Responder Individuality in Red Blood Cell Alloimmunization

Günther F. Körmöczí · Wolfgang R. Mayr

Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Austria

Keywords

Alloimmunization · Red blood cell antibodies ·
Red blood cell antigens

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.

References

- Daniels G: Human Blood Groups, 3rd ed. Oxford, Wiley-Blackwell, 2013.
- Reid ME, Lomas-Francis C, Olsson ML: The Blood Group Antigen Facts Book, 3rd ed. London, Elsevier, 2012.
- Poole J, Daniels G: Blood group antibodies and their significance in transfusion medicine. *Transfus Med Rev* 2007;21:58–71.
- Eder AF: Update on HDFN: New information on long-standing controversies. *Immunohematology* 2006;22:188–195.
- Nordvall M, Dziegiel M, Hegaard HK, Bidstrup M, Jonsbo F, Christensen B, Hedegaard M: Red blood cell antibodies in pregnancy and their clinical consequences: synergistic effects of multiple specificities. *Transfusion* 2009;49:2070–2075.
- Lopez A, de la Rubia J, Arriaga F, Jimenez C, Sanz GF, Carpio N, Marty ML: Severe hemolytic anemia due to multiple red cell alloantibodies after an ABO-incompatible allogeneic bone marrow transplant. *Transfusion* 1998;38:247–251.
- Rourk A, Squires JE: Implications of the Kidd blood group system in renal transplantation. *Immunohematology* 2012;28:90–94.
- Hendrickson JE, Tormey CA, Shaz BH: Red blood cell alloimmunization mitigation strategies. *Transfus Med Rev* 2014;28:137–144.
- Matteucci A, Pierelli L: Red blood cell alloimmunization in sickle cell disease and in thalassaemia: current status, future perspectives and potential role of molecular typing. *Vox Sang* 2014;106:197–208.
- Klein HG, Anstee DJ: Mollison's Blood Transfusion in Clinical Medicine, 11th ed. Oxford, Blackwell, 2005.
- Tormey CA, Stack G: Immunogenicity of blood group antigens: a mathematical model corrected for antibody evanescence with exclusion of naturally occurring and pregnancy-related antibodies. *Blood* 2009;114:4279–4282.
- Frohn C, Dumbgen L, Brand JM, Gorg S, Luhm J, Kirchner H: Probability of anti-D development in D- patients receiving D+ RBCs. *Transfusion* 2003;43:893–898.
- Issitt PD, Anstee DJ: Applied Blood Group Serology, 4th ed. Durham, Montgomery Scientific Publications, 1998.
- Schonewille H, van de Watering LM, Loomans DS, Brand A: Red blood cell alloantibodies after transfusion: factors influencing incidence and specificity. *Transfusion* 2006;46:250–256.
- Tormey CA, Fisk J, Stack G: Red blood cell alloantibody frequency, specificity, and properties in a population of male military veterans. *Transfusion* 2008;48:2069–2076.
- Daniels G: The molecular genetics of blood group polymorphism. *Hum Genet* 2009;126:729–742.
- Ansart-Pirenne H, Rouger P, Noizat-Pirenne F: Cellular mechanisms implicated in anti-erythrocyte alloimmunization. *Transfus Clin Biol* 2005;12:135–141.
- Mintz PD (ed): Transfusion Therapy. Clinical Principles and Practice, 3rd ed. Bethesda, AABB Press, 2011.
- Tormey CA, Stack G: The persistence and evanescence of blood group alloantibodies in men. *Transfusion* 2009;49:505–512.
