Published in final edited form as:

Transfus Med Rev. 2019 October ; 33(4): 217–224. doi:10.1016/j.tmr.2019.09.006.

Examining the Role of Complement in Predicting, Preventing, and Treating Hemolytic Transfusion Reactions

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Abstract

Red blood cell (RBC) transfusion is a critical component of optimal management for a broad range of conditions. Regardless of the indication, pretransfusion testing is required to appropriately match RBC donors and recipients to provide immunologically compatible blood. Although this approach is effective in the vast majority of situations, occasionally, patients will inadvertently receive an incompatible RBC transfusion, which can result in a hemolytic transfusion reaction (HTR). In addition, patients with life-threatening anemia and a complex alloantibody profile, which precludes rapid procurement of compatible RBCs, may also receive incompatible RBCs, placing them at risk for an HTR. Despite the rarity of these clinical situations, when incompatible blood transfusion results in an HTR, the consequences can be devastating. In this review, we will explore the challenges associated with actively preventing and treating acute HTRs following incompatible RBC transfusion. In doing so, we will focus primarily on the role of complement, not only as a key player in HTRs, but also as a potential target for the prevention and treatment of HTRs.

Keywords

Complement; Alloantigen; Sickle cell disease; Hemolytic transfusion reactions; Transfusion medicine, compatibility testing; Alloantibody

The earliest recorded blood transfusion, which occurred nearly 4 centuries ago, demonstrated the potentially devastating consequences of an incompatible blood transfusion. These early reports noted that while some patients benefited from blood transfusion, others experienced poorly understood complications that could be fatal [1,2]. The inability to adequately predict transfusion outcomes, coupled with an incomplete understanding of the cause of severe consequences of transfusion in some recipients, resulted in discontinuation

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Conflicts of Interest
None.

of blood transfusion procedures for centuries [2]. It was not until the discovery of A, B and H blood group antigens (H antigen present in blood group O individuals) and corresponding anti-A and anti-B blood group alloantibodies that the cause of fatal transfusions became understood [3]. Identification of ABO(H) antigens not only represented the first polymorphisms described in the human population, but also suggested that immune reactions following blood transfusion might account for previously reported adverse reactions experienced by some individuals [4]. Subsequent studies demonstrated that the ABO(H) status of a potential blood donor and recipient were important considerations for avoiding what came to be known as hemolytic transfusion reactions (HTRs) [5]. Donor and recipient testing for ABO(H) antigens and anti-ABO(H) alloantibodies quickly became an essential component of transfusion therapy. Because these tests are employed prior to all transfusions, compatibility testing became one of the first clinical tests routinely used in medicine and continues to represent the first and most common example of personalized medicine [6,7].

Following the initial use of ABO(H) antigen and alloantibody testing to guide transfusion therapy, some patients appeared to possess additional alloantibodies that recognized non-ABO(H) alloantigens that were also capable of causing HTRs [8–10]. To account for incompatibility secondary to these additional alloantibodies, procedures were developed to screen for and ultimately define the specificities of potential donor-reactive alloantibodies outside of the ABO(H) blood group, thereby allowing blood products to be matched for these additional alloantigens [11]. In order to avoid HTRs, significant resources have been, and continue to be, employed to refine quality control processes that oversee the integrity of sample acquisition, donor unit labeling, and additional blood bank testing that are necessary to reduce the probability of an incompatible transfusion [12]. Indeed, the vast majority of testing measures in hospital blood banks have been established to ensure that compatible blood products are provided for every patient requiring transfusion [12]. Despite significant efforts devoted to preventing adverse events, quality control measures occasionally fail, resulting in an incompatible transfusion. While rare, incompatible transfusions may result in HTRs that can be fatal. Indeed, HTRs continue to represent one of the most common non-infectious causes of transfusion-related mortality [13–17].

Because most transfusion medicine resources dedicated to HTRs are preventive in nature, very little data are available regarding the optimal management of acute HTRs that do occur. Furthermore, in rare clinical situations when a patient with life-threatening anemia requires immediate transfusion and no compatible blood is available, incompatible RBCs may be intentionally administered [18,19]. In these rare but potentially devastating clinical situations, understanding the pathophysiology of acute HTRs, including potential opportunities to prevent and treat HTRs following transfusion of an incompatible blood product, may be helpful for optimal patient management.

