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Red Blood cell Alloimmunization in Sickle Cell Disease: Pathophysiology, Risk Factors, and Transfusion Management

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Abstract

Red blood cell transfusions have reduced morbidity and mortality for patients with sickle cell disease (SCD). Transfusions can lead to erythrocyte alloimmunization, however, with serious complications for the patient including life-threatening delayed hemolytic transfusion reactions (DHTRs) and difficulty in finding compatible units, which can cause transfusion delays. In this review, we will discuss the risk factors associated with alloimmunization with emphasis on possible mechanisms that can trigger DHTR in SCD, and describe the challenges in transfusion management of these patients, including opportunities and emerging approaches for minimizing this life-threatening complication.

Introduction

Blood transfusion remains a cornerstone of treatment of patients with sickle cell disease (SCD). Despite improved patient outcomes with hydroxyurea administration, indications for chronic transfusions have increased in the last ten years and are associated with considerable reduction in morbidity and mortality, most notably in preventing first stroke in children.¹⁻³ However, transfusions can lead to erythrocyte alloimmunization with serious complications for the patient. These antibodies are often directed against antigens expressed on red blood cells (RBC) of Caucasian individuals, which represent the majority of donors in western countries.⁴ Finding compatible units lacking those antigens can sometimes be difficult, and identifying and characterizing the antibodies can be time-consuming and laborious, causing transfusion delays. Genetic as well as acquired patient-related factors are likely to influence the process of alloimmunization.

The most serious consequence of alloimmunization in SCD patients is the risk of developing a delayed hemolytic transfusion reaction (DHTR), which can be life-threatening. In many cases of DHTR in SCD, the patient's hemoglobin level falls below the pretransfusion level, suggesting that in addition to hemolysis of the transfused RBCs, the patient's own RBCs are lysed, a condition known as hyperhemolysis. Additional transfusions may exacerbate the hemolysis and further worsen the degree of anemia. The destruction of the patient's own RBCs in DHTR of SCD is partly explained by presence of autoantibodies⁵ since alloimmunization is known to trigger autoantibody production. However, DHTR/hyperhemolysis cases have also been reported in the absence of detectable allo- or autoantibodies.

In this review, we will discuss the known risk factors associated with alloimmunization, then emphasize possible mechanisms that can trigger autoimmunization and DHTR in SCD, and

finally describe the challenges in transfusion management of these patients. We will emphasize opportunities and emerging approaches for minimizing this life-threatening complication.

RBC alloimmunization pathobiology

Alloimmunization to erythrocytes involves multiple steps including RBC antigen recognition, processing and presentation of antigen by HLA class II to T cell receptor (TCR), activation of CD4 helper T cells, interaction of T and B cells, and finally B cell differentiation into plasma cells (Fig. 1). Murine and human studies have shown that the process of alloimmunization to RBC antigens can be modulated at each step through acquired and genetic factors, although the relevance of these factors in SCD alloimmunization has not been completely elucidated. Antigenic differences between donor and recipient RBCs are requisite for the initial trigger for alloimmunization. In SCD, multiple studies have shown that alloimmunization risk increases with increasing number of transfusions.⁶⁻¹¹ In addition, women show a higher rate of alloimmunization,¹¹ partially explained by exposure through pregnancy.¹²

Not all patients develop alloantibodies following exposure to transfused RBCs. This fact pertains not only to patients with SCD but also to all transfused recipients. A recent mathematical modeling study has supported the hypothesis that alloimmunized patients represent a genetically distinct group with an increased susceptibility to RBC sensitization.¹³ Within this group only 30% will actually make antibodies, raising the possibility that patient-related factors including the nature of the underlying disease may influence alloimmunization in patients with inherited risks. In the following sections, we aim to describe the antigenic RBC determinants in SCD alloimmunization and identify host-susceptibility factors including those common to any patient population as well as those specific to SCD alloimmunization.

Antigenic differences between donor and recipient RBCs: the initial trigger of alloimmunization

SCD patients are among one of the most frequently alloimmunized transfused population, most likely due to polymorphic differences in immunogenic RBC antigens between the predominantly Caucasian general blood donors and patients of predominantly African descent. In SCD, the published rate of alloimmunization ranges from 20% to 50%.^{4,8} However, SCD patients in Uganda and Jamaica, where donors and patients are racially more homogenous, have reported alloimmunization rates of only 6.1 % and 2.6%, respectively^{14,15} which are comparable to alloimmunization frequencies reported for the general population of these two countries (1-6%).¹⁶ The overall lower use of blood products and blood transfusion therapy for SCD in these countries, in part because of concerns about the safety and availability of blood, also may contribute to these lower alloimmunization rates. Prospective comparison of alloimmunization rates per unit transfused between SCD patients in Uganda and Jamaica with those in western countries will be needed to determine the importance of RBC racial and ethnic differences between donors and recipients on alloimmunization rates. In patients with thalassemia, who are also highly transfused but generally share a more similar ethnic background with blood donors, the rate of alloimmunization is about 10%.¹⁷ But when the general donor pool is mostly Caucasian, Asian patients with thalassemia have an increased rate of alloimmunization compared to Caucasian patients.¹⁸ Together, these observations support the idea that racial antigenic differences account for increased alloimmunization rates.

