RED CELL ANTIBODIES post transfusion - **a review**

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INTRODUCTION

During fetal and early development, T and B-cells learn to recognise the body's own antigens, or 'self'. When non-self or foreign antigens on bacteria or viruses for example, are encountered in later life these initiate an immune response. Antigen presenting cells (APC) isolate the foreign antigen and present it to T or B-cells that will result in either T-cells producing an array of cytokines that lead to the destruction of the foreign antigen bearing cells, or to B-cells transforming into antibody producing plasma cells. More than one plasma cell is activated and after a period of clonal expansion that might take from several days to weeks, antibodies can be detected. The first antibodies produced are IgM, then the cells 'switch class' to secrete IgG antibodies. Unless there is a further exposure to the foreign antigen, the IgM production ceases but IgG antibody continues to be produced, possibly for many years.

If the same antigen is recognised again, because some T and B-cells have become non-antibody secreting 'memory cells', this secondary antibody response does not have a lag-phase and IgG antibodies are produce immediately.

However, some IgM antibodies are produced without any T-cell involvement and these are usually directed to polysaccharide antigens that have multiple identical repeats in their structure, such as A and B antigens. Antibody production continues only when there is continuing exposure to the antigens. These antibodies are normally 'naturally acquired', as opposed to the 'immune antibodies' that are predominately IgG, and produced in response to a recognised sensitising event, such as a blood transfusion.

ABO antibodies are mostly IgM, of the T-cell independent type, but there is often an IgG component that has been stimulated by exposure to antigens on foreign red cells or, classically, A and B like antigens in horse-serum tetanus vaccines. Generally levels of IgG and IgM antibodies are higher in people living in the tropics than in temperate climates as they are exposed to more potential infections, bacterial, viral and protozoal.

Pregnancy and the transfusion of blood can both lead to sensitisation and production of antibodies to foreign antigens on red and white blood cells that enter the blood stream either directly in transfusions or by a leak of fetal cells into the maternal circulation, usually at the time of birth. This does not happen on all occasions someone is pregnant or transfused but the risk of producing antibodies increases with repeated transfusions and pregnancies. The clinical significance of antibodies stimulated by transfusion is considered in this paper.

RED CELL ANTIBODIES

Red cell antigens differ in their ability to initiate an antibody immune response; some are more potent, or *immunogenic,* than others. Based on observations of the frequency with which different antibodies occurred in post-transfused patients, compared with the calculated frequency of the opportunity for immunisation to occur, Giblett in 1969,[1] estimated the relative potency of different antigens (table 1). Although these data were based on observations in a hospital in the United States where the frequency of antigens in that mainly Caucasian population differ from blood group frequencies in Africa, they are still valid. Although more recently Tormey and Stack[2] have observed that some antigens have a greater immunogenicity than proposed by Giblett, especially for Jk^a, which they calculated was five times higher. The prevalence of antibodies found in transfused patients mirrors the estimated immunogenicity, except that in countries where anti-D immunoglobulin is routinely given to RhD negative women delivering a RhD positive infant, anti-D is now relatively less frequent (table 1).

TABLE 1: Relative antigen immunogenicity & antibody frequency

Antigen	Relative Immunogenicity*	Order of antibody frequency
D	0.84	7**
K	0.05	2
E	0.013	1
e	0.008	***
Fy^a	0.005	5
c	0.005	6
Jk^a	0.004	4

* Data from Giblett 1969 (1)
** Since the widespread use of post-partum anti-D immunoglobulin the frequency of anti-D has declined.
*** As only 2% of the population is e-negative the numbers capable of producing anti-e is small

Immune antibodies can cause problems when trying to find compatible blood for future transfusions or could lead to haemolytic disease of the fetus/newborn (HDN) in future pregnancies. But not all antibodies have the same clinical significance; those that are the most immunogenic in initiating an immune response, generally lead to greater increased red cell destruction *in vivo.*

It is because anti-D is readily produced by RhD negative individuals that encounter RhD positive red cells, by transfusion or fetal-maternal haemorrhage, and it is an antibody that can cause severe haemolytic disease of the newborn or a haemolytic transfusion reaction, that we routinely test donors and potential recipients for the D antigen. Where possible, RhD negative patients should be given RhD negative blood; this being of particular importance for females of child bearing potential, to prevent them making anti-D.

CLINICAL SIGNIFICANCE OF ALLOANTIBODIES

Although most antibodies once produced remain detectable for many years, one notable exception to this are Kidd antibodies [anti-Jk^a, -Jk^b], that become undetectable using standard serological techniques within months rather than years. As the antibodies are not detected during pre-transfusion testing, these patients might be given blood that is 'serologically compatible' but 'antigenically incompatible'. In the resultant secondary antibody response, antibodies are quickly produced and bind to the incompatible transfused red cells that are removed from the circulation, leading to a haemolytic transfusion reaction noted 6 to 10 days post-transfusion, with the patients Hb returning to pre-transfusion levels as the incompatible cells are destroyed. This is a typical 'delayed' haemolytic transfusion reaction. Very often the patient presents with no more than slight jaundice and an unexpected fall in haemoglobin but sometimes they will complain of also feeling unwell with a raised temperature. Correction of the Hb with compatible blood is the only treatment normally required but on rare occasions there might be some renal impairment that needs treatment.