Distinct Features of Red Blood Cell Alloantibodies

Because of the high prevalence of anti-ABO(H) alloantibodies, which spontaneously develop in all individuals who lack A or B blood group expression [16,20]. ABO(H)-incompatible RBC transfusions often represent the most common type of HTR following

mis-transfusion. While the stimulus responsible for naturally occurring anti-ABO(H) alloantibody formation remains largely unknown, these antibodies are predominantly, but not exclusively, IgM. The pentameric nature of IgM antibodies not only facilitated the detection of ABO(H) polymorphisms by Landsteiner in 1900 but also continues to allow for compatibility testing by leveraging the ability of these alloantibodies to readily agglutinate red blood cells (RBCs) following engagement. Levels of anti-ABO(H) alloantibodies can vary substantially between individuals and are often used as surrogates when assessing the clinical significance of anti-ABO(H) alloantibodies [21]. For example, anti-ABO antibody titers are routinely employed in some healthcare systems to evaluate the clinical significance of anti-ABO antibodies present in platelet and plasma products [22,23]. Of note, IgG alloantibodies against ABO(H) antigens also develop, and these alloantibodies may contribute to the pathophysiology of ABO(H)-incompatible transfusions. IgG antibodies that react with the A and B antigens, in particular, appear to be the primary contributors to ABO(H)-mediated hemolytic disease of the fetus and newborn [24].

In contrast to anti-ABO(H) alloantibodies, alloantibodies induced following exposure to RBC alloantigens, such as during pregnancy or transfusion, are often initially IgM but ultimately class switch to IgG [25–27]. Unlike the IgM isotype, which includes only one subtype, IgG antibodies can be subtyped into four different categories: IgG1, IgG2, IgG3, and IgG4. As a result, RBC-induced alloantibodies can be composed of differing levels of IgG subclasses, each of which possess distinct abilities to engage IgG receptors (Fc gamma receptors) and/or to fix complement [28]. In addition to potential subclass differences in IgG alloantibodies, IgG glycosylation can significantly influence the ability of a given IgG antibody to engage Fc receptors or fix complement (IgM glycosylation, in contrast, has not reported to have similar effects on antibody function) [29–31]. Furthermore, unlike anti-ABO(H) alloantibody levels which tend to remain relatively stable in most patients [32], IgG alloantibodies can wane over time similar to changes in antigen-specific IgG levels following infection or vaccination [33,34]. Because the initial alloantigen stimulus is likely much weaker than the immune response generated following infection or other stimuli, alloantibodies generated following primary exposure to RBC alloantigens can rapidly evanesce [35]. This can result in failure to detect some RBC-induced alloantibodies following their initial development [33,34]. Because most patients are not routinely matched for alloantigens beyond ABO (H) and RhD, alloantibodies not detected during screening tests raise the possibility of alloantibody recrudescence following re-exposure. RBC-induced anamnestic alloantibody responses can accelerate the removal of transfused RBCs and result in delayed hemolytic transfusion reactions (DHTRs). Although DHTRs can lead to significant complications [36–41], they are the topic of a companion review.

Complement Activation Following Incompatible Transfusion: Examining the Impact of Alloantibodies

Complement regulation is commonly divided into three distinct activation cascades: classical (driven by antibodies), lectin (activated by innate immune lectins), and alternative/nonclassical (constitutively active) [42–46]. Although the lectin and alternative pathways were discovered later, they represent ancient innate immune pathways that evolved prior to

the antibody-dependent, classical pathway of complement activation. While the initial stimulus in each pathway may differ, early activating pathways ultimately converge to form an enzyme complex capable of converting complement component 3 (C3) to C3a and C3b [47]. C3a is a soluble complement split product that engages C3a receptors that are present on many target cells, resulting in immune activation and overall inflammation [47–51]. Because excessive C3 activation can result in sufficient C3a to cause anaphylaxis, C3a is also known as complement anaphylatoxin [48,49]. In addition to generating C3a, C3 cleavage exposes a highly reactive thioester on C3b that allows C3b to covalently attach to the target cell surface [43,47]. When attachment does not occur within 60 microseconds, C3b is rapidly inactivated by hydrolysis [52,53]. This process is thought to control off-target engagement by complement. Once attached to the cell surface, C3b acts as an opsonin by binding to a series of complement receptors, including CR1, CR2, CR3, CR4, and CRIg (expressed on a variety of cells but particularly enriched on phagocytes) [54–59], as well as by facilitating downstream complement effector function [60].