Antigenic differences between donors and SCD patients are represented at 3 levels of increasing complexity (Table 1). First, the prevalence of some common but highly immunogenic antigens differs substantially between donors and transfusion recipients. Specifically, C and E in the Rhesus (RH) blood group, K in the Kell (KEL), Fy^a in the Duffy (FY), Jk^b in the Kidd (JK), and S in the MNS blood groups are more frequently encountered

in Caucasians than in individuals of African descent. Not surprisingly, antibodies against these common antigens are most frequently identified in SCD patients.⁸

Matching for E, C and K reduced the rate of alloimmunization in chronically transfused SCD patients from 3% to 0.5% per unit¹⁹ and is now the standard of care in many western countries, while prophylactic extended matching for RH, KEL, FY, JK and MNS has been shown to be even more effective.^{19,20} However, there are several problems for this approach, including inventory issues in supplying even the limited RH/KEL matched units. The most common RH phenotype in SCD patients is D+C-E-c+e+, which is found in <2% of Caucasians. To avoid anti-C and anti-E alloimmunization, SCD patients are usually transfused with either units of the same phenotype from donors of African descent, or units with the D-C-E-c+e+ phenotype, which are mainly from Caucasian donors. However, two problems can arise from the use of such D negative units for D positive SCD patients. First, it depletes supply of D negative units, which represent <15% of all units. These units are needed for transfusing D negative persons especially pregnant females whose fetus is at risk of hemolytic disease of the newborn (HDN) in order to prevent anti-D immunization, the most frequent cause of HDN. Second, transfusion of D negative units from Caucasian donors, who frequently express other immunogenic common antigens (e.g., Fy^a, Jk^b, S) (Table 1), can expose Black recipients to those other immunogenic RBC antigens and lead to alloimmunization.

It should be also noted that transfusion of RH compatible units to SCD patients does not totally prevent the risk of alloimmunization, due to the presence of numerous RH variants found in individuals of African origin. These RH variant antigens account for the second level of antigenic complexity between donor and patient RBCs (Table 1). Within the five main RH antigens (D, C, E, c, e), two types of variants (“partial” and “weak”) that are encoded by point mutations, multiple missense mutations or hybrid alleles of *RHD* and *RHCE*, have been

described.²¹ Patients with partial variants lack some of the epitopes on RH antigens, and can make alloantibodies against the missing epitopes when exposed to the complete antigen through transfusion or pregnancy. These alloantibodies can be clinically significant and therefore individuals with partial RH antigens should receive RH antigen negative RBCs even though their alloimmunization risk is likely to be less than patients lacking complete RH antigens.²² In contrast, patients with “weak” antigen variants have quantitatively reduced antigen expression but lack no epitopes, so do not routinely become immunized. However, some partial variants may have also a weak antigen expression. For many weak RH antigens, it remains unknown whether patients can make antibodies or not when exposed to the complete antigen.²³ With the elucidation of the molecular background of these RH variants in individuals of African origin, more information regarding the incidence of these associated antibodies should become available.²⁴

Within D variants, the DAR²⁵ antigen, as well as the DIIIa, DIVa and some DAU types can lead to alloimmunization.^{26,27} Some of the variants associated with the *RHCE* gene such as partial C encoded by *(C)ce^S* and *RN* can also induce pathogenic antibodies.²⁸ Amino acid substitutions that cause loss of epitopes in partial RH variants may be also associated with expression of new antigens referred to as “low incidence” antigens because of their prevalence in the Caucasian reference population (Table 1). In Blacks, some are highly prevalent, such as the RH20 (VS) antigen, encoded by the *RHCE*ce^S* allele,²⁹ which is expressed in about 26-40% of individuals of African origin. VS-negative SCD patients receiving blood from donors of same ethnic background can be potentially immunized to VS, but antibodies against VS are not routinely detected by screening tests, since most test RBC reagents do not express VS. Prospective studies are needed to determine the frequency of antibodies to VS, by following the VS antigen status in both donors and recipients using DNA

tests to evaluate the risk of VS alloimmunization, and to determine if a prevention strategy is necessary.

Many other low incidence antigens are described in Blacks and have resulted in alloimmunization, such as the RH32 encoded by the *RN* haplotype, and the DAK encoded by *DIIIa*, *DOL*, and *RN*.^{30,31} Low incidence antigens also exist in other blood group systems, including Js^a and Co^b antigens, which have induced alloantibody production in SCD patients.^{32,33} Antibodies against low incidence antigens are generally not detected in routine antibody screening tests, since test RBCs do not express these antigens. However, they can be detected when a pre-transfusion cross-match is performed between samples of RBC to be transfused and patient serum.

The third level of antigenic complexity between SCD patient and donor RBCs that can account for increased SCD alloimmunization rates arises when the recipient lacks an antigen that is expressed in almost all donor RBCs, otherwise referred to as a “high incidence” antigen. Individuals with such rare blood groups are at increased risk of alloimmunization because of the high prevalence of the missing antigen within the donor population. The main rare blood types in SCD are in the RH (absence of Hr^S, Hr^B or RH46), KEL (absence of Jsb) and MNS (absence of U) blood groups (Table 1), antibodies against which have been shown to cause RBC destruction.³⁴ Transfusion management for these patients, especially once alloimmunization has occurred, can be extremely challenging. This scenario is best illustrated by the U-negative phenotype of the MNS blood group, which is found in at least 1% of the SCD patients, depending on their country of origin. Low blood donation rates, as well as poor donor eligibility due to higher prevalence of infectious markers in individuals from African countries, contribute to the low supply of U-negative and other rare Black blood types. Because of the short blood supply in all western countries, U-negative blood is mainly reserved for patients who have already formed anti-U alloantibodies. For patients with rare

RH blood groups such as Hr^S, Hr^B or RH46 negative phenotypes, the situation is even more complex since few facilities have the corresponding antigen negative supply.