- Fluit CR, Kunst VA, Drenthe-Schonk AM: Incidence of red cell antibodies after multiple blood transfusion. *Transfusion* 1990;30:532–535.
- Zalpur S, Zwaginga JJ, le Cessie S, Elshuis J, Schonewille H, van der Bom JG: Red blood cell alloimmunization and number of red blood cell transfusions. *Vox Sang* 2012;102:144–149.
- Papay P, Hackner K, Vogelsang H, Novacek G, Primas C, Reinisch W, Eser A, Mikulits A, Mayr WR, Körmöczí GF: High risk of transfusion-induced alloimmunization of patients with inflammatory bowel disease. *Am J Med* 2012;125:717 e711–718.
- Sanz C, Nomdedeu M, Belkaid M, Martinez I, Nomdedeu B, Pereira A: Red blood cell alloimmunization in transfused patients with myelodysplastic syndrome or chronic myelomonocytic leukemia. *Transfusion* 2013;53:710–715.
- Zalpur S, Middelburg RA, Schonewille H, de Vooght KM, le Cessie S, van der Bom JG, Zwaginga JJ: Intensive red blood cell transfusions and risk of alloimmunization. *Transfusion* 2014;54:278–284.
- Körmöczí GF, Förstemann E, Gabriel C, Mayr WR, Schönitzer D, Gassner C: Novel weak D types 31 and 32: adsorption/elution-supported D antigen analysis and comparison to prevalent weak D types. *Transfusion* 2005;45:1574–1580.
- Körmöczí GF, Gassner C, Shao CP, Uchikawa M, Legler TJ: A comprehensive analysis of DEL types: partial DEL individuals are prone to anti-D alloimmunization. *Transfusion* 2005;45:1561–1567.
- Wagner T, Körmöczí GF, Buchta C, Vadon M, Lanzer G, Mayr WR, Legler TJ: Anti-D immunization by DEL red blood cells. *Transfusion* 2005;45:520–526.
- Gassner C, Doescher A, Drnovsek TD, Rozman P, Eicher NI, Legler TJ, Lukin S, Garritsen H, Kleirath T, Egger B, Ehling R, Körmöczí GF, Kilgannogler S, Schönitzer D, Petershofen EK: Presence of RHD in serologically D-, C/E+ individuals: a European multicenter study. *Transfusion* 2005;45:527–538.
- Garratty G: Do we need to be more concerned about weak D antigens? *Transfusion* 2005;45:1547–1551.
- Schmidt PJ, Morrison EG, Shohl J: The antigenicity of the Rho (Du) blood factor. *Blood* 1962;20:196–202.
- St-Louis M, Lebrun A, Goldman M, Lavoie M: Alloimmunization of patients by blood units harboring distinct DEL variants. *Immunohematology* 2013;29:136–140.
- Zalpur S, Schonewille H, Middelburg R, van de Watering L, de Vooght K, Zimring J, van der Bom JG, Zwaginga JJ: Effect of storage of red blood cells on alloimmunization. *Transfusion* 2013;53:2795–2800.
- Schonewille H, Doxiadis II, Levering WH, Roelen DL, Claas FH, Brand A: HLA-DRB1 associations in individuals with single and multiple clinically relevant red blood cell antibodies. *Transfusion* 2014;54:1971–1980.
- Sippert EA, Rodrigues C, Gilli SCO, Visentainer JEL, Castilho I: HLA alleles and red blood cell alloimmunization in polytransfused patients with sickle cell disease. *Vox Sang* 2014;107(suppl 1):198.
- Brunker PA: Chimerism in transfusion medicine: the grandmother effect revisited. *Chimerism* 2013;4:119–125.
- Hendrickson JE, Hillyer CD: Noninfectious serious hazards of transfusion. *Anesth Analg* 2009;108:759–769.
- Bauer MP, Wiersum-Osselton J, Schipperus M, Vandenbroucke JP, Briet E: Clinical predictors of alloimmunization after red blood cell transfusion. *Transfusion* 2007;47:2066–2071.
