antibody, called anti-PP1P⁺, is invariably present. Anti-PP1P⁺k usually contains an IgG3 fraction, but it does not cause severe HDFN. However, anti-PP1P⁺k and -P are often associated with habitual abortion, characteristically in the first trimester, the primary target for the antibodies appearing to be the placenta and not the fetus.

Antibodies of the Lutheran system do not cause HDFN, partly because the antigens are expressed only weakly on fetal cells and possibly also because the antibodies are adsorbed by Lutheran antigens on placental cells. Antibodies to the Lewis antigens Le⁺a and Le⁺b are generally not active at 37°C and the antigens are not expressed on fetal cells. In addition, the following have not been implicated in serious HDFN: antigens of the Yt, Dombrock, LW, Chido/Rodgers, Cromer, Knops, and Indian systems, the Cost and Er antibodies, anti-Sce, -Sc2, -Sc3, -Xg⁺, -Kx, -Ok⁺, -MER2, -JMH, -GIL, -Emm, -AnWj, -Sd⁺, -Duclos (only one example known), -PEL, -ABTI, and HLA antibodies. Antibody to the high-frequency antigen Vel usually only causes mild HDFN, probably because anti-Vel is generally IgM and Vel is expressed weakly on fetal cells, but one case of severe HDFN is described. Anti-Lan, -At⁺, and -Jr⁺ have only caused mild HDFN.

**CONCLUDING REMARKS**

Over the course of the last decade the molecular bases for almost all of the clinically significant polymorphisms have been elucidated. Consequently, it is now possible to determine blood group phenotypes from DNA with a reasonable degree of accuracy. Currently, this technology is generally applied to determining blood groups when suitable red cells are not available, such as fetal testing and blood grouping on multiply transfused patients. The development of high-throughput molecular methods means that it may soon be feasible to test large numbers of blood donors for all clinically important blood group polymorphisms. This will provide large databases of fully grouped donors, facilitating the provision of matched blood for transfusion-dependent patients to reduce the chances of them making multiple blood group antibodies, or to provide compatible blood to those who have already made antibodies.

Molecular technology, however, has done little to help us predict the potential clinical importance of an antibody. We still do not understand why some antibodies are hemolytic and clinically significant, whereas others are not. Until we gain more insight into this, the technology of predicting the clinical significance of an antibody will remain, at best, somewhat haphazard.

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