Individual-specific susceptibility factors

Similar to its role in platelet antigen specific alloimmunization,³⁵ the HLA II genotype of the patient is a key predictor of an individual's response to RBC antigens, and is likely to influence predisposition to the RBC antibody responder status.^{36;37} For example, in Caucasians antibody formation to the RBC-specific Fy^a antigen is strongly associated with the DRB1 04 and DRB1 15 alleles, analogous to observations that HLA-DRB3*0101 and HLA-DQB1*0201 are the key players for platelet antigen HPA-1a alloimmunization. Compared to Fy^a, the erythrocyte K antigen is highly immunogenic, probably because the potential K antigen derived peptides can bind to multiple HLA molecules, as indicated by the wide variety of HLA II phenotypes found in individuals producing anti-K.³⁶ The HLA-DRB1*1503 allele has been associated with an increased risk of RBC alloimmunization, regardless of the antibody specificity, while HLA-DRB1*0901 appears to confer protection against alloimmunization.³⁸ These latter data suggest that beyond the direct link between HLA II and antibody specificity, HLA alleles may also modulate alloimmunization at a non-antigen specific level.

Stimulation of helper T cells requires the interaction of peptides presented by HLA II molecules and the cognate TCR on circulating T lymphocytes (Figure 1). T cell activation can be modulated by CD4+ regulatory T cells (Tregs) that are characterized by co- expression of CD25 and FoxP3. Data from mouse models indicate that Tregs inhibit the magnitude and frequency of alloimmunization,³⁹ and that antibody responders have weaker Treg activity and therefore are unable to suppress antibody production as compared to non-responders.⁴⁰

Possible mechanism(s) of Treg-mediated antibody suppression include inhibition of antibody-producing B cells, directly or indirectly via suppression of helper T cell function.

In a small study of chronically transfused patients with SCD, reduced peripheral Treg suppressive function was found in antibody responders compared to non-responders.⁴¹ If confirmed in larger cohorts, this information may help identify T cell associated molecular markers that can accurately predict antibody responders. A higher proportion of Th2 cells, known to regulate humoral immunity, were detected in a subset of alloimmunized SCD patients, documenting a skewed Th2 response in a subgroup of SCD antibody responders (Fig. 1).⁴¹ Since SCD was the only alloimmunized transfused patient population in that study, however, it remains undefined if Th2 skewing is a unique feature of alloimmunized SCD patients or also applicable to other immune responders. Although no differences were evident in Th1 and Th17 cell frequencies between alloimmunized and non-alloimmunized SCD patients, the proportion of Th17 cells, which drive tissue inflammation⁴² as well as circulating TGF- β and IL-6, known to promote human Th17 differentiation⁴³, were higher in transfused SCD cohorts than in healthy race-matched controls,⁴¹ consistent with the proinflammatory nature of SCD (see below).

SCD-specific susceptibility factors

A main feature of SCD is a chronic inflammatory state even in steady state as indicated by increased levels of C-reactive protein,^{44,45} and inflammatory cytokines (IL-1, IL-6 and interferon- γ)^{46,47} compared to healthy controls, as well as an increased white blood cell (WBC) count that is recognized as a risk factor for disease severity.⁴⁸

In mouse models, certain proinflammatory stimuli enhance alloimmunization,^{49,39} most likely due to additional transfused RBC consumption by splenic and liver dendritic cells (DCs), which are potent inducers of alloimmunization.⁵⁰ The recent observation that a previous

febrile reaction to platelets was associated with a higher risk of RBC alloimmunization further supports a role for inflammation promoting alloimmunization in humans,⁵¹ although no SCD patients were studied. Hendrickson et al reported that sickle mice (Berkeley and Townes), with or without pre-treatment with a viral-like inflammatory stimulus, have a similar rate of alloimmunization compared to wild type animals, concluding that perhaps expression of other modifying genes besides HbS may be responsible for enhanced RBC alloimmunization in SCD.⁵² Extrapolating these data to humans must be done with caution, however, since there are inherent differences between clinical features of human SCD and available mouse models,⁵³ and only one example of alloimmunization (to HOD antigen) following a single transfusion was studied.⁵²

It is currently unknown if alloimmunization rates differ depending on the presence or absence of clinical complications of SCD. For example, it remains unclear whether patients during acute vaso-occlusive crisis (VOC) have an altered alloimmunization potential. VOC, compared to steady state SCD, is associated with increased inflammatory factors, such as neutrophil chemotaxis and monocyte phagocytosis, but also increased production of IL-6 and augmentation of TNF- α production.^{54,55} The increased inflammatory state in VOC could, therefore, affect alloimmunization rates. In contrast, transfusion in the absence of inflammation induces antigenic specific tolerance in mouse models.⁵⁶

Children with SCD who are chronically transfused might have less inflammation, which could explain their lower rate of alloimmunization.^{11,57} However, circulating levels of IL-6 were still elevated in a cohort of chronically transfused young SCD patients as compared to healthy controls,⁵⁸ suggesting that the inflammatory state of SCD may continue despite transfusions. Longitudinal studies involving measurements of inflammatory markers are needed to test whether chronic transfusions, especially with concomitant iron chelation, can reduce inflammation and lower the risk of developing alloantibodies.

