

Responder Individuality in Red Blood Cell Alloimmunization

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Summary

Many different factors influence the propensity of transfusion recipients and pregnant women to form red blood cell alloantibodies (RBCA). RBCA may cause hemolytic transfusion reactions, hemolytic disease of the fetus and newborn and may be a complication in transplantation medicine. Antigenic differences between responder and foreign erythrocytes may lead to such an immune answer, in part with suspected specific HLA class II associations. Biochemical and conformational characteristics of red blood cell (RBC) antigens, their dose (number of transfusions and pregnancies, absolute number of antigens per RBC) and the mode of exposure impact on RBCA rates. In addition, individual circumstances determine the risk to form RBCA. Responder individuality in terms of age, sex, severity of underlying disease, disease- or therapy-induced immunosuppression and inflammation are discussed with respect to influencing RBC alloimmunization. For particular high-risk patients, extended phenotype matching of transfusion and recipient efficiently decreases RBCA induction and associated clinical risks.

Introduction

Routinely, red blood cell (RBC) transfusions are administered only after phenotype matching with respect to ABO and RhD type of the recipient. In addition, also unexpected

RBC alloantibodies (RBCA) potentially present in the recipient's plasma have to be considered as transfusion of RBCs carrying corresponding antigens may lead to hemolysis. Immune RBCA are formed upon exposure to allogenic RBCs expressing numerous non-self blood group antigens via transfusion, pregnancy or different types of transplantation. Prerequisites for RBC alloimmunization are that the foreign RBCs express the antigen which is genetically absent in the alloexposed individual and that the HLA class II molecules of the latter are capable of presenting a RBC antigen-derived peptide with a variant amino acid present in the donor or fetus but not in the challenged individual. A total of 35 blood group systems and more than 330 different blood group antigens are responsible for the enormous diversity of RBCA specificities [1]. Especially antibodies against Rh, Kell, Duffy, Kidd, and a number of other blood group antigens are regarded clinically significant: depending on specificity, immunoglobulin class and clinical context, such RBCA may elicit not only hemolytic transfusion reactions but also potentially fatal hemolytic disease of the fetus and newborn [2–5]. RBCA may also complicate hematopoietic stem cell and solid organ transplantation [6, 7]. Generally, hemolytic transfusion reactions due to RBCA are a leading cause of transfusion-related mortality and morbidity [8] and have considerable incidence in particular patient cohorts dependent on regular transfusions [9]. Aside from 'immune' RBCA, also so-called 'naturally occurring' RBCA may develop without any prior exposure to foreign RBCs. The stimulus for most of these antibodies remains obscure; antigenic similarities of environmental or microbial substances with RBC antigens may give rise to their production. Some naturally occurring non-ABO RBCA may be of clinical significance, although many are only cold reactive [10].

Nature and Dose of Red Blood Cell Antigens

The alloantigenic epitopes can exist in carbohydrate or in protein structure. The immune answer against the carbohydrate antigens is T-cell-independent and produces mainly IgM antibodies. This situation will not be considered in this article which limits itself on polypeptide antigens. It is well-known that some blood group antigens are more immunogenic than others, with the highest alloimmunization potential for the D antigen [11, 12]. Among the most commonly reported RBCA specificities in different study cohorts are D, K, E, Fy^a, and Jk^a [10, 13–15]. Generally, the immune response against protein antigens of the erythrocyte surface is not very prominent. A major exception is the immunogenicity of the D antigen which is very high, as a major proportion of D-negative individuals receiving D-positive blood will produce anti-D [10]. The other protein alloantigens show a much lesser immunogenicity: anti-K is only produced in about 10% of incompatibilities, anti-E in 7%, anti-c in 3%, while the immunogenicity of the other specificities is so low that antibodies are only produced in less than 3% of incompatibilities [11, 15].

The probable reason for the high immunogenicity of D and the rather low antigenic strength of the other alloantigens is the fact that D-negative individuals usually carry a deletion of the *RHD* gene in homozygous form and that the RhD polypeptide shows more than 30 amino acids which are not present in the gene product of the *RHCE* gene of D-negative individuals. In the other blood group systems, the differences between alleles are mostly governed by single nucleotide polymorphisms (SNPs) which change only one amino acid, thus giving rise to only minor difference between their gene products [16, 17]. Nevertheless, even among antigens encoded by SNPs, a rank order of immunogenicities exists that shows an astonishingly wide spectrum: for example, Jk^b was calculated to be about 250 and 90 times less immunogenic than K and Jk^a, respectively [18]. The reason for such difference is poorly understood.

