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Current Insights Into K-associated Fetal Anemia and Potential Treatment Strategies for Sensitized Pregnancies

Margaret C. Carpenter[a], Scarlett C. Souter[a], Ronnie J. Zipkin[b], Margaret E. Ackerman[a,b,*]

[a] Thayer School of Engineering, Dartmouth College, Hanover, NH, USA
[b] Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

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ABSTRACT

K-associated anemic disease of the fetus and newborn (K-ADFN) is a rare but life-threatening disease in which maternal alloantibodies cross the placenta and can mediate an immune attack on fetal red blood cells expressing the K antigen. A considerably more common disease, D-associated hemolytic disease of the fetus and newborn (D-HDFN), can be prophylactically treated using polyclonal α-D antibody preparations. Currently, no such prophylactic treatment exists for K-associated fetal anemia, and disease is usually treated with intrauterine blood transfusions. Here we review current understanding of the biology of K-associated fetal anemia, how the maternal immune system is sensitized to fetal red blood cells, and what is understood about potential mechanisms of prophylactic HDFN interventions. Given the apparent challenges associated with preventing alloimmunization, we highlight novel strategies for treating sensitized mothers to prevent fetal anemia that may hold promise not only for K-mediated disease, but also for other pathogenic alloantibody responses.

Introduction

Hemolytic disease of the fetus and newborn (HDFN) can be a life-threatening disease caused by maternal alloantibodies specific for nonself red blood cell (RBC) antigens that can mediate the destruction of fetal RBC and/or erythroid precursors. The resulting reduction in fetal RBC may lead to fetal anemia, hydrops, and death [1]. HDFN occurs in the United States at a rate of 1695/100,000 newborns [2]. Prophylaxis and treatment options for these cases are limited and vary with the type of RBC antigen mismatch [3,4]. Therefore, understanding the underlying biology of each RBC alloantigen has the potential to contribute to identifying pregnancies at the highest risk for this complication, detecting and treating disease, and supporting the discovery of new prophylactic or therapeutic interventions.

In most cases, sensitization of the maternal immune system to RBC alloantigens occurs before the pregnancy that is affected by fetal anemia [4]. Exposure to alloantigen occurs either by transfusion of blood products containing mismatched RBC antigens or during the natural breakdown of placenta or hemorrhaging that leaks fetal blood into the mother's circulation in the course of a previous pregnancy (Figure 1) [5]. This sensitization is most commonly caused by antigens belonging to six blood group systems: Rhesus (Rh), ABO, Kell, Duffy, Kidd, and MNS (Figure 2) [6,7]. The severity of fetal anemia varies with antigen for many reasons, including onset of antigen expression during fetal development, timing and prevalence of antigen expression during RBC development, immunogenicity of the antigen, and characteristics of the maternal antibody response [8]. While α-Rh (anti-Rh antibody), particularly α-D is most common, α-K is the second most common cause of severe fetal anemia [9]. Expression differences between Kell and Rh antigens mean that fetal anemia mediated by the 2 blood systems progresses differently, as α-K antibodies have the added ability to suppress erythrocyte precursors [10]. Unlike Rh-disease, Kell-associated disease can thus present with severe fetal anemia without features associated with hemolysis of mature RBCs [11]. As a result, some have advocated for the broader definition of α-K disease as Kell-associated anemic disease of the fetus and newborn (K-ADFN) [12].

Recently, there have been advances both in our understanding of alloimmunization and in the treatment of women sensitized to different antigens that have the potential to improve the preven-

Abbreviations: α, anti; ADCC, antibody dependent cellular cytotoxicity; ADFN, anemic disease of the fetus and newborn; FcγR, Fcγ receptor; FcRn, neonatal Fc receptor; EGA, estimated gestational age; HDFN, hemolytic disease of the fetus and newborn; HOD, fusion protein of hen lysozyme, ovalbumin, and Duffy human antigen; IUT, intrauterine transfusion; IVIG, intravenous immunoglobulin; K, immunogenic Kell blood group antigen; mAb, monoclonal antibody; MZ, marginal zone; RBC, red blood cell; Rh, Rhesus antigen; D, Rhesus antigen D; RhIg, polyclonal α-Rh IgG, prophylactic treatment for D sensitization.

* Correspondence to: Margaret E. Ackerman, PhD, Thayer School of Engineering, Dartmouth College, 14 Engineering Dr, Hanover, NH 03755.
E-mail address: margaret.e.ackerman@dartmouth.edu (M.E. Ackerman).

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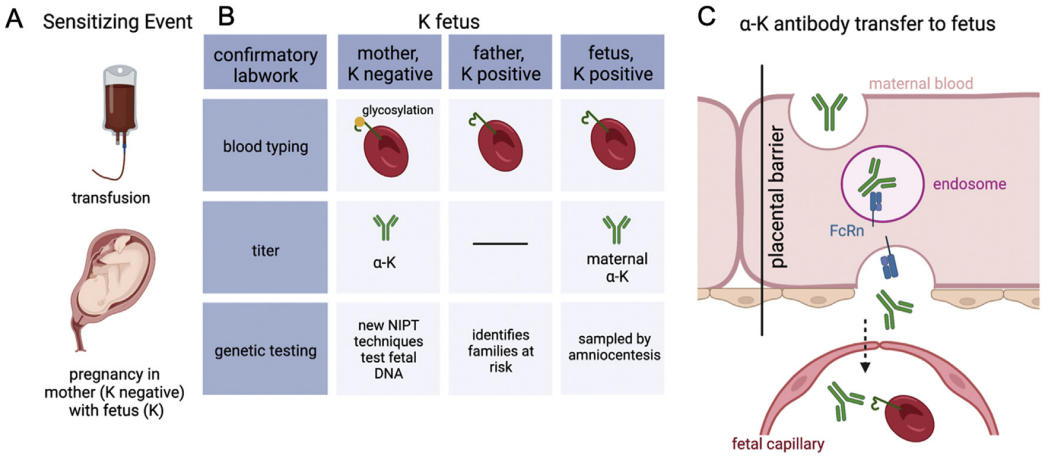
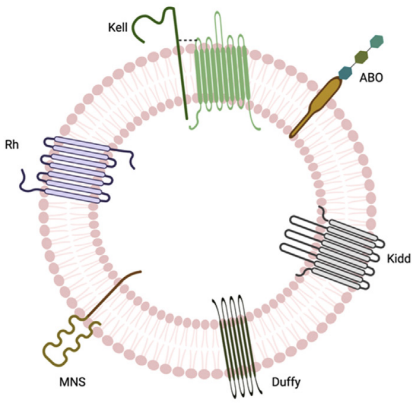


Fig. 1. Maternal K sensitization and α-K antibody mechanism of action. (A) Mothers are usually sensitized either by a transfusion with K blood or by hemorrhage during previous pregnancy. (B) K mismatch or risk of mismatch can be identified with lab work and noninvasive prenatal testing. (C) To cause fetal anemia, α-K IgG must cross the placenta via FcRn.