For an antibody to efficiently initiate complement activation, the first component of the classical pathway, C1q, must be engaged by an antibody bound to a target antigen [61]. Once engaged by antigen-bound antibody, C1q activates C1r, which cleaves and activates C1s [61]. Activated C1s cleaves C2 and C4, producing C2a, C2b, C4a, and C4b. C2b and C4b have a thioester chemistry similar to that of C3b and can therefore quickly attach in a covalent manner to the target surface or become inactivated by hydrolysis. If bound to the cell surface as a complex, C2b and C4b possess the ability to cleave C3, thus functioning as a C3 convertase [47]. This enzymatic process can result in rapid amplification of complement activation, facilitating the release of substantial quantities of C3a and the deposition of C3b. In addition to serving as an opsonin, C3b bound to C4b and C2b forms C5 convertase, which cleaves C5 into C5a and C5b. Like C3a, C5a can act as an anaphylatoxin, with a similarly potent ability to activate immune function [47,50,51]. C5b can bind to C6, C7, and C8, generating a complex that initiates C9 insertion into the plasma membrane. Together, these molecules form the membrane attack complex (MAC) [60]. Activation of the MAC produces a pore in the membrane, leading to lysis of the target cell [60].

Optimal C1q binding occurs when an antibody engages with a target antigen; this reduces continuous antibody-mediated complement activation in solution [61]. C1q-antibody engagement occurs through several mechanisms. For IgM molecules, which are pentameric, one IgM molecule simultaneously bound to several target epitopes results in a conformational change that allows C1q binding [62]. The pentameric nature of IgM also allows the initial antibody engagement to quickly become multivalent, directly increasing the avidity and potential clustering of target antigens on the cell surface [63,64]. Antibody-induced antigen clustering can increase the strength of antibody-antigen interactions, which enhances the conformational changes needed to engage C1q and thereby initiate complement activation [63,64]. Increasing the density of antibody and C1q on the cell surface can also enhance the density of C2b, C3b, and C4b deposited on the cell surface, directly facilitating the activation of downstream complement cascade events. One molecule of IgM can efficiently activate complement; therefore, IgM is one of the most efficient activators of complement among Ig isotypes [65].

In contrast to IgM, IgG antibodies cannot intrinsically activate complement as a single molecule, either free in solution or bound to antigen. Instead, effective C1q engagement by IgG requires two IgG molecules in close proximity, typically within 20 to 30 nm of each other [66]. In addition, distinct IgG subtypes possess different abilities to bind C1q, and therefore activate complement [28], raising the possibility that some IgG alloantibodies may engage the cell surface but not effectively initiate complement activation simply because of the predominant subtype. Although the differential ability of IgG subtypes to fix complement has been long recognized [28], more recent studies have demonstrated that post-translational glycan modification of the Fc domain can also influence IgG function [29–31]. Distinct Fc glycoforms may not only affect binding to Fc receptors, but also impact binding to C1q and subsequent complement activation [29,30]. IgG alloantibodies may thereby represent a mixture of IgG subtypes, each with their own unique glycan signature, which collectively influences the ability of IgG alloantibodies to engage complement and activate other effector functions [67–69].

Complement Activation Following Incompatible Transfusion: Examining the Impact of Target Alloantigens

In addition to the intrinsic abilities of different alloantibodies to fix complement, the target alloantigen itself may influence the consequences of alloantibody binding on the cell surface. For example, although IgM alloantibodies possess the ability to bind five or more target sites following alloantigen engagement on a cell surface, if the cognate alloantigens are not spaced with sufficient density to allow simultaneous IgM binding, multiple epitopes will not be bound [66]. Engagement of one or two epitopes can result in reduced overall interaction affinities and may fail to induce the optimal IgM conformational changes required to engage C1q. This can be particularly important for alloantigen targets directly tethered to the underlying RBC cytoskeleton or complexed with other alloantigens that are similarly immobilized on the cell surface. In contrast, alloantigens that possess the ability to move freely within the plasma membrane allow alloantibody-induced clustering, which enhances the avidity of the interactions and promotes conformational changes that ultimately result in efficient complement activation. Regardless of the mobility of a target antigen in the cell membrane, the relative density of target alloantigens likely plays a substantial role in the ability of alloantibodies to cluster multiple alloantigen targets. Single alloantibody-alloantigen interactions are often relatively weak and may not support a bond half-life sufficient to engage additional antigen targets. These interactions may also result in rapid alloantibody dissociation from the cell surface following initial engagement if additional interactions fail to occur, thereby limiting complement activation. Orientation of the alloantigen can also influence alloantibody binding since alloantigen targets must possess the conformational flexibility and overall availability necessary to support the simultaneous engagement of multiple alloantibody binding events.