It is well known that some antibody specificities are long lasting (e.g., anti-D), while other ones are only detectable during a rather short period of time (e.g., anti-Jk^a). Disappearing RBCA specificities may confound pretransfusion compatibility testing and predispose patients to delayed hemolytic transfusion reactions [19]. Many RBCA specificities escape detection because currently there is no standard procedure to check for the production of alloantibodies after potentially immunizing events (time interval after transfusion or pregnancy, number of tests, intervals between tests).

Aside from the conformational nature of foreign blood group antigens, also their dose is an important denominator for RBC alloimmunization. Its rate is roughly proportional to the number of the transfusions received [20–22]. Not surprisingly, patients suffering from diseases that depend on chronic transfusion support have especially high RBCA rates [18, 23]. However, no significant difference was seen in patients with

intensive transfusion treatment within short time and those with moderate transfusion amount [24].

The blood group antigen dose per RBC may well be a further variable of RBC alloimmunization. Different RBC antigens feature characteristic antigen densities, varying widely from only a few to more than a million antigens per RBC [1]. For example, the highly immunogenic D antigen features an antigen density of 10,000–30,000 D sites per RBC, whereas different weak D variants and DEL types encompass the broad spectrum from a few thousand down to around 30 D sites per cell [25, 26]. Even the weakest D variants were demonstrated to cause anti-D alloimmunization in D-negative individuals [27, 28], but it seems that this happens rarely compared to challenge with normal D [29–31].

In theory, the immunogenicity of RBC antigens upon blood product storage may change. However, in a recent study, no effect of RBC storage on alloimmunization was seen [32].

HLA Genetics and Red Blood Cell Alloimmunization

Briefly, in the immune response against the protein antigens, the erythrocytes are phagocytosed by macrophages, monocytes or dendritic cells, and the proteins carrying the alloantigenic epitopes are processed, mounted in the groove of HLA class II molecules and presented by these cells to the T-cell receptors of CD4-positive T cells. These T cells activate B lymphocytes which are also capable to recognize the antigenic epitopes; the activated B cells subsequently produce antibodies against the correspondent epitopes. In contrast to the peptides presented by HLA class I molecules which encompass 8–10 amino acids, the peptides from HLA class II molecules are longer. For HLA class I peptides, the entire molecule is involved in the binding interaction with HLA while for HLA class II peptides, the region interacting with HLA is nearly in the center of the peptide and contains also 8–10 amino acids.

An association between HLA specificities and the alloimmunization against RBC antigens can therefore be only observed if the HLA molecule is able to present peptides carrying the alloantigenic epitopes. This capability has already been taken into account in the analysis of the influence of HLA on the immune response against erythrocyte alloantigens. Associations between HLA and the alloimmunization against various RBC specificities are listed in table 1.

This table must be considered with great caution: like all other analyses of the association of HLA factors and biological phenomena, many pitfalls have to be avoided, e.g. the ethnic fit between the study population and the controls, statistical problems (the correction according to Bonferroni has to be included in order to avoid false-positive associations), the fact that a positive association should be observed in more than one ethnic group, etc.

Taking into account these considerations, some associations seem to emerge which could have a biologic back-

Table 1. Associations between HLA and the alloimmunization against various red cell antigens