Blood group	Frequency of relevant antigens	Association with Severe HDFN
Kell	K: 2% in Blacks, 9% in Caucasians, up to 25% in Arabs	+++
Rh	D: 85% Caucasians, 92% Blacks, 99% Asians C: 68% Caucasians, 27% Blacks, 93% Asians E: 29% Caucasians, 22% Blacks, 39% Asians c: 80% Caucasians, 96% Blacks, 47% Asians e: 98% Caucasians, 98% Blacks, 96% Asians	+++
ABO	A: 43% Caucasians, 27% Blacks, 28% Asians B: 9% Caucasians, 20% Blacks, 27% Asians A1: 34% Caucasians, 19% Blacks, 27% Asians	+
Kidd	Jk^a: 77% Caucasians, 92% Blacks, 73% Asians Jk^b: 74% Caucasians, 49% Blacks, 76% Asians Jk3: 100% in most populations, >99% in Polynesians	+
Duffy	Fy^a: 66% Caucasians, 10% Blacks, 99% Asians Fy^b: 83% Caucasians, 23% Blacks, 18.5% Asians Fy3: 100% Caucasians, 32% Blacks, 99.9% Asians	++
MNS	M: 78% Caucasians, 74% Blacks N: 72% Caucasians, 75% Blacks S: 55% Caucasians, 31% Blacks s: 89% Caucasians, 93% Black	+

Fig. 2. Blood group antigens associated with maternal sensitization and fetal anemia. Left: Schematic of common RBC blood group antigens associated with HDFN. Right: Antigen classes, allotypic variants and their frequency in different populations, and the degree to which they are associated with severe fetal anemia [4,6-8].

tion and management of alloimmune fetal anemia. Since the *1960s*, the prevalence of α-D-associated HDFN (D-HDFN) has decreased due to successful prevention of maternal sensitization with polyclonal α-D immunoglobulin (RhIg) [13]. No such treatment exists for K-associated fetal anemia, which is rare but often serious when it occurs. As an example, in 1986, a 16-year retrospective study of 127,076 American patients by Caine et al [14], found K sensitization in 127 pregnancies (0.1%). Of the 13 pregnancies with maternal sensitization to fetal antigen, 5 pregnancies (38%) had severe outcomes: 2 neonates suffered hydrops fetalis and 3 died in utero [9,14]. Current treatment for K-associated disease is to treat the mother with IVIG and to manage fetal anemia through intrauterine blood transfusions, suggesting the value of more research into means to reduce either or both the risk and consequences of maternal alloimmunization. This review will focus on recent work investigating the challenges in developing prophylactic recombinant Ab therapeutics to prevent ADFN/HDFN and the development of new treatment strategies to support sensitized pregnancies. We will highlight work related to K-ADFN as it is a common form of severe disease, but many of the mechanistic lessons and treatment strategies may apply across sensitization to a variety of antigens.

Characteristics of the K Antigen and Current Treatment of K-ADFN

A diversity of polymorphic surface proteins are expressed on RBC. Among other factors, these alloantigens cause HDFN with differing prevalence and consequence due to variable characteristics of population genetics and alloantigen biology.

KEL1 Antigen and Immunogenicity

Maternal alloantibodies that mediate K-ADFN are raised against allotypes of a membrane glycoprotein expressed early in RBC development [15–17]. Most often, K-ADFN is associated with the KEL1 allele, in which a single nucleotide polymorphism leads to a substitution of a highly conserved threonine with methionine at amino acid 193 in the Kell protein (Figure 3). This mutation disrupts a glycosylation site, such that an N-linked glycosylation sequon present in the K negative phenotype is absent in the K phenotype, resulting in expression of an immunogenic epitope [15,18,19]. If a mother has been previously exposed to K antigen, she may produce α-K immunoglobulin G (IgG) that then has the potential to mediate de-

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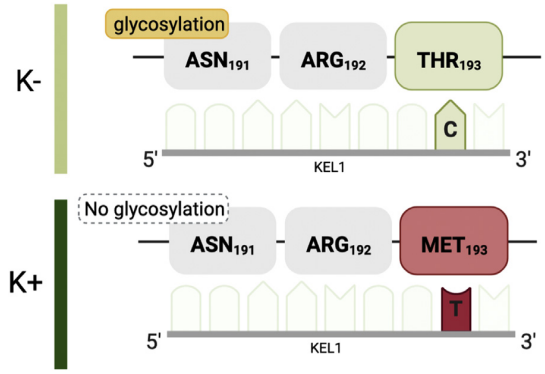

Fig. 3. Amino Acid substitution in K antigen arising from a mutation in the KEL1 gene. An amino acid polymorphism that changes a threonine to methionine eliminates an N-linked glycosylation motif, resulting in presentation of an antigenic epitope.

struction of fetal RBCs when transported across the placenta to the fetus by the neonatal Fc receptor (FcRn) [20], which has important implications for novel approaches to treatment. Based on the incidence of K-sensitized, antigen-mismatched pregnancies reported in retrospective studies [14] as well as global annual pregnancies rates [21], we estimate about 10,000 pregnancies are affected by K-ADFN worldwide per year.

