Some Clinical Consequences of Red Cell Incompatibility
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P. L. MOLLISON, MD, FRCP, FRS
Director, MRC Experimental Haematology Unit, St Mary’s Hospital Medical School, London

There are four circumstances in which incompatibility between red cells and plasma can arise, two related to transfusion and two related to pregnancy (Table 1).

| donor's red cells | recipient's plasma | recipient's red cells |
|-------------------|--------------------|-----------------------|
| donor's plasma    |                    | maternal plasma       |
| maternal plasma   |                    | Fetal red cells       |

Transfused red cells can be incompatible with the recipient's plasma, or transfused plasma can be incompatible with the recipient's red cells. In pregnancy, incompatible fetal red cells can enter the maternal circulation, or antibodies from the mother can cross the placenta and damage fetal red cells. In considering the clinical consequences of these various interactions, ABO incompatibility dominates the scene, excepting conditions damaging to fetal red cells, in which Rh(D) incompatibility is the most important.

Transfusion of Incompatible Red Cells

Red cell destruction may be predominantly intravascular or extravascular. By intravascular destruction is meant the lysis of red cells within the plasma with consequent haemoglobinemia, and by extravascular destruction is meant the engulfment of red cells by macrophages with the subsequent release of bilirubin into the plasma.

Intravascular Lysis

Antibody-mediated intravascular lysis occurs only with those antibodies that activate the whole complement sequence, since it is only the terminal components of complement, C8 and 9, that cause damage to the red cell membrane. Virtually all examples of strongly lytic red cell alloantibodies found in man have the specificity anti-A or anti-B. Since almost all deaths from the transfusion of incompatible blood are associated with strongly lytic antibodies, ABO-incompatibility is of predominant importance. The frequencies of the different ABO groups are such that if donor or recipient are mis-identified, so that the wrong blood is given, the transfusion will prove incompatible in almost one case in three.

Symptoms and Signs from ABO-incompatible Red Cells

The transfusion of ABO-incompatible red cells is often, in the recipient, associated with acute symptoms such as sternal pain, lumbar pain, a feeling of heat in the vein into which the transfusion has been given, flushing of the face and, sometimes, itching of the skin. Hypotension is a common sign and there may be urticaria. These effects are believed to be mediated by fragments of complement, C3a and C5a, that are liberated into the plasma during activation of the complement cascade. These small peptides cause histamine release from mast cells and contraction of smooth muscle, and they also have chemotactic effects on neutrophils. Judged by the ability to evoke oedema and erythema in human skin, C5a, on a molar basis, is 1,000 times more powerful than C3a (Müller-Eberhard, 1975). It seems probable it is C5a that produces the severe symptoms that may follow the injection of even 1 ml of ABO-incompatible red cells.

When ABO-incompatible transfusions prove fatal they do so either by causing uncontrollable bleeding secondary to disseminated intravascular coagulation (DIC), or by causing renal failure, which may also be a consequence of DIC. Although DIC can be triggered off by the intravascular lysis of autologous red cells, due presumably to the release of thromboplastic substances from red cell stroma, there is some evidence that it is less severe when it is caused by non-immunological mechanisms. For example, in a patient with Clostridium perfringens septicaemia with very severe intravascular haemolysis (plasma Hb 4 g/dl), only very mild signs of DIC developed (Slotki et al., 1976).

If it is true that for a given level of intravascular lysis DIC is more severe when immune mechanisms are involved, it may be because the antigen-antibody complexes interact with leucocytes to release procoagulant substances and also possibly interact with platelets (see review by Zimmerman et al., 1977). It is also suspected that complement activation helps to trigger DIC, although the details of the interaction are unknown.