Another important issue, recently addressed by Verduzo and Nathan, relates to the effects of age at transfusion initiation on alloimmunization rates in SCD.⁵⁹ Chronic transfusion protocols for prevention of primary stroke typically begin in childhood, but at an older age than children with thalassemia who begin chronic transfusions in infancy. Multiple studies have shown that the number of cumulative transfusions increases the rate of alloimmunization,⁶⁻¹¹ but it is not known whether chronic transfusions initiated at an early age can lower alloimmunization rates in SCD, perhaps by inducing immune tolerance.

Besides these acquired factors, identification of genetic markers predictive of immunization in SCD is an important area of investigation. A polymorphism in the immunoregulatory TRIM21 gene, in close proximity to the beta-globin locus, was recently shown to be associated with an increased rate of SCD alloimmunization, especially in early childhood.⁶⁰ In mice lacking TRIM21, no significant increases in alloimmunization frequency against RBC or platelets were observed, although the mouse model did not have SCD.⁶¹ Genome-wide association studies and whole exome sequencing of large cohorts of patients with SCD receiving transfusions should facilitate the identification of genetic predictors of alloimmunization, but these studies will only be informative if performed in conjunction with an accurate laboratory phenotype, which requires routine and thorough testing for RBC alloantibodies.

Autoantibody formation following alloimmunization

Development of RBC autoantibodies following alloimmunization occurs in transfused populations without hemoglobinopathies, although the rates are much higher in transfused patients with thalassemia¹⁸ and SCD⁶²⁻⁶⁶ with a cumulative incidence of about 6 to 10%. Possible theories to explain this phenomenon include failure to regulate alloantibody-induced

lymphoproliferation, as well as altered processing and presentation of alloantigens to T cells. Using a mouse model of autoimmune hemolytic anemia (AIHA),⁶⁷⁻⁷⁰ a crucial role for Tregs has been identified for dampening the autoantibody response. AIHA may result from an imbalance between pathogenic and regulatory arms of the immune response, since antigen-specific IL-10 secreting regulatory T cells can be detected during periods of remission,⁷¹ whereas Th1 responses are present during active disease.⁶⁹ Hall et al⁷¹ have proposed that AIHA arises when antigen presentation changes as a result of altered cytokine environment due to infection or inflammation.⁷⁰ This not only leads to activation of autoaggressive helper T cells, but also lack of presentation of protective peptides that induce Tregs, thus tipping the balance from regulatory toward pathogenic autoreactive T cells.

From the clinical perspective, autoantibodies against red cell antigens can be pathological with shortened RBC survival, and can cause hyperhemolysis through DHTR. In a retrospective study, Castellino et al documented autoantibody-mediated hemolysis in 4 out of 14 chronically transfused patients who developed detectable autoantibodies.⁶² In each case featuring hemolysis, IgG and C3 were present on RBCs as detected by the direct antiglobulin test. Cold-reactive IgM autoantibodies of the IgM isotype also occurred but were less pathogenic. Aygun et al also reported possible involvement of autoantibodies in DHTR/hyperhemolysis.⁶⁴ In some cases, autoantibodies have been prominent and likely responsible for the development of DHTR, with a clinical picture resembling typical AIHA.^{5,72,73} In these life threatening cases, the autoantibodies appear as panagglutinins,^{74,75} making the identification of compatible blood and characterization of underlying alloantibodies even more difficult. Low titers of autoantibodies can be detected with increased sensitivity using enzyme-treated RBCs that enhance Rh antibody reactivity. The clinical significance of these RBC autoantibodies, especially if they do not bind complement, is

unclear at this time. Their presence does indicate that autoimmunization is underway, however, suggesting that careful serial monitoring of these patients is warranted.

Delayed hemolytic transfusion reaction (DHTR)

The most life-threatening consequence of alloimmunization in SCD is the development of DHTR with hyperhemolysis. This type of hemolytic reaction is unpredictable and potentially under-recognized because its clinical presentation may resemble a VOC.^{76,77} DHTR usually occurs between 5 to 15 days following a transfusion, and is characterized by a marked drop in the hemoglobin level with the destruction of both transfused and autologous RBCs, and exacerbation of SCD symptoms. Profound reticulocytopenia can worsen the degree of anemia and thus lead to additional transfusions, which further exacerbates the process and result in life-threatening anemia.

Main features and hypothesized mechanisms in SCD

Classically in DHTR, alloantibodies have developed against antigens on transfused RBCs. These antibodies are often not detected in the serum during the pre-transfusion screening test, and therefore presumably result from remote antigenic exposure and waning of alloantibody titers, followed by current immune restimulation.⁷⁸ This scenario occurs commonly when detailed and longitudinal transfusion records are not maintained for patients with SCD.

In SCD, cases also exist where the newly formed antibodies were not known to be classically hemolytic, but their pathogenicity was possibly mediated by activated effector cells in SCD such as hyper-reactive macrophages or NK cells with increased FcR expression.⁷⁹ In most reported cases (Table S1), patients harboring these antibodies were transfused because of an

acute complication of SCD or during surgery, both associated with inflammatory cytokine release that can potentially activate effector cells. The SCD milieu may also affect the RBC membrane integrity of transfused cells, making sensitized RBCs more susceptible to hemolysis. Phosphatidylserine (PS)-exposure on RBCs is significantly increased after incubation of donor RBCs with pre-transfusion plasma from SCD patients in crisis compared to steady-state patients.⁸⁰ PS-exposure on donor RBCs can increase their binding to complement,⁸¹ and also potentiate their destruction by macrophages through PS receptors.