Clinically, the potential impact of the target antigen in dictating the preferential use of alloantibody effector systems has been observed for decades. ABO(H)-mediated HTRs are thought to almost always result in complement activation, which often proceeds to MAC formation and intravascular hemolysis [70]. This is presumably due to not only the

predominance of the IgM subclass within anti-ABO(H) alloantibodies but also the high density, relative simplicity, significant lateral mobility (many ABO(H) antigens are on glycolipids), and overall accessibility of the target alloantigens [71]. Alloantibodies directed toward Kidd and Duffy alloantigens have also been shown to fix complement and induce intravascular hemolysis [70,72], suggesting that significant complement fixation is not limited to ABO(H) HTRs. Although this ability may reflect unique antigen features that enable IgG alloantibodies to fix complement following alloantigen engagement, the results of one study suggest that IgM anti-Kidd alloantibodies may primarily account for complement fixation following Kidd-incompatible RBC transfusion [73].

In contrast to alloantibodies against ABO(H), Duffy, and Kidd, alloantibodies directed toward other alloantigens, such as RhD, have rarely been shown to fix complement. This does not appear to reflect the development of anti-RhD alloantibody subclasses that are simply unable to fix complement, as these subtypes can activate complement in other settings [67]. Similarly, recent studies have demonstrated that therapeutic antibodies directed against certain RBC surface antigens, such as CD38 and RhD, also fail to induce detectable complement fixation [74,75]. It should be noted that, given the infrequency of HTRs, most reports seeking to define the relative contributions of complement have used C3 deposition on the cell surface as a surrogate for potential complement-mediated hemolysis. However, C3 can be quickly inactivated by a series of complement inhibitory pathways [56,76–81]. As a result, C3 can be deposited on the cell surface at levels that are insufficient to adequately facilitate RBC removal via complement receptors or MAC complex formation [82]. Therefore, C3 detected on the RBC surface should not be interpreted as direct evidence of complement involvement in antibody-mediated RBC removal [70]. In contrast, when extensive hemolysis occurs, complete removal of transfused cells will also prevent the detection of RBCs with C3 deposition, preventing a lack of detectable C3 on RBCs from accurately reflecting a C3-dependent process. As direct antiglobulin tests only examine IgG and C3 and, therefore, would be unable to detect IgM bound to the cell surface in the absence of C3 deposition, IgM-engagement of RBCs that may result in RBC clearance through a C3-independent process may likewise go undetected given current testing algorithms [11].

Consequences of Complement Activation Following a Hemolytic Transfusion Reaction

Complement activation may not only directly facilitate RBC clearance through extravascular and intravascular pathways but also lead to systemic consequences through the effects of complement split products C3a and C5a on immune and vascular activity [47,50]. For example, C3a and C5a can directly alter vascular permeability and induce a variety of immune cell populations to produce pro-inflammatory cytokines [47,50]. Alterations in vascular tone and permeability caused by the direct effects of complement split products, coupled with the production of inflammatory mediators in response to C3a and C5a exposure on immune cells, can contribute to hemodynamic instability [47,50]. Vascular injury itself can also induce widespread thrombin activation, facilitating the development of disseminated intravascular coagulation [83]. These changes, in addition to complement-

induced endothelial injury [47,50], can produce substantial end organ injury, including acute lung and kidney injury. Thus, the byproducts of complement activation on the cell surface can influence the pathophysiology of HTRs (Fig 1).