Current Treatment of K-ADFN

Early detection of K=mediated fetal anemia is essential for positive outcomes. Thirty years ago, a study of 208 pregnancies reported that 89% of fetuses expressing D antigen survived using intrauterine transfusion as treatment for fetal anemia [22]. The same study reported that only 56% of those expressing K survived, likely in part because of delayed recognition and referral for intrauterine transfusion [22]. Because K is expressed in primitive erythrocytes, maternal α-K alloantibodies inhibit erythroid progenitor cell growth [10,11,23,24]. *In vitro* assays comparing the effect of α-D or α-K IgG monoclonal antibodies on progenitor cell proliferation found α-K IgG specifically inhibited growth of K-expressing erythroid progenitor cells and had no effect on the proliferation of either K negative erythroid progenitor cells or progenitor cells of other lineages [10]. Perhaps because of this early expression of the antigen, there is much less hemolysis in K-associated fetal anemia than in the more common Rh-associated HDFN [12], making it challenging to diagnose. Historically, the severity of fetal hemolysis in Rh-associated disease can be determined with spectrophotometric measurement of bilirubin in amniotic fluid, but in the case of α-K=mediated disease, bilirubin concentration is low, and diagnosis is less accurate for detecting severe K=associated anemia (83%-89% accurate) than for Rh-associated disease (95%) [9,25]. Cordocentesis-based measurement of fetal hemoglobin can provide a better indicator of severity of K=associated fetal anemia [26]. Because of the invasiveness of both amniocentesis and cordocentesis, Doppler ultrasonography measurements of fetal middle cerebral artery peak systolic velocity have been correlated to cordocentesis measurements [27–29]. These ultrasonography based measurements allow for noninvasive monitoring of fetal anemia mediated by many antigens [27–29]. In the case of a K-sensitized mother with potential for a K fetus, the most effective course is to monitor the fetus for signs of anemia and hydrops every 1 to 2 weeks by ultrasound starting at 17 weeks [30].

When identified, fetal anemia is typically treated with intrauterine blood transfusions (IUT). IUT is effective but carries a rate of fetal loss of 8.5% before 20 weeks estimated gestational age (EGA), and 0.9% after 20 weeks [31]. In K=associated disease, maternal antibody titer does not significantly correlate well with the severity of fetal anemia or the EGA of the first necessary IUT [30]. This lack of correlation suggests that qualitative aspects of the antibody response may have an important influence on its pathogenicity [32,33], and results in conflicting practices concerning the influence of antibody titers in guiding clinical response [33]. Fortunately, for children who survive with IUT treatment, cognitive and physical outcomes are good [34].

Recent Work to Define the Mechanisms of Sensitization to RBC Antigens in Mouse Models

Recent studies in preclinical models have shed light on processes involved in alloimmunization [35,36]. An important fundamental consideration in understanding alloimmunization is to identify the immune cells that first recognize the antigenic RBC. A recent study in mice indicated that upon transfusion, RBC expressing a model antigen comprised of hen lysozyme, ovalbumin, and human Duffy antigen (HOD), colocalized with marginal zone (MZ) B cells [37]. In mice depleted of MZ B cells, lower titers of α-HOD antibody (IgM and IgG) were detected, whereas depletion of follicular B cells had no effect on α-HOD titer [37]. This identification of MZ B cells as an important cell type in the process of alloimmunization suggests a new potential target for prophylactic treatment. This model also points to a role for CD4+ T cells in sensitization, as mice depleted of CD4+ T cells did not raise α-HOD responses. In addition, α-HOD responses were durable, lasting for more than 3 months, suggesting CD4+ T cell-driven differentiation of B cells to antibody secreting cells [37]. An analogous study that focused on Kell sensitization in mice uncovered similar MZ B cell-dependent sensitization responses, though these were independent of $CD4^+$ T cells, again highlighting that the mechanism of alloimmune responses may vary by antigen [38,39].

Other host factors also play a role. In a model of K sensitization in which healthy mice can be protected from sensitization with α-K prophylactic and K^+ RBC [38], researchers observed increased rates of sensitization when they also induced viral-like inflammation in mice by intraperitoneal administration of the adjuvant poly (I:C) [40]. This sensitization was dependent on the complement pathway, as mice lacking complement pathway component proteins C3 or C1q did not show increased sensitization [40]. In *in vitro* studies, B cells isolated from complement knock-out mice were less likely to bind complement-fixed, opsonized RBC compared to B cells from wild-type mice [40]. This study highlights the potential roles of inflammation state and complement in mediating B cell activation and maternal sensitization to antigenic RBCs, suggesting further targets that may be relevant to strategies to prevent alloimmunization.

Overall, while these and other preclinical studies modeling RBC sensitization vary in biological relevance, they are consistent in identifying that multiple factors play a role in the likelihood and characteristics of humoral alloimmunity.

Developing New Treatments for HDFN

To review new strategies for treatment of HDFN, we consider the prophylaxis of HDFN by RhIg (eg, RhoGAM), which has changed the landscape of D-mediated disease. Interest in the mechanism of RhIg has also motivated work to better understand alloimmunization more broadly [41–46]. The success of RhIg is tempered by challenges in reproducing the effect with monoclonal antibodies (mAbs). These challenges mean that while expanding prophylactic treatment to HDFN mediated by other antigens is of high interest, this goal may not be achievable. Because antigen-specific prophylactics are likely to be difficult to develop, alternative treatments

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to better manage pregnancies in women who are already sensitized that are agnostic to the RBC antigen involved would be of high clinical value.

Prophylaxis of HDFN

In principle, HDFN can not only be treated to reduce disease severity and sequelae, but also can be prevented by interventions that reduce the likelihood of alloimmunization. This strategy has been enormously successful in the case of D-HDFN. Worldwide, D-HDFN is estimated to occur in around 276 out of 100,000 pregnancies [47]. Of those that develop D-HDFN and go untreated, 50% will either die or develop brain damage as a result [47,48]. The introduction of RhIg in 1968 led to the decrease of D-associated alloimmunization in pregnancies by 95% [49,50]. In a Dutch study, Koelewijn and colleagues reported antenatal RhIg treatment lowered the frequency of pregnancies in D negative mothers resulting in Rh sensitization from 0.66% in pregnancies given only postpartum RhIg (200 µg dose) to 0.31% in pregnancies given both antepartum and postpartum RhIg (both 200 µg doses) [51], a rate of sensitization that represents a 50-fold decrease from the risk of sensitization without any treatment [52]. A corresponding halving of the rate of severe HDFN was also seen in mothers given antenatal RhIg [51]. Generally, RhIg treatment is safe, effective, and specific, successfully reducing the frequency of D sensitization with minimal risk of side effects [53,54]. Successful prevention of D-HDFN using RhIg is a major achievement in the fields of fetal care and immunotherapy. The efficacy of this treatment might guide the development of new treatments for K-mediated disease [53,55].