- Ameen R, Al-Shemmari S, Al-Humood S, Chowdhury RI, Al-Eyaadi O, Al-Bashir A: RBC alloimmunization and autoimmunization among transfusion-dependent Arab thalassemia patients. *Transfusion* 2003;43:1604–1610.
- Verduin EP, Brand A, Schonewille H: Is female sex a risk factor for red blood cell alloimmunization after transfusion? A systematic review. *Transfus Med Rev* 2012;26:342–353, 353 e1–5.
- Winters JL, Pineda AA, Gorden LD, Bryant SC, Melton LJ 3rd, Vamvakas EC, Moore SB: RBC alloantibody specificity and antigen potency in Olmsted County, Minnesota. *Transfusion* 2001;41:1413–1420.
- Heddle NM, Soutar RL, O'Hoski PL, Singer J, McBride JA, Ali MA, Kelton JG: A prospective study to determine the frequency and clinical significance of alloimmunization post-transfusion. *Br J Haematol* 1995;91:1000–1005.
- Redman M, Regan F, Contreras M: A prospective study of the incidence of red cell allo-immunisation following transfusion. *Vox Sang* 1996;71:216–220.
- Blumberg N, Heal JM, Gettings KF: WBC reduction of RBC transfusions is associated with a decreased incidence of RBC alloimmunization. *Transfusion* 2003;43:945–952.
- Stiegler G, Sperr W, Lorber C, Fabrizio V, Hocker P, Panzer S: Red cell antibodies in frequently transfused patients with myelodysplastic syndrome. *Ann Hematol* 2001;80:330–333.
- Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP: Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. *Blood* 2000;96:3369–3373.
- Lasalle-Williams M, Nuss R, Le T, Cole L, Hassell K, Murphy JR, Ambruso DR: Extended red blood cell antigen matching for transfusions in sickle cell disease: a review of a 14-year experience from a single center. *Transfusion* 2011;51:1732–1739.
- Boctor FN, Ali NM, Mohandas K, Uehlinger J: Absence of D alloimmunization in AIDS patients receiving D-mismatched RBCs. *Transfusion* 2003;43:173–176.
- Zalpur S, Evers D, Zwaginga JJ, Schonewille H, de Vooght KM, le Cessie S, van der Bom JG: Immunosuppressants and alloimmunization against red blood cell transfusions. *Transfusion* 2014;54:1981–1987.
- Casanueva M, Valdes MD, Ribera MC: Lack of alloimmunization to D antigen in D-negative immunosuppressed liver transplant recipients. *Transfusion* 1994;34:570–572.
- Yuan S, Davis R, Lu Q, Goldfinger D, Ziman AF: Low risk of alloimmunization to the D antigen in D- orthotopic liver transplant recipients receiving D+ RBCs perioperatively. *Transfusion* 2008;48:2653–2655.
- Cummins D, Contreras M, Amin S, Halil O, Downham B, Yacoub MH: Red cell alloantibody development associated with heart and lung transplantation. *Transplantation* 1995;59:1432–1435.
- Habibi B, Lecolier B: Inconvenience of planned prophylaxis of transfusion-induced alloimmunization to red cells in chronic renal insufficiency. *Rev Fr Transfus Immunohematol* 1983;26:267–277.
- Gunson HH, Stratton F, Cooper DG, Rawlinson VI: Primary immunization of Rh-negative volunteers. *Br Med J* 1970;1:593–595.
- Pollack W, Ascari WQ, Crispen JF, O'Connor RR, Ho TY: Studies on Rh prophylaxis. II. Rh immune prophylaxis after transfusion with Rh-positive blood. *Transfusion* 1971;11:340–344.