RBC alloimmunization: anti-	Associated HLA	Population	Peptide binding prediction	Reference
D	DRB1*15	Caucasoid	yes	Quoted by Hall et al. 2005 [64]
D	DRB1*06	Caucasoid	no	Darke et al. 1983 [65]
D	DRB1*06	Caucasoid	no	Wojtulewicz-Kurkus et al. 1981 [66]
D – high titer	DOB1*02:01	Caucasoid	no	Hilden et al. 1995 [67]
D – high titer	DRB1*15	Caucasoid	no	Verhagen et al. 2013 [68]
D	Nil	Caucasoid	no	Hors et al. 1974 [69]
D	Nil	Caucasoid	no	Petranyi et al. 1975 [70]
D	Nil (with corrected p)	Indian	no	Kumar et al. 2002 [71]
E	DRB1*09	Oriental	no	Lin et al. 2014 [72]
K	DRB1*11, DRB1*13	Caucasoid	yes	Chiaroni et al. 2006 [73]
K	DRB1*13	Caucasoid	yes	Noizat-Pirenne et al. 2006 [74]
Fy ^a	DRB1*04	Caucasoid	yes	Noizat-Pirenne et al. 2006 [74]
Fy ^a	DRB1*04	Caucasoid	no	Raos et al. 2014 [75]
Jk ^a	DRB1*01	Caucasoid	yes	Ansart-Pirenne et al. 2004 [76]
Jk ^a	DRB1*07:01	Caucasoid	yes	Reviron et al. 2005 [77]
S	DRB1*07	Caucasoid	no	Schonewille et al. 2014 [33]
Mi ^a	DRB1*09:01	Oriental	yes	Chu et al. 2009 [78]
Di ^a	DRB1*07:01	Brazilian	yes	Baleotti Jr et al. 2014 [79]

ground: alloimmunization against D and HLA-DRB1*15, K and DRB1*13, as well as Fy^a and DRB1*04. These associations are corroborated by the analysis of the peptides obtained from the immunizing alloantigens which could be presented by the relevant HLA class II molecules. Other associations have to be confirmed in further studies.

Besides these specific associations, some investigations indicate that HLA class II genes could influence the general responsiveness to alloantigens: HLA-DRB1*15 seems to be a marker for multiple antibody responders and high responders [33, 34]. Other HLA genes could have a protective function, e.g. HLA-A*23 [35].

The ‘Grandmother Effect’

Due to a high frequency of microchimerism in the mother and her newborn child, a possible effect of non-inherited maternal antigens (NIMAs) on the immune response has been of some interest [36]. Concerning the alloimmunization against RBC antigens, it would be important to see whether the immunization of a D-negative mother by the D antigen of her child depends on the RhD status of the grandmother. Theoretically, a *RHD*-heterozygous grandmother could reduce via the NIMA effect the rate of anti-D immunization of the D-negative mother (the D-positive erythrocytes of the grandmother carrying the NIMA could induce a state of low responsiveness against D in the mother which considers this antigen as self; in contrast, such an effect could not be induced by the RBCs of a D-negative grandmother). Until now, several studies have been published which, however, could not

demonstrate the existence of a ‘Grandmother Effect’; the alloimmunization against RBC antigens therefore seems to be independent of the influence of NIMAs.

Clinical Factors Influencing Red Blood Cell Alloimmunization

Transfusion-induced alloimmunization depends not only on dose and immunogenicity of the antigen but also on clinical patient-related variables [37]. Female sex was reported to be associated with a higher alloimmunization rate [38, 39]. However, a generally higher post-transfusion response rate for women could not be confirmed in a recent meta-analysis but was rather attributed to more allogenic exposure mainly because of pregnancy [40].

The clinical condition of the recipient appears to profoundly influence the rate of RBC alloimmunization. Less than 0.3% of apparently healthy blood donors have RBCA in their plasma [3, 10], as compared to 1–2% of mixed patient cohorts [10, 15, 41]. The posttransfusion alloimmunization rate of surgery patients is in the range of 2.5–3.3% [10, 42], and may approach 6% if naturally occurring RBCA of doubtful clinical significance are included [43]. Likewise, 2.8% of acute myelogenous leukemia patients exhibit RBCA after transfusion [44]. Extremely high RBCA prevalences (up to more than 30%) are seen in chronically transfused patients suffering from myelodysplastic syndrome, thalassemia, sickle cell, or autoimmune hemolytic disease [9, 10, 18, 39, 45]. Extended phenotype matching of transfusions with respect to antigens like Rh CcEe, K, Fy and Jk antigens results in considerable reduction

of the RBCA incidence of such chronically transfused patients [46, 47]. Further risk factors for RBCA formation include diabetes mellitus, solid malignancy as well as allogenic hematopoietic stem cell transplantation with establishment of a functional foreign immune system [20, 38].

Conversely, disease- or therapy-associated immunosuppression results in decreased or even abolished tendency to develop RBCA [22, 48, 49]. Especially immunosuppressed solid organ transplant recipients subjected to immunosuppressive protocols to avoid or treat graft rejection have a markedly impaired alloimmune response with minimal RBCA rates [50–52]. This holds true also for chronic renal failure patients on hemodialysis, even when chronically transfused [45, 53].