By contrast, the shortcomings of RhIg - the difficulty of manufacture, the mysterious mechanism of action, and the challenges of developing a recombinant version of the therapy – temper the feasibility of identifying antigen-specific prophylactics. RhIg is pooled, polyclonal IgG antibody from D-negative donors sensitized to D-positive RBC [56]. Therefore, RhIg is limited in supply, variable in nature, somewhat expensive to produce, and carries a small risk of transmission of blood borne disease [56]. The exact dosing of RhIg varies by country and case, but most women receive 2 to 3 doses between 100 and 300 µg RhIg [51]. Globally, about 2.7 million women are treated with RhIg per year [57]. Still, much of the need in poorer countries remains unmet, and recent work suggests a gap in needed D prophylaxis of about 2.9 million doses [57]. Therefore, the development of prophylatic α-D monoclonal antibody (mAb) treatments that could be mass-produced to replace RhIg is of significant interest. Despite the prevalence of mAb therapies in diverse disease settings and the long-standing, widespread use of polyclonal RhIg, a monoclonal form of RhIg (RhoClone), has only recently been approved and is available in relatively few countries [58]. Lessons from efforts to develop prophylactic α-D mAbs illustrate the complexity of developing mAb therapeutics in the context of prevention of alloimmunization.

Challenges in Developing mAbs to Prevent Sensitization

Until the early *2000s*, clearance of antigenic RBC was a favored hypothesis for the efficacy of RhIg, as clearance of antigenic fetal red blood cells from maternal circulation is an effective metric for ensuring the correct dosing of RhIg [59,60]. Clearance of RBC was used as a surrogate measure to identify promising candidate monoclonal D-specific antibodies, but the majority of these mAbs had disappointingly variable effects on sensitization. Candidates with varying IgG subclasses and profiles for recognition of antigen, lysis or phagocytosis of antigen-expressing cells, and clearance of RBC *in vivo* have been considered [61,62], and recent work has sought to clone novel candidates from RhIg donors [63]. In the late *1990s* and early *2000s*, at least 15 first-in-human trials of mAb and recombinant Ab RhIg replacement candidates were completed [50]. In addition to safety, these studies assessed the ability of treatments to clear antigenic RBC, and in some cases, the ability to reduce the probability of alloimmunization [50]. Although candidates were initially identified through similar performance in model clearance assays, a wide variety of outcomes were seen in the clinic [50]. Sadly, one proposed mAb treatment, chosen for rapid RBC clearance, led to enhanced alloimmunization in a small human clinical trial [50,59]. Other treatments had low bioavailability and variable *in vivo* effects. Perhaps most intriguingly, performance of 2 mAbs, BRAD-3 and BRAD-5, changed when they were manufactured using a human B-lymphoblastoid cell line or Chinese Hamster Ovary cells, leading to the hypothesis that antibody glycosylation patterns, which vary by expression host and affect binding to FcγR, were essential to mAb activity [50].

The importance of mAb glycosylation has been demonstrated in a few contexts. Broadly, galactosylated and asialylated IgG's have been shown to be proinflammatory and to increase ADCC activity through FcγRIIIa interactions [64], and increased levels of IgG galactosylation and sialylation are associated with onset of rheumatoid arthritis remission during pregnancy [65]. Characterization of 23 α-D mAbs, many investigated clinically, demonstrated a diversity of glycosylation states [66]. Across this panel of mAbs, differences in fucose and galactose content resulted in differences in FcγRIIIa binding and activity in a cell-based antibody-dependent cellular cytotoxicity assay (ADCC), as well as in *in vivo* clearance assays in humans [66]. In another study to probe the importance of glycosylation state to prophylaxis, D-specific antibodies were purified from a polyclonal RhIg treatment and glycoprofiled [67]. The D-specific antibody fraction was more highly galactosylated and sialylated than the total IgG found in an RhIg product, indicating that immunomodulatory and lower ADCC activity may be preferable for prophylaxis [67]. This increase in galactosylation of D-specific IgG was also seen in a survey of RhIg from different manufacturers [68]. Glycosylation patterns of α-K, particularly the fucose content of the Fc domain glycans, have been shown to correlate with severity of disease in K-ADFN [69,70]. Similar observations have been made in α-D[71], α-c HDFN[70], and thrombocytopenia [72]. These glycosylation patterns are stable through the pregnancy suggesting that, in addition to being an important factor in the design of prophylactic mAbs, glycosylation patterns of α-antigen antibodies could be leveraged in developing treatment plans for affected pregnancies [69,72].

Work to extend prophylactics to K sensitization in animal models has hinted at similarly complex responses to prophylactic mAbs and the weakness of RBC clearance as a surrogate measure of efficacy. The importance of prophylactic mAb subclass to sensitization, but not RBC clearance, was systematically demonstrated in a K antigen mouse model of alloimmunization. While this is a useful model in the study of sensitization, an important caveat is that recipient mice lack the Kell protein expression altogether and are then transfused with K^+ RBC, whereas in human disease, mother and baby express 2 allotypes of the same protein (Figure 3). A strength of this study is that mice were treated with antibodies of identical specificity to a single Kell epitope and recombinantly expressed in the complete panel of mouse IgG subclasses (IgG1, IgG2a, IgG2b, IgG2c, and IgG3), which exhibit variable effector function activities. The mice were then sensitized with mouse RBCs engineered to express Kell. Each subclass-switched mAb treatment led to clearance of K^+ RBCs, but mAb-treated mice raised differential titers of α-K antibody relative to those treated with a buffer control [73]. Subclasses with high affinity for FcγR tended to enhance alloimmunization, and those with decreased effector function generally decreased alloimmunization compared to buffer control [73]. IgG subclass and FcγR-dependent sensitization was also