Cases of DHTR also exist where no detectable antibodies are found in the post-transfusion screening test (see below and Table S1). The mechanism of hyperhemolysis without detectable antibodies is poorly understood, and therefore difficult to explain, prevent, or treat. In SCD, the process has been attributed to macrophage activation, bystander hemolysis, reactive hemolysis, and possible continuation of hemolysis of autologous RBCs during painful VOC.^{74,82} Incubation of stored donor RBCs in the pre-transfusion plasma from SCD patients encountering DHTR without detectable antibodies caused the stored RBCs to undergo eryptosis,⁸⁰ an antibody-independent phenomenon characterized by erythrocyte shrinkage, membrane blebbing, and phosphatidylserine exposure, all classical features of apoptotic death of nucleated cells and observed in aging or damaged erythrocytes.⁸³ Transfused RBCs may undergo eryptosis in the SCD milieu, potentially due to presence of circulating toxic inflammatory factors that preferentially target and bind receptors present on transfused RBCs but not on autologous RBCs. One such candidate is the chemokine binding protein, Duffy (FY) expressed on RBCs of Caucasian donors, but rarely on RBCs of patients of African origin (Table 1). In sickle mice, HOD RBCs expressing FY antigens were cleared more rapidly than FY-null RBCs,⁵² suggesting a role for FY protein in hyperhemolytic reactions without detectable antibodies.

Many cases of DHTR present with vaso-occlusive sickle cell symptoms. One possible explanation for this observation is a cytokine storm that normally accompanies transfusion-associated hemolysis,⁸⁴⁻⁸⁶ which can contribute to VOC by inducing adherence of sickled erythrocytes to endothelium, directly or through activation of neutrophils. In support of this theory, VOC occurred following induction of hemolysis in SCD mice and was hindered by inhibitors of inflammatory cytokine receptors.⁸⁷ Another possibility is that PS-exposure, which is increased on autologous RBCs during post transfusion hemolysis,⁸⁰ can contribute to VOC since PS-exposed sickle RBCs have increased adhesiveness.⁸⁸

Clinical and biological characteristics of the antibodies in DHTR

A literature review of DHTR since 1984 reveals that new antibodies or additional antibodies have been found on post-DHTR screening tests in the majority of cases (50 of 73 cases, see supplemental Table 1). The most frequently encountered antibodies were against Fy^a, Jk^b, and S.⁸⁹⁻⁹³ In about half of the cases, 2 or more antibodies were found, typically in patients who were previously alloimmunized. These observations support the use of RBCs lacking additional immunogenic antigens, against which alloimmunized SCD patients have not yet been immunized, to decrease the incidence of DHTR. In some cases, the patients had a documented history of the offending antibodies, but the information was not known to the transfusion center. This fact highlights the need for well-maintained patient files and communication between centers that provide transfusion support for patients with SCD.

Several antibodies produced in RH variant carriers have also been associated with alloimmunization and DHTR in SCD. Within D variants, it is known that DAR²⁵ antigen, as well as the DIIIa, DIVa and some DAU types can lead to alloimmunization,^{26,27} although only DAR has been implicated in DHTR.^{24,94} Some variants associated with the *RHCE* gene such as partial C encoded by (*C*)*ce*^S and *RN* can also induce antibodies that have caused DHTR.²⁸

The low incidence Go^a antigen encoded by the partial DIVa has been associated with DHTR in SCD,⁹⁵ as well as low incidence antigens in other blood group systems including Js^a and Co^b antigens.^{32,33} Patients with rare Rh blood groups such as Hr^S, Hr^B or RH46 negative phenotypes have also been involved in serious cases of DHTR.^{10,94}

In 20 out of 73 cases, no additional antibodies were identified in the post-DHTR screening tests. These cases included patients without alloantibodies and documented alloimmunized patients who received matched units for those target antigens. The lack of detectable new antibodies in the post-transfusion screening test is consistent with a non-antibody dependent mechanism of post-transfusion hemolysis as described above.

Diagnosis and management of DHTR

The diagnosis of DHTR in SCD is frequently missed because clinical symptoms resembling VOC presentation may occur up to 15 days post-transfusion. Therefore, DHTR should be suspected whenever patients develop vaso-occlusive symptoms following a recent transfusion. Besides the classical biological and clinical symptoms of post-transfusion hemolysis, the diagnosis of DHTR is often characterized by the coincident disappearance of transfused HbA donor RBCs.

