CLINICALLY SIGNIFICANT RED CELL ANTIBODIES MEDIATING HAEMOLYTIC TRANSFUSION REACTIONS

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The transfusion of red cells is a very safe procedure in the vast majority of cases. In fact, if patients are transfused with blood of the same ABO and Rh D groups as themselves, approximately 98-99% will experience the expected rise in Hb levels. This means that only 1-2% of recipients of blood have - other than anti-A, -B, anti-A,B, and anti- D - clinically significant red cell alloantibodies, such as anti-c, -K, -Fy^a, capable of binding to red cells in vivo and causing frank haemolytic transfusion reactions. These induce premature destruction of transfused red cells or cause haemolytic disease of the fetus/newborn. However, since more than 2% of potential recipients of blood are found, during pretransfusion antibody screening, to have atypical red cell alloantibodies, the blood banker must decide which of these antibodies, reactive in vitro, are clinically significant. In practice, the clinical importance of different red cell alloantibodies depends partly on the mechanisms of red cell destruction which they mediate and partly on their frequency (e.g., ABO antibodies as well as ABO antigens are present in most subjects whilst Kell antibodies are rare).

Depending on the nature of the antibodies, the mechanisms of immunological in vivo red cell destruction can be classified into intravascular or extravascular.

a) Intravascular (e.g., caused by anti-A; -B; -A,B; anti-PP1Pk) with activation of the full cascade of the classical pathway of complement, C1 to C9, by IgM antibodies leading to haemolysis with haemoglobinaemia and haemoglobinuria. C1 to C9 activation leads to liberation of anaphylatoxins C3a and C5a, responsible for a significant proportion of the signs and symptoms of immune intravascular haemolysis (e.g., hypotension, shock, renal failure, disseminated intravascular coagulation (DIC)), which are usually far more serious than those of non-immune intravascular haemolysis or than those of immune extravascular haemolysis. C3a and C5a act on mononuclear phagocytic cells and neutrophils to stimulate the respiratory burst and to enhance the expression of C3b receptors on these cells. As anaphylotoxins, C3a and C5a trigger the mast cell and basophil release of mediators already preformed in their granules (e.g., histamine, platelet-activating factor, tumor necrosis factor (TNF), IL-1, IL-3, IL-4, IL-5 and IL-6) or newly synthesized through the metabolism of arachidonic acid (e.g., leukotrienes, prostaglandins). In addition, mononuclear phagocytic cells are activated by phagocytosis per se and by C5a with the consequent secretion of mediators of the acute inflammatory response: TNF, IL-1, IL-8, PGE2, neutrophil-

activating factor (NAP-1), neutrophil chemotactic factor (NCF). Thromboplastic substances released by haemolysis and the activation of complement lead to activation of the extrinsic pathway of the coagulation cascade, contributing to DIC.

b) Extravascular (caused by anti-Rh; -K; etc.): adherence of red cells coated with IgG1 or IgG3 antibodies and/or C3b to Fc receptors (FcγR1, FcγR2 and FcγR3) and to complement (C3b) receptors on mononuclear phagocytic cells or lymphocytes leads to either phagocytosis and/or cytotoxicity of red cells. Cytotoxicity is mediated mostly by lysozymal enzymes released by the mononuclear phagocytic cells when red cells are heavily coated with IgG1 and/or IgG3 antibodies. Moderate coating of red cells leads to phagocytosis. C3b alone does not mediate phagocytosis but greatly enhances IgG-induced phagocytosis through adherence to the complement receptors (for C3b only) on mononuclear cells. Free IgG in plasma inhibits the opsonizing effect of IgG red cell antibodies on mononuclear phagocytic cells. Hence, red cells coated with non-complement fixing IgG1 or IgG3 antibodies (e.g., anti-D, -E, -c) are destroyed mainly in the spleen where there is haemoconcentration and large numbers of macrophages. C3b abolishes the inhibitory effect of free IgG in plasma on Fc receptors of mononuclear cells. For this reason, red cells coated with IgG and C3b (e.g., most anti-Jka, -Jkb, many anti-K, many anti-Fy^a, etc.) are destroyed predominantly in the liver, where there are abundant phagocytic cells and a good blood flow. This destruction occurs generally more rapidly and efficiently than when cells are coated only with IgG antibodies. Most clinically important IgG red cell antibodies are composed of subclasses 1 and 3, which are the subclasses with greatest destructive power because Fcg receptors recognise only these two subclasses. Those IgM antibodies which do not activate complement do not seem to cause red cell destruction. Red cell alloantibodies composed only of IgA have not been found.