In addition to the direct impact of complement split products on HTRs, free hemoglobin (Hb) released during intravascular hemolysis can also contribute to the pathophysiology of HTRs. Although haptoglobin can scavenge free Hb, haptoglobin is often quickly saturated, allowing Hb to directly induce coagulopathy and kidney injury [84–88]. Breakdown of Hb to free heme also produces a toxic metabolite that can itself induce substantial endothelial injury and coagulopathy following the rapid saturation of hemopexin, the scavenger of free heme [83,89]. Extravascular hemolysis secondary to removal of alloantibody and complement-coated RBCs can also result in cytokine secretion by phagocytes, which may contribute to a systemic inflammatory response, altering hemodynamic function and contributing to end organ dysfunction [90]. Recent studies suggest that heme itself can facilitate complement activation [91], thereby triggering additional complement activation that may further exacerbate complement-related pathology in the setting of an HTR. In patients with sickle cell disease, sickle erythrocytes appear to be particularly prone to antibody-independent hemolysis [41], possibly due to inadequate complement regulatory pathways that evolved to normally protect RBCs from complement activation [92]. Thus, patients with sickle cell disease may be particularly sensitive to the effects of initial complement activation, heme release, and subsequent alternative complement activation that results in enhanced hemolysis of their own sickle erythrocytes. This positive feedback loop may contribute to the pathophysiology of hyperhemolysis [93–95], which can cause substantial morbidity and mortality [96].

Predicting, Preventing, and Treating Complement-Related Hemolytic Transfusion Reactions

Because alloantibody engagement of a cognate alloantigen on the RBC surface can result in variable outcomes, it can be difficult to predict whether an incompatible transfusion will even result in an HTR, let alone whether complement-dependent hemolysis or generalized complement activation will occur. In contrast to alloantibody identification strategies in the setting of hemophilia and other conditions which use activity assays to test alloantibody function [97], alloantibody screening and identification approaches employed by transfusion services do not routinely examine alloantibody activity. Instead, these tests are almost entirely designed to detect and characterize the specificity of an alloantibody that may be present. As such, there is often little intrinsic information in the alloantibody identification process that allows a clinician to know whether a given alloantibody will cause an HTR and whether this will occur through a complement-influenced process. Instead, transfusion medicine services are often confined to using the historical likelihood of specific alloantibodies (eg, anti-Jk^a) to cause an HTR following incompatible transfusion when seeking to predict the clinical significance of that alloantibody in a specific patient.

Because HTRs can be fatal, carefully designed prospective studies defining the mechanisms whereby alloantibodies clear incompatible RBCs following different alloantibody and

alloantigen combinations are often unethical. To address this issue, various animal models have been employed that use distinct alloantigen targets to define key factors that govern HTRs. Although these models are limited to only a few alloantigens, data obtained using these pre-clinical models suggests that a variety of different antibody effector pathways can be engaged following incompatible RBC transfusion. For example, depending on the model, complement may be required, Fc receptors may be involved, both Fc receptors and complement may be needed, or neither complement nor Fc receptors may be involved in the ability of an alloantibody to effectively eliminate RBCs [68,90,98–100]. The potential requirement of different antibody effector systems appears to reflect the type of antibody involved and the target alloantigen. Additional studies suggest that antibodies can actually induce the removal of the target antigen from the RBC surface independent of RBC hemolysis, a phenomenon that can require complement and has recently been corroborated in several clinical studies [69,74,75,99,100]. While these data may provide some insight into the complexities of antibody-mediated HTRs, additional studies are needed to fully characterize the distinct consequences of an incompatible transfusion following unique alloantigen and alloantibody combinations.

As nearly half of incompatible RBC transfusions do not result in an HTR, even when the implicated alloantibody is deemed on a historical basis to be clinically significant, several functional tests have been employed in an effort to determine a priori the clinical relevance of a given alloantibody in difficult to transfuse patients. The monocyte monolayer assay (MMA) is the most commonly used test, and assesses the ability of alloantibodies to bind to cognate RBCs and facilitate phagocytosis of alloantibody-coated cells following incubation with monocytes [101,102]. The major limitation of this assay is that it does not directly test the ability of alloantibodies to induce RBC hemolysis. Nevertheless, the MMA has been shown to predict the likelihood of an HTR following incompatible RBC transfusion in numerous settings [101,102], suggesting that extravascular hemolysis following a non-ABO(H) incompatible transfusion may be the dominant mechanism of RBC clearance. However, it is also possible, at least in some scenarios, that the ability of the MMA to predict the clinical significance of a given alloantibody reflects the overall ability of an alloantibody to effectively engage multiple antibody effector systems. This may be especially important when considering that some antibody subclasses and glycoforms exhibit enhanced ability to engage Fc receptors and fix complement [28–31,103].