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seen in a second model in which mice were passively immunized with α-Duffy IgG, and then exposed to RBCs expressing a fusion protein (HOD) including part of the human Duffy antigen [74]. In this study, sensitization in the context of Duffy-specific mAb was also associated with epitope spreading—the induction of antibodies toward other, co-expressed RBC antigens [74]. Beyond supporting the finding that subclass is important to modulating immune response, experiments here identify $CD8^+$ $CD11b^+$ dendritic cells (DC) as essential to antigenic RBC clearance and show that this clearance coincides with increased $CD4^+$ T cell proliferation [74]. Hoping to isolate RBC clearance and sensitization to a particular FcγR and immune compartment, an ambitious series of conditional FcR γ chain knockout mice strains were created to attempt to modulate FcγR activity in subsets of immune cells. Treatment and sensitization of mice with variation in FcγR expression successfully decoupled RBC clearance from alloantibody response in this model [74], and made apparent that the phagocytic cells that clear antigenic RBCs are distinct from the antigen presenting cells that drive sensitization leading to increased alloantibody titers [74]. In a follow up study, either genetic knockout or mAb blocking of murine FcγRIV prevented sensitization and RBC clearance by IgG2c subclass α-HOD [75]. This finding is consistent with the identification of the role of DCs in clearance as in mice FcγRIV is expressed on splenic DCs [76].

In a similar K antigen mouse model, Stowell and coworkers proposed modulation of antigen expression rather than RBC clearance as the mechanism of antibody prophylaxis to alloimmunization [38]. They observed that alloimmunization could be prevented when mice transfused with mouse RBCs expressing K were treated with α-K sera or polyclonal α-K IgG, alloimmunization could be prevented. Mice were not protected when sera or polyclonal antibody specificity were mismatched with transfused RBC antigen. Intriguingly, by monitoring transfused RBC using either a tracking dye or GFP expression rather than antigen expression, about 50% of the transfused RBCs remained in circulation for 20 days post-transfusion, but detection of the K antigen expression was nearly entirely diminished within 24 hours of antibody treatment. In contrast, K antigen remained detectable over the same time period in mice transfused with K RBC alone and was stable for weeks in agammaglobulinemic mice [38]. In a recent study, antibodies that lead to the removal of HOD antigen from RBC suppressed sensitization, confirming antigen loss rather than RBC clearance as an important mechanism in tolerance [77]. Together these studies point to the importance of α-antigen mAb effector function in sensitization and have uncovered new insight into the effects of antigen dose and antigen loss on RBCs, although care should be taken in generalizing lessons across epitopes and from mouse models to the human immune system.

Overall, results from prophylactic mAb studies in animal and human experiments certainly reinforce the complexity of the biology of alloimmunization, as well as the likelihood that multiple mechanisms are at play in sensitization to RBC antigens. Nonetheless, they may also suggest some generalizable concepts. Namely, that clearance of antigen, whether by antigenic modulation, RBC clearance, or other mechanisms is desirable, but outcomes may be best if this clearance is achieved in the absence of inflammation, such as may be induced by non-cytolytic mAbs. Additional effects on B and T cell immunomodulation, including changes that may be associated with pregnancy, appear difficult to model, but may be important factors clinically.

Antigen-Independent Treatments

Beyond the open questions in understanding therapies in the absence of clear mechanism of action, typing the mother-infant dyad can be difficult, and the occurrence of fetal anemia caused by antigens other than D is rare. Therefore, there is a clinical need for alternative interventions that could prevent or treat fetal anemia across different alloantigens in the context of existing sensitization.

Motivation, Benefits, and Risks of Antigen-Independent Treatment

Preventing maternal sensitization requires blood group typing of mothers, and potentially genotyping of the father and fetus. Noninvasive prenatal testing, in which fetal (cell-free) DNA is sequenced from a maternal blood sample, has improved screening for D+ fetuses and emerging technologies may make it possible to screen for other blood antigens including Kell blood group antigens [78]. Given the rarity of K-mediated fetal anemia, even if effective strategies to prevent K alloimmunization were available, implementation of testing would likely face challenges to its cost-effectiveness.

In contrast, novel approaches to prevent anemia after sensitization have the potential to improve outcomes. Additionally, these strategies could potentially be employed for diverse cases of fetal anemia, independent of the identity of the specific blood antigen mismatch. These strategies include removing maternal alloantibodies from circulation, blocking alloantibody transfer across the placenta, blocking alloantibody and RBC antigen interactions, and reducing maternal antibody production [37,79]. A downside to these treatments is that they also affect the transfer of most or all maternal antibodies, which is expected to leave the neonate more vulnerable to infectious disease. The importance of maternal-fetal antibody transfer to health in early life has been demonstrated in both the protection of neonates through maternal vaccination [80] and in the increased risk of neonatal infection when maternal antibody transfer is impaired [81].

Blocking Alloantibody Transfer

Because maternal alloantibodies must be transferred to the fetus via the placenta to cause ADFN/HDFN, manipulating maternal-fetal antibody transfer may lead to generalizable, or antigen-independent treatment modalities. All molecules passing from maternal to fetal blood must cross this barrier, which is made up of 2 layers of cells, the first being multinucleated syncytiotrophoblasts and the second the endothelial cells of fetal capillaries [20]. Molecules less than 500 Da can usually passively diffuse across the placenta, while molecules of higher molecular weight generally do not transfer at all [20]. IgG antibodies, with molecular weight around 160 kDa, are an exception to this rule and are the sole antibody isotype to be transferred across the placenta in significant amounts [20]. IgG transfer occurs via pH-dependent binding of IgG to the neonatal Fc receptor (FcRn), which is an integral membrane glycoprotein that supports both the long plasma half-life of IgG and its transport across the placenta from acidifying endosomes [82,83]. Though transfer efficiency varies by IgG subclass, IgG is actively transported across the placenta [84]. Therefore, when the mother's blood exhibits high concentrations of IgG such as α-K, these antibodies will readily be transported through the placenta and into the fetal blood where they may have detrimental effects, suggesting the value of interventions that would restrict placental transfer of antibodies [85].