The binding of IgG (and probably IgM) antibodies does not damage red cells directly. Several important factors determine the rate, site and mechanism of immune haemolysis. These factors pertain to (i) the **antibody**: thermal range, specificity, plasma concentration and avidity, immunoglobulin class and subclass and ability to activate complement; (ii) the **antigen**: nature and density of the antigen on the red cell membrane; presence of antigen in plasma; (iii) **activity of the mononuclear phagocyte system (MPS)** macrophages and other mononuclear cells; and (iv) the **amount of incompatible red cells transfused**. All these factors must be considered in evaluating *in vivo* and *in vitro* data in specific cases of immune haemolysis. Routine blood bank techniques can predict in most cases whether transfused red cells will survive adequately in the presence of the relevant antibody. Of the criteria available, the most useful are thermal range and lytic capacity. Antibodies which fail to react *in vitro* above 30°C (most anti-A_1, -P_1, -HI, -Le^b) cause no destruction *in vivo* and should be ignored for practical

blood transfusion purposes. On the other hand, not all antibodies reacting *in vitro* at 37°C will cause in vivo red cell destruction, and there are some antibody specificities such as anti-Xg^a, Kn^a, Yk^a which, though reactive at 37°C, usually by the indirect antiglobulin test (IAT), never lead to red cell destruction.

In those extremely rare instances when it is impossible to establish with *in vitro* techniques whether an antibody is clinically significant, and the patient is in need of transfusion of red cells, survival studies with ^{51}Cr or ^{99m}Tc-labelled red cells are indicated. The rate and pattern of clearance of small volumes of cells are important in predicting destructive power, although often the destruction of a small volume of red cells is much greater than the destruction of whole units of blood. The results obtained with *in vitro* monocyte/macrophage binding or phagocytic assays and/or antibody-dependent cellular cytotoxicity (ADCC) tests may also help.

In routine pretransfusion testing, it is recommended to type the patientÕs red cells for ABO and RhD and to screen the patient's serum for the presence of red cell alloantibodies using at least two sensitive techniques (e.g. two-stage enzyme at 37°C or polybrene, or polyethylene glycol (PEG), and IAT). The IAT is imperative and may be used as the conventional IAT in tubes (in NISS or LISS) or in gels, beads or solid phase. The unpooled screening cells should be 2 or 3 and, between them, they should carry the antigens, ideally in double dose, that will detect the vast majority of clinically significant antibodies. This antibody screening should be performed on the samples of all patients who will possibly need blood. If a positive reaction is found, the antibody/ies should be identified with the aid of an identification red cell panel and antigen-negative cells used for the crossmatch. Blood should be crossmatched for all patients with clinically significant antibodies even if they are having an operation where the possibility of requiring blood is minimal (e.g., cholecystectomy, Caesarean section). On the other hand, for those patients without red cell alloantibodies, blood should be crossmatched only when there is a high probability of a requirement for blood transfusion and according to a locally agreed maximal surgical blood ordering scheme (MSBOS). If the antibody screening has been performed as recommended, the crossmatch should be very simple and consist merely of a one-tube test with an immediate spin (check on ABO compatibility) followed by the indirect antiglobulin technique after incubation at 37°C. If the head of the laboratory has confidence in the performance of his/her staff with antibody screening, then an immediate spin should suffice.

Despite advances in the sensitivity of serological techniques (e.g., LIP, solid phase, ELISA, gel test, PEG), we still occasionally fail to detect antibodies capable of causing haemolytic transfusion reactions. On the other hand, it is meaningless to aim at perfect compatibility testing if we neglect other important aspects of transfusion such as donor selection and identification, appropriate labelling and identification of patients' samples and adequate documentation of pretransfusion testing and of the transfusion procedure. Transfusion practice

should be clearly laid down in standard operating procedures, and compliance with such procedures should be subjected regularly to clinical audit.

The commonest cause of incompatible haemolytic transfusion reactions and transfusion fatalities is the failure to identify the recipient correctly either when the samples for pretransfusion testing are drawn or at the time of transfusion, in the operating theatre, the intensive care unit or the ward. It follows that alloantibodies which occur most frequently are the most important provided they are capable of causing severe red cell destruction and that blood containing the corresponding antigens is also common; ABO antibodies meet all these criteria.

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