Current management of DHTR in SCD remains controversial since the exact mechanisms remain unclear, especially when antibodies cannot be detected, and also because some of the treatments used in DHTR may be deleterious for SCD patients. For example, corticosteroids can reduce antibody-mediated hemolysis, but may lead to a rebound phenomenon with an increase of SCD-related symptoms.⁹⁸ IVIg, also commonly used for DHTR,^{5,8,9,19} carries a small risk of thromboembolic complications due to hyperviscosity;⁹⁹ however, IVIg is effective for DHTR even in cases where no antibody or new antibody is detected,¹⁰⁰ probably

due to the inhibitory effects of IVIg on the cellular immune response, including inflammation and macrophage phagocytic function associated with SCD (Fig. 1). Erythropoiesis stimulating agents can also be given to reticulocytopenic patients.¹⁰⁰ Rituximab may be another potential treatment option, especially when hemolysis occurs in a known immunized patient. In one reported case, rituximab was used prophylactically in a SCD patient with prior DHTR episodes, to prevent recurrence of autoantibodies and appearance of new alloantibodies.⁵ Rituximab given with steroids and darbopoietin alpha was also effective in treating SCD antibody-mediated hemolysis.¹⁰¹ However, the risk to benefit ratio has not been defined in this setting, and prospective studies are warranted. In most cases of DHTR, further transfusions should be withheld to avoid exacerbating the ongoing hemolytic reaction. Since withholding transfusions for patients with cerebral vasculopathy could increase the likelihood of stroke, the risks and benefits of additional transfusions should be carefully evaluated for each patient with DHTR.

Transfusion management strategies to prevent alloimmunization and DHTR

Recommendations for clinical care

Transfusion of leukoreduced RBC units, which are phenotypically matched for immunogenic RH/KEL blood groups and then cross-matched with a recent serum sample, should be the minimum standard of care for patients with SCD (Fig. 2). Despite the associated costs and effort, an additional extended phenotype to other blood groups performed at diagnosis can save valuable time in the transfusion management of patients with multiple allo- and autoantibodies during acute situations. The utility and cost-effectiveness of an early extended phenotype matching has not been reported.

Molecular tools are already available to genotype patients for common antigens, but also for variant antigens and rare blood groups. Such tools are increasingly used in major transfusion reference laboratories. Typing of weak antigens and partial variants by molecular analysis in SCD patients before initiation of transfusion therapy will enable advance preparation of appropriate units for transfusion, especially if the recipient develops an alloantibody and requires further transfusion in an emergency setting. In situations when the SCD patient has developed antibodies against an expressed antigen (such as anti-D in a D positive patient), prior knowledge of the presence of variants by molecular typing can help distinguish an autoantibody from an alloantibody, which influences the clinical decision about issuing antigen negative RBC units.

With technological advances in genomics, high throughput DNA typing platforms will become cheaper for donor typing, and should reduce the need for rare serological reagents to find rare compatible donors.¹⁰² This approach will still depend on increasing the pool of donors with African origin, and strategies to promote blood donation in these communities should be an ongoing priority (see below).

For every transfusion, all known antibodies in the patient's history must be considered to minimize the risk of antibody-mediated DHTR that follows immune restimulation. It is critical to have well-maintained patient records, and if possible, to monitor patients with antibody screening following every transfusion, testing for development of antibodies that may become undetectable before the next transfusion. Based on the study by Schonewille et al.,¹² post-transfusion screening tests should ideally be performed twice, shortly after transfusion (about 3-7 days) and also a longer period of time after transfusion (4-8 weeks) for optimal detection of antibodies. Figure 2 illustrates an algorithm describing the interplay between antibody screening tests and the patient's own RBC phenotype.

RBC donations from individuals of African descent

Strategies to increase RBC donations from individuals of African descent are critical for tackling the issue of RBC shortage for SCD patients, but also should decrease the rate of alloimmunization. Different cultural approaches to blood donation exist among African-Americans, African-Caribbeans, and Whites with a well-documented disparity in donor eligibility depending on ethnicity.¹⁰³ Some programs, such as the “Cooperative Sickle Cell Donor Program” have been launched with the goal of increasing donation from African-Americans through active recruitment strategies.

Additional problems and questions will arise if African American donations grow, however, mainly because a high proportion (up to 10% in the United States) of these donors will have sickle cell trait (SCT). In the majority of countries, including the US, individuals with SCT are eligible for blood donation. It is not known whether SCT blood products can have side effects when transfused into SCD patients, but generally the policy is to avoid transfusion of these blood products into this patient population. From a practical standpoint, transfusing RBC units from SCT donors into SCD patients also confounds routine laboratory monitoring of HbA and HbS levels. Leukoreduced blood products that reduce HLA antibody production, febrile reactions, and CMV transmission, are also problematic for donors with SCT.¹⁰⁴ In addition to clogging the filters, leukoreduced SCT units do not always reach accepted standards of $<1 \times 10^6$ leukocytes per unit; these units may escape leukoreduction quality control checks that are performed mostly on random units. Finally, low pH conditions due to citrate anticoagulation can promote HbS polymerization during filtration, thereby increasing adhesion of SCT RBCs to the filters. Attempts to reduce failure of leukoreduction have included a metered anticoagulant device to prevent citrate-mediated HbS polymerization.¹⁰⁵