Although it was believed that haemoglobin was nephrotoxic and that the renal tubular necrosis which sometimes follows the transfusion of ABO-incompatible
disturbance in renal haemoglobin traces prepared blood Nevertheless, circumstances are aetiologies. The sometimes wrong ABO group is overlooked. haemoglobinaemia. During appreciable concentration on whose Day 4. Figure 1 shows very mild haemolytic reaction following the transfusion of 7.5 units of group A blood to a group O subject whose plasma contained a relatively low-titred anti-A (32). The only immediate sign of red cell destruction was transient haemoglobinaemia. During the following 3 days no anti-A could be detected in the plasma although there was appreciable destruction of group A red cells from day 2 onwards and the serum bilirubin concentration reached a peak on day 4. Following the reappearance of anti-A, red cell destruction accelerated and was complete by day 7 (Mollison, 1978).
reached 100 mg/dl and there was no haemoglobinuria; during this period the anti-A titre in the recipient's plasma fell from 32 to 1. After the transfusion of further units of group A blood no anti-A could be detected in the recipient's plasma. Although the patient developed fever, headache, and various pains during the transfusion of the first unit, no untoward effects were noted during the transfusion of the remaining units. As the figure shows, there was relatively little destruction of the transfused A cells during the first 3 days but thereafter anti-A reappeared in the patient's plasma but steadily increased in concentration. Concomitantly, there was a rise in the serum bilirubin concentration and all the group A red cells were eliminated within 7 days of the transfusion. It was necessary to transfuse the patient with group O red cells during this latter period to prevent the development of severe anaemia, but otherwise there were no problems.

An estimate of the prevalence of ABO-incompatible transfusions was provided by Wallace (1977). In a series of 130,000 recipients, transfused with 400,000 units of blood, 40 cases of ABO-incompatibility were detected. In another series (personal communication from a colleague in the USA), among 75,000 recipients transfused with 300,000 units of blood, 13 cases of ABO-incompatibility were found. One may summarise these two experiences by saying that ABO-incompatible blood was transfused to approximately 1 in 5,000 recipients and that approximately 1 in 15,000 units of blood transfused were ABO-incompatible. If donors and recipients were not ABO-grouped, only about 30 per cent of transfusions would be ABO-incompatible. The fact that ABO-incompatible blood was transfused to approximately 1 in 5,000 recipients therefore implies that donors or recipients were mis-identified in approximately 1 in 1,500 transfusions, a very disconcerting figure.

Mortality from ABO-incompatible Transfusions

In the two series referred to above, there were 4 deaths out of 40 and none out of 13 respectively; in another ten cases seen by the present author there were no deaths. It therefore seems likely that mortality from the transfusion of ABO-incompatible blood does not exceed 10 per cent. If this is correct, the overall mortality from ABO-incompatible transfusions must be no more than 1 in 50,000 transfusions. In England and Wales, about 1,350,000 units of blood are transfused each year (based on figures for 1976 kindly supplied by Sir William Maycock). Assuming that these units are given to approximately 400,000 recipients, not more than about 8 deaths would be expected a year from ABO-incompatibility. It is evident that there is at least one more important cause of death following transfusion, namely post-transfusion hepatitis, from which the death rate seems to be between about 1 in 400 (MRC, 1974) and 1 in 2,500 (Seeff et al., 1977). It is possible that deaths from the transfusion of blood heavily contaminated with bacteria may be almost as frequent as those from ABO-incompatibility.

Passage of ABO-incompatible red Cells from Fetus to Mother

Before leaving the subject of the effect of introducing ABO-incompatible red cells into the circulation, it is worth mentioning that, very rarely, a group O woman carrying a group A or B infant may suffer an incompatible transfusion from her infant following some traumatic episode. Cases in which this has caused haemoglobinuria and even renal tubular necrosis in the mother have been described (Glasser et al., 1970; Samet and Bowman, 1961).

Predominantly Extravascular Red Cell Destruction

Virtually all red cell alloantibodies except anti-A and anti-B bring about red cell destruction via the agency of macrophages. All mature cells of the mononuclear phagocyte system possess receptors for the Fc fragment of IgG and for C3b, the activated form of the third component of complement. It is believed that the role of C3b is to bring about the attachment of coated macrophages whereas IgG mediates erythrophagocytosis (Mantovani et al., 1972). IgG red cell alloantibodies that fail to bind complement, e.g. anti-Rh, bring about red cell destruction predominantly in the spleen, whereas those that activate complement also bring about clearance in the liver. In both cases the process of clearance of coated cells from the blood stream is accompanied by some degree of haemoglobinanaemia. This appears to be due to the fact that when coated red cells come into contact with macrophages they may lose part of their membrane by engulfment and then break away, reseal, and circulate as microspherocytes (see review by Brown, 1974). Presumably, some of these microspherocytes rupture in the bloodstream.

Incompatible transfusions due to non-lytic antibodies are not associated with the acute symptoms that characterise the transfusion of ABO-incompatible blood. When reactions are due to Rh antibodies, which do not activate complement, the lack of symptoms is not unexpected since neither C3a nor C5a is liberated. On the other hand, when antibodies are complement-activating but non-lytic, C3a must be liberated although C5a may not be; C3a is far less potent than C5a in mediating vasoactive effects. Antibody-mediated extravascular red cell destruction is invariably associated with fever and leucopenia (Jandl and Tomlinson, 1958).