If a patient already has serologically detectable antibodies directed to antigens on the red cells being transfused, i.e. the blood is incompatible, then red cells destruction will commence immediately - an immediate or acute haemolytic transfusion reaction. If the transfused blood is ABO incompatible then then recipient's IgM anti-A/B will activate complement and the cells will be lysed within the circulation – intravascular heamolysis. In severe cases this can result in renal failure, disseminated intravascular coagulation (DIC) or death. If a recipient has an pre-existing IgG antibody, such as anti-S, that was not detected in the pre-transfusion testing, the red cell destruction will not be so dramatic, as the incompatible cells, coated with the IgG antibody will be removed more slowly by the spleen – extravascular haemolysis. However, there can be serious complications such as renal failure that needs urgent treatment but more commonly other signs of haemolysis are observed, such as anaemia and jaundice.

Hyperhaemolyic transfusion reaction (HHTR) or *hyperhaemolysis syndrome* (HHS) is a rare but sometimes fatal complication of red cell transfusions in which both the transfused and the recipients own red cells are destroyed causing the Hb to fall below pre-transfusion levels. Most cases reported have been in patients with sickle cell disease; in 2013 six such cases were reported in the UK to SHOT – the national haemovigilance scheme.[3] In the acute form haemolysis occurs within 7 days of transfusion but a causative antibody is not found. In the chronic form, occurring after 7 days, newly formed, causative antibodies can be found. The mechanism for the haemolysis of both the donor and recipient cells is not fully understood, but it is usually accompanied by a low reticulocyte count and high ferritin levels that resolve during recovery.[4]

Another consequence of receiving antigenically incompatible blood is that the recipient is stimulated to produce a new antibody. As there is a lag phase, of some six weeks, before antibody is detectable in the blood then the red cells that stimulated the antibody will have been removed naturally from the circulation and therefore, not affected by this new antibody. However, if this person receives another antigenically incompatible transfusion then a haemolytic reaction could result. This is why it is important that blood is crossmatched by an indirect antiglobulin technique to detect even weak antibodies, and only serologically compatible blood is transfused.

ANTIBODY PREVALENCE WORLDWIDE

There have been many papers published from Europe or North America with data on antibody production post-transfusion, but only a few African studies. In a study from Uganda,[5] of 214 multi-transfused patients, 13 [6.1%] had antibodies, including 6 with anti-E and 2 anti-S. Eleven of the patients with antibodies had experienced up to 10 transfusion episodes. In a smaller series of 58 cases in Cameroon,[6] alloantibodies were detected in 10 out of 23 multiply transfused individuals, and 2 of 35 multiparous women. No details of the specificity of the antibodies were given.

A study of 1,000 randomly selected general hospital patients in Malawi[7] showed that 1.1% had antibodies; anti-D and anti-S being the significant antibodies. However, three of these patients had not been transfused but were multiparous, with just one case of anti-S in a patient who had received a single transfusion and two pregnancies. A recent European study[8] showed that 1.8% of patients who had never been previously transfused produced an antibody after transfusion. Overall the incidence of antibody formation was 1.0% at five units of blood transfused; 2.4% at 10 units; 3.4% at 20 and 6.5% at 40 units.

In a 2013 UK study[9] of 590 transfused patients who between them received 2,253 units of blood, 20 [4.4%] produced a new clinically significant antibody with anti-E, anti-c and anti-K predominating. [Anti-c and anti-E often occur together; anti-c+E].

An earlier study from 1996[10] found that in 319 patients, who were not immunocompromised, with no previous history of pregnancy or transfusion, the rate was 5%, with 75% of the antibodies having Rh and K specificity. Most antibodies did not appear until after 6 weeks post-transfusion, some at late as 24 weeks. In patients who have been previously transfused and already sensitised, antibodies can be detected within 2 weeks of transfusion.

UK SHOT data for 2013[3] on alloimmunisation showed as similar picture, with 1-2 % of all recipients producing a new antibody; anti-E was found in 21% of cases, anti-K in 17%, Rh antibody mixtures in 17%, anti-c in 6%, anti-Jk^a in 16%, and other specificities in 1 or 2% (table 2). Therefore, apart from anti-D, the prevalence of antibodies has changed little over the years. The same 2013 SHOT report also presented data on delayed haemolytic reactions. In 58% of cases, Kidd antibodies were implicated [9 examples of anti-Jk^a, 5 of anti-Jk^b], again consistent with previous data.