- 56 Strauss RG, Cordle DG, Quijana J, Goeken NE: Comparing alloimmunization in preterm infants after transfusion of fresh unmodified versus stored leukocyte-reduced red blood cells. *J Pediatr Hematol Oncol* 1999;21:224–230.
- 57 Hendrickson JE, Desmarests M, Deshpande SS, Chadwick TE, Hillyer CD, Roback JD, Zimring JC: Recipient inflammation affects the frequency and magnitude of immunization to transfused red blood cells. *Transfusion* 2006;46:1526–1536.
- 58 Hendrickson JE, Chadwick TE, Roback JD, Hillyer CD, Zimring JC: Inflammation enhances consumption and presentation of transfused RBC antigens by dendritic cells. *Blood* 2007;110:2736–2743.
- 59 Smith NH, Hod EA, Spitalnik SL, Zimring JC, Hendrickson JE: Transfusion in the absence of inflammation induces antigen-specific tolerance to murine RBCs. *Blood* 2012;119:1566–1569.
- 60 Yazer MH, Triulzi DJ, Shaz B, Kraus T, Zimring JC: Does a febrile reaction to platelets predispose recipients to red blood cell alloimmunization? *Transfusion* 2009;49:1070–1075.
- 61 Goodhand JR, Kamperidis N, Rao A, Laskaratos F, McDermott A, Wahed M, Naik S, Croft NM, Lindsay JO, Sanderson IR, Rampton DS: Prevalence and management of anemia in children, adolescents, and adults with inflammatory bowel disease. *Inflamm Bowel Dis* 2012;18:513–519.
- 62 Petz LD, Garratty G: *Immune Hemolytic Anemias*, 2nd ed. Philadelphia, Churchill Livingstone, 2004.
- 63 Yazdanbakhsh K, Ware RE, Noizat-Pirenne F: Red blood cell alloimmunization in sickle cell disease: pathophysiology, risk factors, and transfusion management. *Blood* 2012;120:528–537.
- 64 Hall AM, Cairns LS, Altmann DM, Barker RN, Urbaniak SJ: Immune responses and tolerance to the RhD blood group protein in HLA-transgenic mice. *Blood* 2005;105:2175–2179.
- 65 Darke C, Street J, Sargeant C, Dyer PA: HLA-DR antigens and properdin factor B allotypes in responders and non-responders to the Rhesus-D antigen. *Tissue Antigens* 1983;21:333–335.
- 66 Wojtulewicz-Kurkus J, Zupanska B, Podobinska I, Brojer E: Preliminary evaluation of DR antigens in responders and nonresponders to antigen D from the Rh blood group system. *Arch Immunol Ther Exp* 1981;29:447–450.
- 67 Hilden JO, Gottvall T, Lindblom B: HLA phenotypes and severe Rh(D) immunization. *Tissue Antigens* 1995;46:313–315.
- 68 Verhagen OJHM, Della Valle L, Dohmen SE, Swelsen W, Vidarsson G, de Haas M, van der Schoot E: HLA-DRB1*15 is not strongly linked to RhD immunization risk, but associated with high anti-D titers after hyperimmunisation. *Vox Sang* 2013;105(suppl 1):235.
- 69 Hors J, Dausset J, Gerbal A, Salmon C, Ropartz C, Lanset S: HL-A phenotype and anti-RhO(D) immunization. *Haematologia* 1974;8:217–221.
- 70 Petranyi GG, Ivanyi P, Hollan Z: Genetic relationships between the HI-A system, the blood group systems (Rh, ABO) and the immune reactivity (in German). *Folia Haematol Int Mag Klin Morphol Blutforsch* 1975;102:29–34.
- 71 Kumar US, Ghosh K, Gupte SS, Gupte SC, Mohanty D: Role of HLA antigens in Rh (D) alloimmunized pregnant women from Mumbai, Maharashtra, India. *J Biosci* 2002;27:135–141.
- 72 Lin WC, Perng CL, Chang JS: HLA-DR associations in individuals with red blood cell antibody anti-E. *Vox Sang* 2014;107(suppl 1):200.
- 73 Chiaroni J, Dettori I, Ferrera V, Legrand D, Touinssi M, Mercier P, de Micco P, Reviron D: HLA-DRB1 polymorphism is associated with Kell immunisation. *Br J Haematol* 2006;132:374–378.
- 74 Noizat-Pirenne F, Tournamille C, Bierling P, Roudot-Thoraval F, Le Pennec PY, Rouger P, Ansart-Pirenne H: Relative immunogenicity of Fya and K antigens in a Caucasian population, based on HLA class II restriction analysis. *Transfusion* 2006;46:1328–1333.
- 75 Raos M, Zunec R, Grojceta K, Lukic M, Plankovic F, Golubic Cepulic B: The association of the HLA system polymorphisms with alloimmunization to FyA red blood cell antigens. *Vox Sang* 2014;107(suppl 1):245.
- 76 Ansart-Pirenne H, Zeliszewski D, Lee K, Martin-Blanc S, Rouger P, Noizat-Pirenne F: Identification of immunodominant alloreactive T-cell epitopes on the Jka red blood cell protein inducing either Th1 or Th2 cytokine expression. *Blood* 2004;104:3409–3410.
- 77 Reviron D, Dettori I, Ferrera V, Legrand D, Touinssi M, Mercier P, de Micco P, Chiaroni J: HLA-DRB1 alleles and Jk(a) immunization. *Transfusion* 2005;45:956–959.
- 78 Chu CC, Ho HT, Lee HL, Chan YS, Chang FJ, Wang CL, Lin M: Anti-‘Mi(a)’ immunization is associated with HLA-DRB1*0901. *Transfusion* 2009;49:472–478.
- 79 Baleotti W Jr, Ruiz MO, Fabron A Jr, Castilho L, Giuliatti S, Donadi EA: HLA-DRB1*07:01 allele is primarily associated with the Diego a alloimmunization in a Brazilian population. *Transfusion* 2014;54:2468–2476.