Risks from Rh Incompatibility

In screening the plasma of prospective recipients for transfusion in 1974-75, anti-D was found in 0.52 per cent of subjects (Giblett, 1977). Evidently, if the risk of misidentifying donor or recipient is 1 in 1,500 (see above) the overall risk of accidentally transfusing Rh-positive blood to a recipient whose plasma contains anti-D would be only 1 in 300,000. In practice, the risk is substantially greater than this, mainly because mistakes in Rh grouping are commoner than mistakes in ABO grouping. The transfusion of Rh-positive blood to a patient whose plasma contains anti-D seldom causes a life-threatening reaction.

A far more serious risk arises from the inadvertent transfusion of Rh-positive red cells to an Rh-negative woman, namely that the transfusion may induce Rh immunisation and make it impossible for her to bear a live-born Rh-positive infant subsequently. When no Rh grouping tests are carried out the chance that Rh-positive blood will be given to an Rh-negative recipient is about 1
in 14 and since only about 70 per cent of Rh-negative recipients can respond to Rh, the risk that Rh-immunisation will occur is about 1 in 10. Clearly, this is the risk of Rh immunisation when donor or recipient are mis-identified, so that accidental Rh immunisation might be expected in approximately 1 in 15,000 transfusions. Again, the risk in practice must be greater than this since errors in Rh grouping are, unfortunately, relatively common.

Rh immunisation can almost certainly be prevented even when a whole unit (Pollack et al., 1971), or even perhaps several units, of Rh-positive blood have been transfused, provided that an adequate dose of anti-D is given within a few days.Suppressive therapy, at least against small amounts of Rh-positive red cells, may be effective even when the interval between the injection of red cells and anti-D is as long as two weeks (Samson and Mollison, 1975).

**Risks of Incompatibility from Antibodies other than Anti-A, -B and -D**

Giblett (1977) noted that whereas in 1956-57 antibodies other than anti-A, -B and -D were found in only 0.35 per cent of recipients, the frequency had risen to 1.12 per cent by 1974-75, due presumably to an increase in the number of patients having repeated transfusions. It is routine practice in most hospital laboratories to carry out screening tests for a very wide variety of red cell alloantibodies rather than to rely simply on the crossmatching procedure for their detection. For this reason haemolytic transfusion reactions from these other antibodies have become relatively rare. When haemolytic reactions do occur they are often due to antibodies that are difficult to detect in vitro but cause severe red cell destruction in vivo. Anti-Jk^*^ is the prime example of an antibody of this kind.

**Delayed Haemolytic Transfusion Reactions**

Although immediate haemolytic transfusion reactions from antibodies other than anti-A and anti-B are relatively rare, delayed haemolytic transfusion reactions are encountered more commonly. In this type of reaction the recipient is sensitised to some red cell antigen due to previous transfusions, but the concentration of antibody in the plasma is too low to be detectable. Following a further transfusion there is a secondary response leading to the production of readily-demonstrable antibody within a few days. Typically, the patient develops anaemia, fever, jaundice and/or haemoglobinuria approximately seven days after transfusion. This type of reaction has been estimated to occur after the transfusion of about 1 in 12,000 units (Pineda et al., 1978), implying that it may occur in about 1 in 4,000 recipients.

**The Unimportance of Cold Alloagglutinins**

Blood transfusions are frequently postponed because it has been found that the patient's serum contains an agglutinin reacting with some samples of red cells at room temperature. Substantial time and effort is usually then invested in identifying the specificity of the agglutinin and an effort may be made to find donors whose red cells do not react with the agglutinin. It is coming to be realised that far too much attention is paid to antibodies of this nature. In the great majority of cases the agglutinins are not active at 30°C and are very seldom active at 37°C. Unless they are active at or near 37°C they cause no red cell destruction. Even when they are active at 37°C their concentration in the serum is usually so low that although they may be able to bring about the rapid destruction of a small dose of red cells, such as may be injected for test purposes, they are scarcely ever able to cause sufficient red cell destruction to be of clinical importance. A great deal of time would be saved in transfusion laboratories and a great many operations would no longer have to be postponed if room temperature crossmatching tests were omitted and tests were carried out only at 37°C.