TABLE 2: New alloantibodies produced post-transfusion - UK SHOT data 2013[3]

Specificity: anti-	Number [n=113]	Percentage [%]
E	21	18.5
K	17	15.2
Rh mixture	17	15.2
Jka	16	14.2
Fya	8	7.2
c (+E)	6	5.3
D	3	2.6
Fyb	3	2.6
Lua	3	2.6
Kpa	3	2.6
Jkb	2	1.7
Cw	2	1.7
S, C, Lea	1 of each	2.6
Other mixtures	9	8.0

In studies on groups of patients that have received many transfusions, such as in sickle cell disease, the prevalence of antibodies is much higher than for a general hospital patient population. Figures of 18 -47% have been reported for sickle cell patients[11] and between 5.2 and 23% for thalassaemia patients,[12] in both groups most antibodies having Rh and K specificities.

Data from the Far East show there is more variation in antibody prevalence than in Europe or Africa. In a seminal paper from Taiwan[13] the authors reported that most blood group antigens are very homozygous amongst Chinese people and that clinically significant antibodies were only found in 0.146% on pre-transfusion testing; anti E being the most common, followed by anti-c+E or anti-c. However, it was subsequently found that the incidence of what is normally regarded as a low frequency antigen of the Miltenberger group, MiIII, was 7.3% in Taiwan and the corresponding antibody anti-"Mi^a" was the most frequently occurring antibody, found in 0.28% of Chinese patients[14] and capable of causing haemolytic disease of the new born and a haemolytic transfusion reaction. Red cells used for antibody screening in some Far Eastern countries are selected so that will detect these antibodies whereas those used for Caucasian populations do not. There is no evidence that antibodies of these specificities are important in Africa.

However some Rh antigens, such as VS, are found predominately in Black Africans and the antibodies, as with most Rh antibodies, are clinically significant causing both HDN and transfusion reactions. The lack of common antigens can cause problems in all racial groups if a patient produces an antibody that will only be compatible with those lacking the same antigen. In Africans the frequency of the S-s-U- phenotype is about 1% (<0.1% in Caucasians) but as these individuals can make anti-U, a clinically significant antibody, finding compatible blood is difficult.

Another major difference between African and Caucasian blood group distribution is the phenotype Fy(a-b-); this is very rare in Caucasians but reported to be 68% in Black African populations, but a much higher figure of 97.2% in Malawi.[7] These individuals can produce an antibody called anti-Fy^3, a clinically significant IgG antibody. However finding compatible blood among Black African donors would be far less difficult than among European donors.

In the limited African studies, it has been found that anti-S is more prevalent than in European or North American transfused populations. As the incidence of the K antigen in Black Africans is low, <1% in Malawi,[7] then the likelihood of anti-K being stimulated is also low, whereas in the UK it is the commonest antibody after anti-E. Antibody prevalence in general hospital populations depends of the type of patients being treated. In Europe many patients are elderly with malignant diseases; in some parts of Africa a large proportion are HIV/AIDS cases receiving ART, many of whom need transfusions. In other sub-Saharan African countries, children with malaria and women with obstetric complications predominate. It is generally held that few people in Africa have repeated transfusions, however, the Malawi study[7] showed 10% of the hospital patients had been previously transfused.

DISCUSSION

Where donors and recipients are ethnically homogeneous there are less blood group antigen differences than in ethnically diverse populations such as those found in some European countries and the USA, and the risk of being stimulated to a 'foreign' antigen is less. In some countries patients with conditions requiring repeated transfusions, such as sickle cell disease and thalasaemia, routinely receive units matched for their Rh and K status to reduce exposure to 'foreign' antigens and antibody production. Pre-transfusion testing would also include an antibody screen which if positive, would be followed-up with tests to determine the antibody specificity. Units of blood negative for the corresponding antigen, would then be selected and crossmatched by an indirect antiglobulin technique [IAT]. However, not all Blood Services are able to perform these tests therefore, it is essential that blood is crossmatched using an IAT, and if incompatible, further units tested to find some that are compatible. If problems in finding compatible blood have been previously encountered, then adequate notice should be given by the requesting medical officer to enable sufficient units to be fully crossmatched and compatible blood found. Except in cases where the Hb is below 4 g/dL, a delay in transfusing will not usually harm the patient but giving incompatible blood could have serious sequalae.

Planned and appropriate transfusions, with enough time to perform full pre-transfusion tests and select compatible blood, will reduce untoward or adverse side effects and improve transfusion safety. When devising national transfusion policies, especially for the selection of blood and for pre-transfusion testing, knowledge of the local prevalence of antibodies in patients is important, as are the reasons for transfusion and the reports of transfusion reactions. These data will also inform of the need, or otherwise, for Blood Services to make available blood phenotyped for antigens other than ABO and RhD for patients who are multiply transfused. These studies need to be undertaken in Africa and the results published.

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