Future strategies to prevent alloimmunization and DHTR

Ongoing studies should explore novel approaches to inhibit alloimmunization in SCD. Immunomodulatory therapies such as the use of immune-cell-depleting agents, costimulatory blockade, and cytokine blockade may be effective in suppressing alloimmunization (Fig. 1), although their use should be carefully balanced against the risk of infections for SCD patients. Rituximab, a mouse-human chimeric antihuman monoclonal antibody that binds CD20 expressed on all B cell, has been used successfully to treat autoantibody production and hemolysis in SCD,^{101,106} presumably by depleting pathogenic antibody-producing B cells.⁵ TNF blockade, through the use of neutralizing antibodies to TNF- α , was recently shown to inhibit alloimmunization in a transplant model.¹⁰⁷ TNF inhibition has anti-inflammatory effects on multiple pathways including endothelial activation and leukocyte recruitment, both known to be involved in VOC^{108;109} and may therefore be effective in SCD for suppression of alloimmunization. Similarly, agonists of adenosine 2A receptors have shown efficacy for treatment of pulmonary inflammation and VOC in SCD mouse models, through inhibition of activation of invariant natural killer cells and other leukocytes¹¹⁰ and may represent an alternative strategy for limiting alloimmunization by downregulation of lymphocyte activation. Blockade of co-stimulatory interactions between T and B cells, for example by inhibiting the CD40-CD40 ligand pathway with anti-CD40 ligand monoclonal antibody or the B7 pathway with CTLA-4Ig/Abatacept¹¹¹ are other possible options. Finally, induction of tolerance through the use of immunodominant peptides derived from the immunogenic polypeptides¹¹² or Treg immunotherapy³⁹ have shown feasibility in mouse studies for inhibition of alloantibody production, some of which are being actively pursued as additional therapeutic approaches for prevention of alloimmunization. Ideally, when genetic modifiers and risk factors become available, transfusion recipients who are genetic predisposed should be carefully matched and monitored to avoid development of alloimmunization.

Summary

Challenges remain for the diagnosis, prevention, and management of alloimmunization and DHTR in SCD. Understanding the mechanisms and associated risk factors will aid in developing strategies to prevent and inhibit production of antibodies in transfused patients, and to minimize its life-threatening complications. Studies in murine models are central to dissection of immune molecular mechanisms of alloimmunization and DHTR. In parallel, careful epidemiologic and prospective studies are needed to investigate critical topics including the optimal age for initial exposure to RBC antigens and whether alloimmunization rates differ in patients during VOC. Ongoing studies should clarify the role of genetic modifiers of alloimmunization and help identify susceptibility genes that contribute to SCD alloimmunization. Little is known about the etiology of DHTRs without detectable antibodies in SCD and studies to elucidate their underlying mechanism are critical for SCD patient care. With regard to current transfusion management of SCD patients, we recommend performing an extended phenotype for all patients with SCD at diagnosis, careful monitoring of laboratory tests before and following every transfusion, and a well-maintained electronic system of patient transfusion history. Molecular tools to type most blood group variants have been developed,¹¹² and ongoing studies to determine the incidence and clinical significance of antibodies against variants are needed to develop cost-effective genotyping tests for those antigens whose associated antibodies are clinically significant. In parallel, strategies to promote blood donation amongst individuals of African origin should remain a high priority for increasing the donor pool of antigen-matched blood for SCD patients.

Authorship

K.Y. and F.N-P. conceived, wrote and R.E.W. edited the manuscript.

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Table 1. Main RBC antigenic differences between Caucasian donors and Black recipients

All blood group and antigen frequencies have been obtained from The Blood Group Antigen “Facts Book”, From Marion E. Reid and Christine Lomas-Francis. Elsevier Academic Press.

Table 1

Blood group differences between donors and recipients

		% in Caucasian donors	% in Black recipients
Common antigens			
ABO groups	A	43	27
	B	9	20
	O	44	49
	AB	4	4
RH	D	85	92
	C	68	27
	E	29	20
	c	80	96
	e	98	98
KEL	K	9	2
FY	Fy^a	66	10
	Fy^b	83	23
JK	Jk ^a	77	92
	Jk ^b	74	49
MNS	S	51	31
	s	89	93
Partial RH antigens			
partial D among D+		1	7
partial C among C+		0	30
partial e among e+		0	2
Low incidence antigens			
VS (RH20)		0.01	26-40
Js ^a (KEL6)		0.01	20
Rare blood groups			
U neg- (MNS:-5)		0	1
Hr ^S neg- (RH:-18)		0	0.1
Hr ^B neg (RH:-34)		0	0.1
RN (RH:-46)		0	0.1
Js ^b neg (KEL:-7)		0	1

Figure Legends

Figure 1. Hypothetical schema of immune response to RBC antigens in alloimmunized versus non-alloimmunized patients. Multiple participants in alloimmunization versus no alloimmunization states are outlined. Preventive interventions and the specific steps in prevention of alloimmunization (see text) are shown in yellow. In addition, the possible modes of action of steroids and IVIg for treatment of DHTR are also shown in yellow. The hypothetical model predicts that the chronic inflammatory state in SCD creates a microenvironment with increased inflammatory cytokines, which favors antigen presenting cells (APCs) such as macrophages and dendritic cells to increase consumption of the transfused allogenic RBCs and also favor generation of immunogenic peptides in APCs. The individual's HLA repertoire will then dictate whether these peptides are presented to the naïve CD4⁺ T helper (Th) cells or not. Alloimmunized sickle patients have increased Th2 frequency, normally associated with humoral immune response, or decreased Treg activity associated with alloimmunization. The skewing toward Th2 or decreased Treg is proposed to be associated with B cell activation and antibody production. In SCD patients who may have a genetic predisposition to be non-responders, or patients who do not produce antibodies following allogeneic transfusions, the APCs may be less immunostimulatory such that the peptides presented to the naïve CD4 cells are tolerizing and result in the induction of Tregs that can reduce Th2 and/or B cell and APC activation (indicated by question mark). Abbreviations: TCR, T cell receptor; HLA, human leukocyte antigen.

Figure 2. Recommended transfusion strategy for SCD patients.