**The Transfusion of Incompatible Plasma**

**Anti-A and Anti-B**

The transfusion of plasma containing anti-A or anti-B to a group A or B recipient is expected on theoretical grounds to have far less serious consequences than the transfusion of incompatible red cells. First, there is the dilution factor; when one unit of group A red cells is transfused to a group O subject the ratio of incompatible plasma to red cells is about 15:1. On the other hand, when one unit of group O blood is transfused to a group A recipient, the ratio of incompatible plasma to red cells is about 1:8. Overall then, the ratio of incompatible plasma to red cells is more than 100 times less when a unit of blood with incompatible plasma is transfused than when a unit with incompatible red cells is transfused. Secondly, there is the protective mechanism provided by the presence of A (and B) substance in virtually all tissues and secretions, so that much transfused anti-A is diverted away from the red cells. This point is illustrated later in considering the serological findings in haemolytic disease of the newborn due to ABO- and Rh-incompatibility respectively. In practice, serious haemolytic reactions from the transfusion of single units of group O blood to group A (or B) recipients are very rare, although mild haemolytic syndromes are quite commonly seen when large volumes of plasma containing anti-A are transfused to A recipients (Ebert and Emerson, 1946; Topley et al., 1963). The effect of cumulative doses of anti-A was well illustrated by a case described by Gasser (1945). The transfusion of 80 ml of group O plasma to a group A infant aged 8 months caused no ill-effects but the transfusion of the same amount from the same donor five days later caused a sharp drop in haemoglobin concentration and the development of jaundice.

The effects of transfusing very large amounts of group O plasma to a group A recipient were dramatically demonstrated in an extraordinary case described by Lauer (1941). In an attempt to obtain tissue to repair a girl's badly injured foot, the foot was grafted on to the thigh of her sister. The injured sister was group A and the uninjured sister group O. After four or five days it was obvious that a connection had been established between the circulations because the group O sister developed jaundice, fever and haemoglobinuria. The injured sister developed vomiting on the seventh day; by day 8 only group O cells could be found in her circulation and her serum contained anti-A with a titre of 32. Meanwhile, the
anti-A titre in the group O sister had risen to 2.048. The sisters were separated and recovered uneventfully.

Anti-Rh

Haemolytic reactions due to the accidental transfusion of plasma containing anti-Rh are unknown, although the effects of deliberately transfusing plasma containing exceptionally potent anti-D to a volunteer have been described (Bowman et al., 1961). In addition, there is now substantial experience of the deliberate injection of large amounts of anti-D into D-negative women who have been inadvertently transfused with D-positive blood, the object of the injection being, of course, to suppress Rh immunisation. My experience is that when a suitable preparation of anti-D is given intravenously in a dose of about 10 μg/ml D-positive red cells following the transfusion of 1-3 units of blood, the ensuing red cell destruction occurs over a period of two or more days and is not usually accompanied by clinical jaundice. Haemoglobinuria occurred in one patient but there was no disturbance of renal function and the patient made an uneventful recovery (Mollison, 1978).

Haemolytic Disease of the Newborn

IgG is the only immunoglobulin transferred across the placenta. Anti-A and anti-B are wholly or predominantly IgM in A and B subjects, but in O subjects are often partly IgG. Thus, for all practical purposes it is only infants born to group O mothers who are at risk from ABO haemolytic disease. If haemolytic disease affected all the A and B infants born to group O mothers almost 25 per cent of all infants would be affected. If a sufficiently sensitive method is used, IgG anti-A or anti-B can in fact be found on the red cells of virtually all A or B infants born to group O mothers (Hsu et al., 1974) but there is usually less than about 0.2 μg antibody per ml red cells. Only about one infant in 5000 has a sufficiently severe haemolytic process to require exchange transfusion. In such cases the amount of the antibody on the red cells is usually about 0.6 μg/ml or more (Romano et al., 1973). It is doubtful if ABO-incompatibility between fetus and mother ever leads to stillbirth.