One such FcRn blocking strategy is treatment with nipocalimab (also called M281), which is an aglycosylated recombinant human IgG1 mAb with an affinity for FcRn 1,000 times that of endogenous IgG Fc [79], developed with treatment of both auto- and alloimmune antibody-dependent diseases as potential indications. Preliminary studies from animal models, human placenta studies, and human trials suggest blocking FcRn with this antibody drug potentially protects the fetus in 2 ways [79,86-89]. First, this inter-

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vention prevents maternal IgG from crossing the placenta [86,88], and second, it inhibits the IgG recycling function of FcRn, thereby decreasing the circulating concentration of maternal IgG, including pathogenic IgG alloantibodies [79,89]. Phase I trials of nipocalimab proved that it was safe in humans and did not increase the risk of infection [90]. In June of 2023, results from UNITY (NCT03842189), a phase II clinical trial to assess the ability of nipocalimab to result in live birth at 32 weeks of gestation or later without the need for IUT in alloimmunized pregnancies at high risk of early-onset severe HDFN were announced. Whereas only 10% of reference pregnancies met this outcome, 54% of patients who received nipocalimab did, with a mean gestational age greater than 37 weeks [91]. While inclusion criteria included α-D titers $\geq$32 or α-K titers $\geq$4, it is not yet clear how many participants exhibited which alloreactivity. While promising, this study relied on published and unpublished data as a historical reference for comparison to outcomes with the 13 subjects enrolled. A more rigorous randomized and placebo controlled phase III study in 120 participants is planned (NCT05912517). Other FcRn blocking mAbs, such as efgartigimod [92] and rozanolixizumab [93], which have been approved for treatment of generalized myasthenia gravis based on reducing circulating antibody and are in development for treatment of disease mediated by other autoantibodies, may hold similar promise in preventing or treating ADFN/HDFN [94,95].

Reducing Levels of Maternal Alloantibody

Blocking FcRn is not the only means to reduce the levels of pathogenic maternal alloantibodies. In fact, in a small case study, 2 cases of severe K=mediated disease were successfully treated with 3 such strategies: immunoadsorption, intravenous immunoglobulins (IVIG), and rituximab. Treatment was started at 14 and 21 weeks EGA, at which point the mothers had α-K antibody titers of 2000 and 8000 respectively [96]. Each component of this treatment mitigates the severity of fetal anemia. First, immunoadsorption is an apheresis technique that selectively removes immunoglobulins and immune complexes from the blood. In the context of HDFN, the goal of this intervention would be to lower the titer of pathogenic IgG by removal of IgG from circulation [79]. This process removes beneficial antibodies as well, and requires highly skilled technicians and specialized equipment, making it unlikely to be widely adopted [79]. Second, though used in many indications, the mechanism of action of IVIG is unclear, but in this context, it is thought to dilute maternal IgG and to occupy FcRn binding sites. This occupancy is thought to result in reduced transfer of endogenous maternal IgG to the fetus, and/or to reduce antibody expression by plasmablasts through a rheostat-style mechanism [31]. A fraction of antibodies included in IVIG, likely those with Fc sialylation, may also be immunomodulatory [97]. Studies of both RhIg and IVIG point to antigen-independent action of these treatments that modulate cytokine expression likely impacting T and B cell activity [97–101]. These antibody Fc domain-based interactions are likely context-dependent and the receptors involved in signaling in humans have yet to be identified [102]. Lastly, rituximab mediates CD20+ B cell killing, and significantly reduces the number of circulating plasmablasts, resulting in temporary reduction in α-K [96,103,104]. This combination of treatments lowered the α-K antibody titers to 32 in both mothers [96]. Though Rituximab treatment was discontinued early in third trimester, after delivery one baby had about a 5% decrease in B cells, while the other baby had a complete loss of B cells [96]. However, in both cases, babies were delivered at full term and required relatively minor follow-up care after delivery [96], suggesting the promise of these interventions individually or in combination. Because such case-studies are by nature anecdotal, future studies will be needed to rigorously establish the safety and benefit of these and other strategies to reduce HDFN.

Other approaches realizing success in allo- or auto-immune states that work by reducing levels or blocking the function of the pathogenic antibodies in circulation could also hold promise. To this end, Imlifidase, or IdeS, a selective IgG Fc domain protease repurposed from *Streptococcus pyogenes* has been used to desensitize organ transplant recipients who are positive for antibodies to donor organ antigens [105–108]. If employed in the context of HDFN, this drug would be anticipated to both reduce maternal antibody levels as well as restrict placental transfer. Although care should be taken in trials of IdeS, as patients experienced unexpected severe complications in a small trial for treatment of immune thrombotic thrombocytopenic purpura (iTTP) [108]. Another future prospect might be the selective clearance or blockade of pathogenic afucosylated antibodies, such as has been recently described in a mouse model of severe dengue infection using a nanobody specific for afucosylated IgG Fc [109]. In sum, as compared to the paucity of data suggesting optimism for the prospects for prevention of sensitization, the pipeline of therapies that might reduce HDFN in the context of sensitized pregnancies clearly holds considerable promise.

Conclusion

The seriousness of K=mediated fetal anemia, the lack of prophylactic treatment to prevent K sensitization, and the limited tools available for treating K= mediated fetal anemia create a difficult situation for affected families. While a prophylactic polyclonal serum Ig preparation or mAb capable of preventing sensitization is desirable, the challenges to successful development and even deployment of such a treatment are significant. Despite decades of use, the mechanism by which RhIg protects remains unclear. Surrogate measures of reduced likelihood of sensitization have not been dependable [59], and translation from animal models to humans is not straightforward [110]. On the other hand, many characteristics, including epitope specificity, antibody subclass, and glycosylation state appear to affect mAb prophylactic action [66,73], leading to interesting new understanding of maternal and fetal immunology and antibody activity. Fortunately, new strategies for treating K-ADFN include blocking FcRn and reducing alloantibody in maternal circulation, which have the potential to treat multiple causes of antibody-mediated HDFN. Together these new developments point to a more hopeful future for affected families.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Consent for publication of this paper has been obtained from all authors.