All recommendations are based on the results of the serum antibody screening test results and the extended RBC phenotype of the patient. For patients with no detectable antibodies, no

known previous antibodies, and no abnormal RBC phenotype, transfusion of leukoreduced, cross-matched RH/KEL compatible RBC units is recommended.

When antibodies are identified at the screening test or known by history, 2 scenarios can be encountered: (i) the corresponding antigen is not expressed on patient RBCs and therefore the antibody is an alloantibody ($Ab = Allo$), and hence antigen-negative RBCs should be transfused; (ii) the corresponding antigen is expressed on patient RBCs and serological and/or molecular studies are needed to determine whether the antibody is an alloantibody produced in a partial antigen carrier or an autoantibody ($Ab = Auto$). If an alloantibody, and there are available donor units, compatibility should extend to other common non-expressed antigens. If an autoantibody, matching for the corresponding antigen is unnecessary. For the phenotype of the patient, 2 situations should be considered: (i) Presence of a weak antigen, where molecular analysis will be needed to determine whether it is a partial antigen. If the patient has no detectable antibodies, transfusion with an antigen negative unit is preferred although antigen positive RBCs can also be issued, since the risk of alloimmunization in patients with weak antigens has not yet been determined (see text). However, close monitoring of possible immunization is needed if antigen-positive units are transfused; (ii) Presence of a rare phenotype, where availability of high frequency antigen negative units is the determining factor for the transfusion strategy. If the patient is already immunized against the high frequency antigen, antigen-negative units are recommended for transfusion. If no rare units are available, the benefit/risk ratio of transfusion has to be considered. For patients who are not yet immunized against high incidence antigens, transfusion of antigen negative units are also recommended if such units are not in short supply. Otherwise, the standard RBCs for SCD can be issued, and the patients should be monitored closely. Given that antigen negative units for patients with rare phenotype are by definition in short supply (see text), transfusion management of these patients can become extremely challenging and we recommend that the

risk/benefit ratio of alternative treatments, such as hydroxyurea (hydroxycarbamide) for adult patients or bone marrow or cord blood transplantation for children, is discussed with such patients.

FIGURE 1

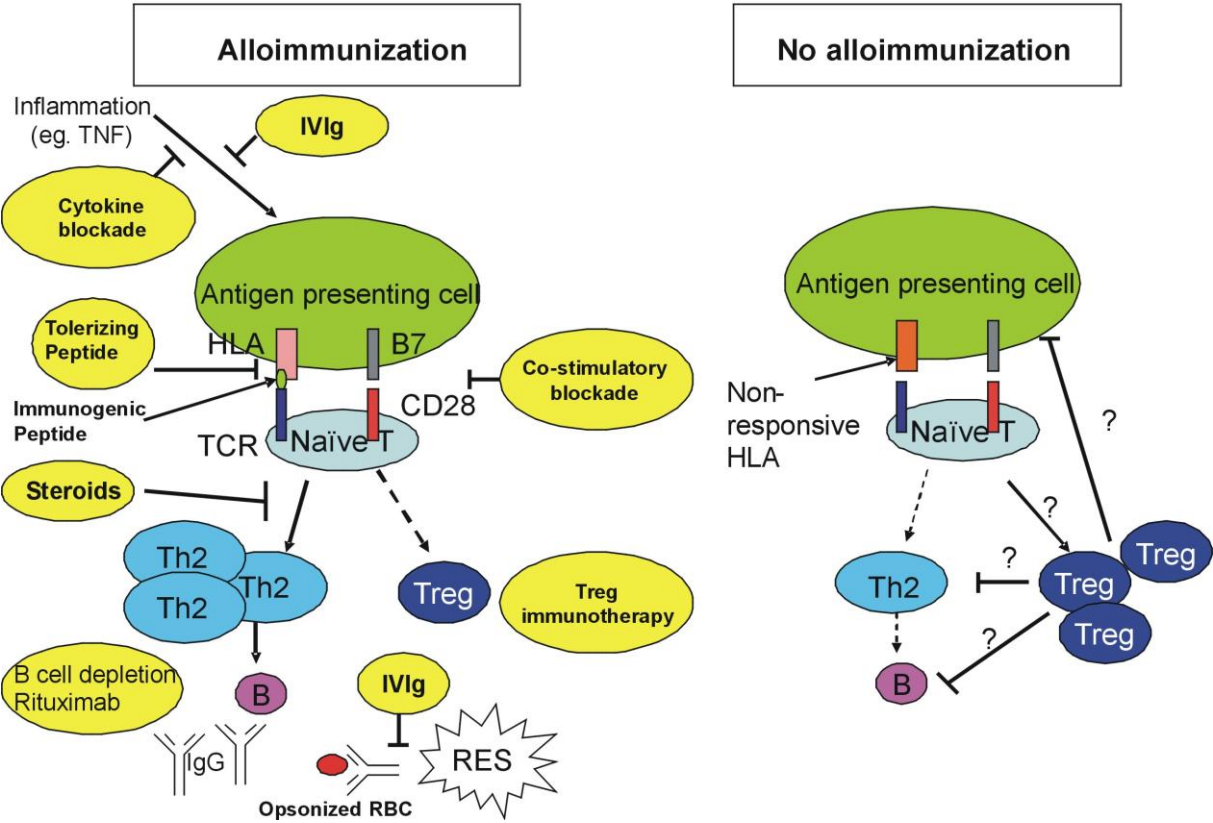


FIGURE 2