Rh-haemolytic disease of the newborn is, of course, far more severe than ABO-haemolytic disease, despite the fact that anti-Rh is not a complement-binding antibody. Evidently there must be very effective mechanisms protecting the infant against anti-A and anti-B. These mechanisms are, first, the weakness of A and B antigens at the time of birth and, secondly, the widespread distribution of A and B substances, present in virtually all the secretions and tissues of the body, which divert much of the IgG anti-A or anti-B that crosses the placenta. This latter protective effect is well illustrated by contrasting the serological findings in haemolytic disease of the newborn due to anti-A and anti-B, on the one hand, and to anti-Rh, on the other. As Table 2 shows, in Rh haemolytic disease the concentrations of antibody in the mother's plasma and on the infant's red cells are similar, whereas in ABO-haemolytic disease the concentration of antibody on the infant's red cells is far lower than that in the mother's plasma. It has been shown that complement activation by anti-A or anti-B occurs only when there is about 14 μg of antibody per ml red cells (Romano and Mollison, 1975) and the amounts found on the red cells of infants with ABO haemolytic disease are far below this level.

The number of deaths from Rh-haemolytic disease has been falling for at least 25 years (Figure 2). Following the introduction of exchange transfusion in about 1950 there was a substantial fall in the death rate among live-born infants. From about 1960 onwards there was a large decline in the stillbirth rate due, among other factors, to the increasing tendency towards smaller families (Knox, 1976), since severe haemolytic disease is uncommon in the first two Rh-positive infants born to an Rh-negative

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**Table 2. Typical values for the amount of antibody on the infant's red cells and in the mother's plasma in haemolytic disease of the newborn due (1) to anti-Rh and (2) to anti-A (or -B).**

| Haemolytic disease of the newborn due to | Amount of antibody (μg/ml) | On infant's red cells | In mother's plasma |
|----------------------------------------|-----------------------------|-----------------------|-------------------|
| anti-Rh                                | 6                          | 4                     |                   |
| anti-A (or -B)                         | 0.5                        | 0.5†                  | 20‡               |

*Hughes-Jones et al. (1967) †Romano et al. (1973)

†Deduced from data of Polley et al. (1965), assuming that the relation between the indirect antiglobulin titre and antibody concentration is the same for IgG anti-A as for IgG anti-Rh.

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**Figure 2. Deaths from haemolytic disease in fetuses and newborn infants during 1950-77, based on reports of the Registrar General (supplemented by a personal communication from Sir Cyril Clarke). For many reasons the figures can be regarded as only approximate but they indicate clearly the fall in neonatal death rate following the introduction of exchange transfusion (Ex.) in about 1950, the fall in stillbirths starting about 1960 and the hope of elimination of deaths from the disease following the introduction of suppressive therapy (Suppr.) about 1970.**
woman. Nevertheless, it was only in the 1960s that hope emerged of abolishing the disease altogether. In 1963 it was shown that primary Rh immunisation could be prevented by giving a suitable dose of anti-D at the time when Rh-positive red cells were introduced into the circulation of an Rh-negative subject (Clarke et al., 1963). The routine administration of anti-D postnatally to Rh-negative women who had given birth led to further reduction in deaths from haemolytic disease.

A current enquiry into stillbirths and live-born deaths from Rh haemolytic disease in 1977 indicates that only a small proportion of deaths in that year was due to true failures of treatment, the majority being due to Rh immunisation of women before treatment became available or to failure to administer anti-Rh postnatally (Sir Cyril Clarke, personal communication). It is evident that if anti-Rh were administered postnatally to all Rh-negative women who had been delivered of an Rh-positive infant, deaths from haemolytic disease of the newborn would fall to a very low level indeed. In order to abolish Rh-haemolytic disease completely it is necessary to administer anti-Rh antenatally to those Rh-negative women who suffer substantial transplacental haemorrhages during pregnancy, as may happen during amnioncensis; it may be necessary to introduce routine antenatal administration of anti-D because of the small proportion of Rh-negative women who become immunised during apparently uneventful pregnancies (Bowman et al., 1978).

Summary

Most deaths from red cell incompatibility are due either to the transfusion of ABO-incompatible red cells or to Rh-haemolytic disease of the newborn; almost all the deaths are preventible. It seems that approximately 1 in 5,000 recipients receive ABO-incompatible blood, a figure which implies that donor and/or recipient may be mis-identified in as many as 1 in 1,500 transfusions. Another serious consequence of mis-identification is the transfusion of Rh-positive blood to an Rh-negative female who has no children and is not past the age of childbearing. The need for rigorous checking procedures to ensure that patients are transfused with the ‘right’ blood cannot be stated too often.

Some deaths from Rh-haemolytic disease of the newborn are due to the fact that the mother has not received suppressive therapy after a previous pregnancy. Wider recognition of the great importance of administering anti-D to women who might otherwise become immunised would save many lives.

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