In addition to the MMA, several investigators have recently developed an antibody activity assay focused on complement activation. The complement hemolysis using human erythrocytes (CHUHE) assay mixes a transfusion recipient's alloantibodies with target RBCs [104,105]. RBCs are then incubated and evaluated for potential induction of hemolysis in vitro. Using this approach, in vitro complement-mediated hemolysis does not entirely correlate with RBC agglutination titers, suggesting that a functional approach to assessing complement activation may be more useful than prior methods when seeking to determine the clinical importance of an alloantibody [104,105]. Therefore, coupling the CHUHE assay and the MMA to determine the overall functionality of an alloantibody may represent a more complete and accurate in vitro approach to determining the clinical significance of a given antibody [106]. However, current limitations in the availability and turn-around time for each of these assays often limit their clinical utility in urgent situations.

Ultimately, the best approach may be an in vivo crossmatch, in which patients are transfused with a small volume of incompatible RBCs (using a volume that is unlikely to produce major complications) to directly determine whether the patients' alloantibodies induce the clearance of RBCs isolated from a particular donor unit. Although this approach has been used previously and would be ideal for predicting whether a given patient is likely to experience an HTR following RBC transfusion [107], logistical limitations often preclude implementation of this strategy in most hospital settings.

The ability to determine the clinical significance and potential involvement of alloantibody effector systems following a potential HTR has prophylactic implications when a patient presents with life-threatening anemia, and no compatible RBCs are readily available [18,108–116]. Such patients may have an alloantibody against a high incidence alloantigen or possess a constellation of alloantibodies that likewise lead to difficulty procuring compatible RBCs [18,108–116]. In addition, some patients experience recurrent HTRs despite the lack of a clearly defined alloantibody specificity [114]. In these situations, ABO(H) incompatibility is rarely, if ever, implicated. Instead, the antibodies are predominantly IgG alloantibodies against minor alloantigen targets. As such, a variety of approaches have been used, including administration of corticosteroids and intravenous immunoglobulin in an attempt to suppress reticuloendothelial cell function and thereby reduce extravascular hemolysis [33,34,39,40,117–122]. However, as some alloantibodies, including alloantibodies against Kidd and Duffy, have historically been thought to cause hemolysis through a complement-dependent process, inclusion of a complement inhibitor, such as eculizumab, may also be warranted [93,94]. Ultimately, the development and more routine use of CHUHE or similar assays may be helpful when seeking to determine whether an alloantibody may fix complement and, therefore, guide the use of complement inhibitory approaches when seeking to prevent HTRs in this setting (Table 1).

Once an HTR occurs, either after intentional transfusion in the presence of an alloantibody or following an inadvertent incompatible transfusion, optimal treatment strategies may vary depending on whether the HTR is predominately extravascular, intravascular, or both. Initial RBC clearance most often has already occurred by the time an unexpected acute HTR has been recognized, reducing the probability that interventions designed to inhibit alloantibody effector activities will favorably modulate HTR outcomes. In contrast, in the setting of a DHTR in which alloantibodies evolve more slowly, interventions designed to reduce or prevent ongoing alloantibody-mediated hemolysis may be beneficial [33,34]. Regardless of whether a patient has just developed an acute HTR or whether the HTR is ongoing, when excessive Hb release occurs, heme-induced complement activation can contribute to the ongoing pathophysiology of the HTR. This may manifest as ongoing systemic end organ function and/or hyperhemolysis, which may reflect additional heme release and complement activation [91,96]. Consistent with this, activation of the alternative complement pathway has been documented in sickle cell patients experiencing hyperhemolysis [93–95]. In this setting, inclusion of a complement inhibitor, such as eculizumab, may be warranted to prevent ongoing complement-related pathology as supportive measures are continued [93–95] (Table 1). Such an approach has been recently shown to actively inhibit ongoing complement activation and hyperhemolysis in SCD patients with DHTRs [93–95]. However, it should be noted that currently available complement inhibitors, such as eculizumab, while

capable of blocking C5 activation and subsequent MAC formation, do not prevent C3 deposition. C3 deposition can therefore occur unabated even in the presence of C5 inhibitors and possibly contribute to extravascular hemolysis through C3 receptor-facilitated erythrophagocytosis. Newer approaches aimed at preventing complement upstream of C5, such as C3 inhibitors, may therefore be even more effective at preventing all forms of complement-mediated hemolysis than currently available complement inhibitor approaches. Regardless of the approach, potential infectious risks associated with complement inhibition should be considered; these risks may be more apparent the more proximal the target of the complement inhibitor approach that is employed. These additional complement inhibitor approaches, including their advantages and disadvantages are outlined in an accompanying review.