Availability of Data and Materials

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Author's Contributions

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Birchenall KA, Illanes SE, Lopez F, Overton T, Liebling R, Soothill PW, et al. Neonatal outcomes of pregnancies affected by haemolytic disease of the foetus and newborn and managed with intrauterine transfusion: a service evaluation. Blood Transfus 2013;11:548–52.
[2] Yu D, Ling LE, Krumme AA, Tjoa ML, Moise KJ Jr. Live birth prevalence of hemolytic disease of the fetus and newborn in the United States from 1996 to 2010. AJOG Glob Rep 2023;3(2):100203. doi:10.1016/j.xagr.2023.100203.
[3] Ree IMC, Smits-Wintjens VEHJ, van der Bom JG, van Klink JMM, Oepkes D, Lopriore E. Neonatal management and outcome in alloimmune hemolytic disease. Expert Rev Hematol 2017;10:607–16.
[4] Hendrickson JE, Delaney M. Hemolytic disease of the fetus and newborn: modern practice and future investigations. Transfus Med Rev 2016;30:159–64.
[5] Webb J, Delaney M. Red blood cell alloimmunization in the pregnant patient. Transfus Med Rev 2018;32:213–19.
[6] Dean L. Blood Groups and Red Cell Antigens. In: Beck B, editor. National Center for Biotechnology Information. Bethesda: MD; 2005. p. 81.
[7] Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. Amsterdam: Academic Press; 2012.
[8] Kumawat V, Kulkarni K, Goyal M, Lokadas V. ABO hemolytic disease of fetus and newborn; still a diagnostic dilemma: a case report. *Indian J* Hematol Blood Transfus 2018;34:183–4.
[9] Moise KJ. Non-anti-D antibodies in red-cell alloimmunization. Eur J Obstet Gynecol Reprod Biol 2000;92:75–81.
[10] Vaughan JI, Manning M, Warwick RM, Letsky EA, Murray NA, Roberts IAG. Inhibition of erythroid progenitor cells by anti-kell antibodies in fetal alloimmune anemia. NEJM 1998;338:798–803.
[11] Weiner CP, Widness JA. Decreased fetal erythropoiesis and hemolysis in Kell hemolytic anemia. Am J Obstet Gynecol 1996;174:547–51.
[12] Ohto H, Denomme GA, Ito S, Ishida A, Nollet KE, Yasuda H. Three non-classical mechanisms for anemic disease of the fetus and newborn, based on maternal anti-Kell, anti-Ge3, anti-M, and anti-Jra cases. Transfus Apher Sci 2020;59:102949.
[13] Ling L, Yu D, Gleeson CD, Moise K Jr. 968 Estimation of hemolytic disease of the newborn in the United States from 1996-2010. Am J Obstet Gynecol 2021;224:S600–S6S1.
[14] Caine ME, Mueller-Heubach E. Kell sensitization in pregnancy. Am J Obstet Gynecol 1986;154:85–90.
[15] Lee S, Wu X, Reid M, Zelinski T, Redman C. Molecular Basis of the Kell (Kl) Phenotype. Blood 1995;85:912–16.
[16] Redman CM, Russo D, Lee S. Kell, Kx and the McLeod syndrome. Best Pract Res Clin Haematol 1999;12:621–35.
[17] Pu JJ, Redman CM, Visser JWM, Lee S. Onset of expression of the components of the Kell blood group complex. Transfusion 2005;45:969–74.
[18] Lee S, Bennett PR, Overton T, Warwick R, Wu X, Redman CM. Prenatal diagnosis of Kell blood group genotypes: KEL1 and KEL2. AJOG 1996;175:455–9.
[19] Hessner MJ, McFarland JG, Endean DJ. Genotyping of KEL1 and KEL2 of the human Kell blood group system by the polymerase chain reaction with sequence-specific primers. Transfusion 1996;36:495–9.
[20] Palmeira P, Quinello C, Silveira-Lessa AL, Zago CA, Carneiro-Sampaio M. IgG placental transfer in healthy and pathological pregnancies. Clin Dev Immunol 2012;2012:985646.
[21] Sedgh G, Singh S, Hussain R. Intended and unintended pregnancies worldwide in 2012 and recent trends. Studies in family planning 2014;45:301–14.
[22] van Kamp IL, Klumper FJ, Meerman RH, Oepkes D, Scherjon SA, Kanhai HH. Treatment of fetal anemia due to red-cell alloimmunization with intrauterine transfusions in the Netherlands, 1988-1999. Acta Obstet Gynecol Scand 2004;83:731–7.
[23] Daniels G, Hadley A, Green CA. Causes of fetal anemia in hemolytic disease due to anti-K. Transfusion 2003;43:115–16.
[24] Vaughan JI, Warwick R, Letsky E, Nicolini U, Rodeck CH, Fisk NM. Erythropoietic suppression in fetal anemia because of Kell alloimmunization. Am J Obstet Gynecol 1994;171:247–52.
[25] Bowman JM, Pollock JM, Manning FA, Harman CR, Menticoglou S. Maternal Kell blood group alloimmunization. Obstet Gynecol 1992;79:239–44.
[26] Nicolaides KH, Clewell WH, Mibashan RS, Soothill PW, Rodeck CH, Campbell S. Fetal haemoglobin measurement in the assessment of red cell isoimmunisation. Lancet 1988;331:1073–5.
[27] Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ Jr, et al. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. N Engl J Med 2000;342:9–14.
[28] Oepkes D, Seaward PG, Vandenbussche FPHA, Windrim R, Kingdom J, Beyene J, et al. Doppler ultrasonography versus amniocentesis to predict fetal anemia. N Engl J Med 2006;355:156–64.
[29] van Dongen H, Klumper FJCM, Sikkel E, Vandenbussche FPHA, Oepkes D. Non-invasive tests to predict fetal anemia in Kell-alloimmunized pregnancies. Ultrasound in Obstet Gyn 2005;25:341–5.
[30] van Wamelen DJ, Klumper FJ, de Haas M, Meerman RH, van Kamp IL, Oepkes D. Obstetric history and antibody titer in estimating severity of kell alloimmunization in pregnancy. Obstet Gyn 2007;109:1093–8.
[31] D'Mello RJ, Hsu C-D, Chaiworapongsa P, Chaiworapongsa T. Update on the use of intravenous immunoglobulin in pregnancy. NeoReviews 2021;22:e7.
[32] Pittala S, Bagley K, Schwartz JA, Brown EP, Weiner JA, Prado IJ, et al. Antibody Fab-Fc properties outperform titer in predictive models of SIV vaccine-induced protection. Mol Syst Biol 2019;15:e8747.
[33] Kilby MD, Johnson A, Oepkes D. Fetal therapy: scientific basis and critical appraisal of clinical benefits. Cambridge: Cambridge University Press; 2020.