Obviously, individual immune competence determines the potential to respond to RBC antigen challenge. After administration of D-positive RBC transfusions, D-negative apparently healthy volunteers formed anti-D in more than 80% [54, 55]. In patients, the anti-D alloimmunization rate is far lower, probably depending on the severity of their disease or immunosuppression. Only 16 of 78 (21%) D-negative patients of a mixed disease cohort showed anti-D after D-positive transfusions [12]. AIDS patients may not form anti-D after D-mismatched transfusions at all, possibly attributable to their immunosuppressed state with CD4-positive T-cell deficiency [48]. Likewise, also preterm infants known to have an immature immune system are unlikely to form RBCA [56]. It can be suspected that also very old patients have a blunted immune response towards RBC antigens; however, part of such reduced alloimmunization tendency will be counterbalanced by a higher risk for secondary immunization based on anamnestic exposure throughout earlier decades of life.

The Effect of Inflammation on Red Blood Cell Alloimmunization

Generally, alloimmunization against RBC antigens is one of the most frequent adverse reactions of transfusion. Recent evidence points towards an essential role of inflammation in RBCA acquisition. Animal studies indicate that the inflammatory status of the recipient at the time of transfusion has a pivotal role in triggering RBC alloimmunization. In murine models of RBC transfusion, artificially induced inflammation showed an association with increased RBCA production [57]. Moreover, also enhanced RBC antigen presentation by dendritic cells and more pronounced proliferative responses of CD4-positive T cells were encountered, compared to animals without inflammation [58]. Recipient inflammation also transformed RBC alloimmune nonresponders into responders, while transfusion in the absence of inflammation seems to result in tolerance to RBC antigens [59].

Importantly, more recent studies in humans also demonstrate an association of acute inflammation with a higher propensity for RBCA acquisition: as many as 8% of transfusion

recipients with a cytokine-mediated febrile reaction formed RBCA, as compared to only 3% of recipients without signs of inflammation [60].

Recently, the influence of chronic inflammation on RBCA induction was investigated [22]. Patients with inflammatory bowel disease (IBD) exhibit long-term or intermittent immune activation and frequently feature anemia, often with the necessity for transfusion [61]. IBD patients with a history of transfusion or pregnancy were found to have an exceptionally high risk of RBC alloimmunization, compared to transfused patients with noninflammatory diseases (8.4% vs. 3.4%). Of note, this striking difference was observed despite the fact that the IBD patients had received significantly fewer transfusions; moreover, there was a marked age difference between IBD patients and controls (median 31 years vs. 65 years), inevitably with a higher life-time exposure risk regarding transfusion and pregnancy of the latter. It may be suspected that chronic inflammation and possibly a higher immune competence of the younger IBD patients could trigger alloimmunization. On the other hand, therapeutic immunosuppression appeared to mitigate RBCA formation in this patient cohort [22]. Based on this data, extended phenotype matching of transfusions for IBD patients may be advisable to minimize RBCA induction and associated clinical risks.

Aberrant activation of the immune system may well be responsible for the fact that also patients with different other autoimmune diseases such as systemic lupus erythematosus or warm autoimmune hemolytic anemia are prone to RBC alloimmunization [10, 62]. Moreover, the particularly high RBCA prevalence of patients suffering from sickle cell disease may not only be caused by chronic transfusion need but also by their chronic inflammatory state with increased inflammatory cytokines stimulating antigen-presenting cell activities [63].

Conclusion

Summing up the data, it can be stated that many genetic and non-genetic factors influence the alloimmunization against RBC antigens. Due to the low immunogenicity of all gene products with the exception of A, B and D, the matching for these factors seems to be sufficient for sporadic transfusions in the majority of cases. In order to avoid the induction of RBCA, an extended matching of Rh CcEe, K, Fy and Jk antigens can be considered for patients at risk, i.e. polytransfused patients, female individuals younger than 50 years, or probably patients with chronic inflammation. For these patient cohorts, such extended phenotype matching would increase transfusion safety by minimizing the clinical risks associated with RBCA.

Disclosure Statement

The authors have no conflict of interest relating to this work.

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