Future Directions

Although complement inhibition has directly altered the way a variety of hemolysis-related diseases are treated [95], its use in the management of HTRs is only beginning to be realized. As recent studies suggest that complications of HTRs in patients with SCD, in particular, are much more common than previously appreciated [38], the judicious use of complement inhibitors, such as eculizumab, may provide an important approach when seeking to potentially prevent and treat these complications, as opposed to providing only supportive care [93–95]. In addition to potentially regulating alloantibody formation [123,124], the role of complement in mediating HTRs following incompatible RBC transfusion warrants further study. In the event that studies demonstrate that complement inhibition is beneficial in treating transfusion-related complications, the relative efficacy of different forms of complement inhibition will need to be defined. However, because alloantigen targets are diverse and pre-clinical models suggest that alloantibodies can induce RBC hemolysis through a variety of different pathways [68,90,98,99,125–127], additional approaches are required to determine the most effective strategies for preventing and treating all types of HTRs. As a result, future investigations are necessary to more thoroughly define the factors that contribute to the pathophysiology of HTRs and, in so doing, identify useful targets that may be employed to more accurately prevent and treat these transfusion reactions.

Acknowledgements

This work was supported in part by the National Institutes of Health Early Independence grant DP5OD019892 and the National Heart Lung and Blood Institute grant R01HL13557501 and P01HL132819 to S.R.S.

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Fig 1. Consequences of an incompatible RBC transfusion:

Antibody engagement of an antigen on an RBC surface can cause complement activation, which initially results in C3b deposition on the RBC surface and the release of C3a. C3b can bind additional complement proteins to initiate the membrane attack complex (MAC), which can ultimately result in intravascular hemolysis. C3b can also serve as a direct ligand for complement receptors, which, in addition to Fc receptor engagement of bound antibodies, can facilitate erythrophagocytosis. Receptor engagement of antibodies, C3b, C3a and Hemoglobin (Hb) (and its metabolites) can result in the activation of monocytes/macrophages and many other cells involved in immune function, directly contributing to the pathophysiology of a hemolytic transfusion reaction.

Table 1

Predicting, preventing and treating acute hemolytic transfusion reactions

**Predicting:*

- 1. The use of an *in vivo* RBC clearance assay in patients is preferable if available
- 2. If an *in vivo* assay is not available, the clinical significance of an alloantibody can be evaluated using surrogate approaches such as the MMA or CHUHE assay

Preventing:

- 1. If the patients is experiencing life-threatening anemia and an incompatible transfusion is deemed necessary, consideration of the implicated alloantibody-antigen combination should be employed when seeking to determine the optimal immunoprophylaxis approach.
 - a. For IgG incompatibilities, IVIg and steroids are often employed
 - b. For all incompatibilities (regardless of antibody isotype) where complement is implicated (either due to historical data or a CHUHE test result), consideration of a complement inhibitor is warranted.
- 2. Regardless of the incompatibility, treatment with complement inhibitors should be considered if hyperhemolysis develops post-transfusion

Treating an active hemolytic transfusion reaction

- 1. Supportive care
- 2. IVIg and steroids
- 3. Consider treatment with complement inhibitors should hyperhemolysis develop

* The data demonstrating that any of these approaches accurately predict, prevent or treat an acute hemolytic transfusion reaction are very limited. When considering an incompatible RC transfusion in a patient with life-threatening anemia (and no compatible RBCs are available), no prophylactic or treatment approach has been demonstrated to prevent the deleterious and occasionally fatal consequences of an acute hemolytic transfusion reaction. When considering options to prevent or treat a HTR, previous reports have employed the following dosing approaches and general considerations: Methylprednisolone or prednisone 1-4 mg/kg per day. IVIg. 0.4-1 g/kg per day for 3-5 days (not to exceed 2 g/kg total). Eculizumab 900 mg (weight based for children) on days 1 and 7, followed by repeat doses if rebound/breakthrough hemolysis occurs. When considering eculizumab, start vaccination with Menveo (MenACWY) and either Bexsero or Trumenba (MenB) immediately while also starting ciprofloxacin (500 mg PO BID) for 14 days for meningococcal infection prophylaxis. Consider consultation with an infectious disease specialist.