[34] Ree IMC, van 't Oever RM, Jansen L, Lopriore E, de Haas M, van Klink JMM. School performance and behavioral functioning in children after intrauterine transfusions for hemolytic disease of the fetus and newborn. Early Hum Dev 2021;157:105381.
[35] Hendrickson JE, Tormey CA. Understanding red blood cell alloimmunization triggers. Hematology Am Soc Hematol Educ Program 2016;2016:446–51. doi:10.1182/asheducation-2016.1.446.
[36] Ryder AB, Zimring JC, Hendrickson JE. Factors influencing RBC alloimmunization: lessons learned from murine models. Transfus Med Hemother 2014;41:406–19. doi:10.1159/000368995.
[37] Zerra PE, Patel SR, Jajosky RP, Arthur CM, McCoy JW, Allen JWL, et al. Marginal zone B cells mediate a CD4 T-cell–dependent extrafollicular antibody response following RBC transfusion in mice. Blood 2021;138:706–21.
[38] Stowell SR, Arthur CM, Girard-Pierce KR, Sullivan HC, Santhanakrishnan M, Natarajan P, et al. Anti-KEL sera prevents alloimmunization to transfused KEL RBCs in a murine model. Haematologica 2015;100:e394–e3e7.
[39] Yazdanbakhsh K. "Allo" from the (marginal) zone. Blood 2021;138:595–6.
[40] Escamilla-Rivera V, Santhanakrishnan M, Liu J, Gibb DR, Forsmo JE, Foxman EF, et al. Complement plays a critical role in inflammation-induced immunoprophylaxis failure in mice. Front Immunol 2021;12:704072.
[41] Brinc D, Lazarus AH. Mechanisms of anti-D action in the prevention of hemolytic disease of the fetus and newborn. Hematology 2009;2009:185–91.
[42] Smith T. Active immunity produced by so called balanced or neutral mixtures of diphtheria toxin and antitoxin. J Exp Med 1909;11:241–56.
[43] Kumpel BM. On the immunologic basis of Rh immune globulin (anti-D) prophylaxis. Transfusion 2006;46:1652–6.
[44] Pollack W, Ascari WQ, Kochesky RJ, O'Connor RR, Ho TY, Tripodi D. Studies on Rh Prophylaxis. Transfusion 1971;11:333–9.
[45] Karlsson Mikael CI, Wernersson S, Diaz de Ståhl T, Gustavsson S, Heyman B. Efficient IgG-mediated suppression of primary antibody responses in Fcγ receptor-deficient mice. PNAS 1999;96:2244–9.
[46] Yu H, Stowell SR, Bernardo L, Hendrickson JE, Zimring JC, Amash A, et al. Antibody-mediated immune suppression of erythrocyte alloimmunization can occur independently from red cell clearance or epitope masking in a murine model. J Immunol 2014;193:2902.
[47] Costumbrado J, Mansour T, Ghassemzadeh S. Rh Incompatibility. [Updated 2022 Dec 11]. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459353/.
[48] de Haas M, Thurik FF, Koelewijn JM, van der Schoot CE. Haemolytic disease of the fetus and newborn. Vox Sang 2015;109:99–113.
[49] Freda VJ. Rh immunization–experience with full-term pregnancies. Clin Obstet Gynecol 1971;14:594–610.
[50] Kumpel BM. Lessons learnt from many years of experience using anti-D in humans for prevention of RhD immunization and haemolytic disease of the fetus and newborn. Clin Exp Immunol 2008;154:1–5.
[51] Koelewijn JM, de Haas M, Vrijkotte TG, Bonsel GJ, van der Schoot CE. One single dose of 200 microg of antenatal RhIG halves the risk of anti-D immunization and hemolytic disease of the fetus and newborn in the next pregnancy. Transfusion 2008;48:1721–9.
[52] Qureshi H, Massey E, Kirwan D, Davies T, Robson S, White J, et al. BCSH guideline for the use of anti-D immunoglobulin for the prevention of hemolytic disease of the fetus and newborn. Transfus Med 2014;24:8–20.
[53] Keith L, Halloway M, Stepto RC. The multiple use of RhoGAM. J Natl Med Assoc 1973;65:40–4.
[54] Legler TJ. Rhlg for the prevention Rh immunization and IVIg for the treatment of affected neonates. Transfus Apher Sci 2020;59:102950.
[55] Tiller H, Killie MK, Chen P, Eksteen M, Husebekk A, Skogen B, et al. Toward a prophylaxis against fetal and neonatal alloimmune thrombocytopenia: induction of antibody-mediated immune suppression and prevention of severe clinical complications in a murine model. Transfusion 2012;52:1446–57.
[56] Kent J, Farrell A-M, Soothill P. Routine administration of Anti-D: the ethical case for offering pregnant women fetal RHDgenotyping and a review of policy and practice. BMC Pregnancy Childbirth 2014;14:87.
[57] Pegoraro V, Urbinati D, Visser GHA, Di Renzo GC, Zipursky A, Stotler BA, et al. Hemolytic disease of the fetus and newborn due to Rh(D) incompatibility: a preventable disease that still produces significant morbidity and mortality in children. PloS One 2020;15:e0235807.

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[58] Mayekar RV, Paradkar GV, Bhosale AA, Sachan R, Beeram S, Anand AR, et al. Recombinant anti-D for prevention of maternal-foetal Rh(D) alloimmunization: a randomized multi-centre clinical trial. Obstet Gynecol Sci 2020;63:315–22.
[59] Kumpel BM. Efficacy of RhD monoclonal antibodies in clinical trials as replacement therapy for prophylactic anti-D immunoglobulin: more questions than answers. Vox Sang 2007;93:99–111.
[60] Murphy MFBritish Committee for Standards in Haematology, Blood Transfusion Task Force. Guidelines for the clinical use of red cell transfusions. Br J Haematol 2001;113:24–31.
[61] Kumpel BM. In vitro functional activity of IgG1 and IgG3 polyclonal and monoclonal anti-D. Vox Sang 1997;72:45–51.
[62] Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol 2014;5:520.
[63] McGowan EC, Flower RL, Jones ML, Irving DO, Barnard RT, Hyland CA, et al. Aggregates in blood filter chambers used from the plasma donations of anti-D donors: evaluation for monoclonal antibody discovery using phage display. Blood Transfus 2021;19:64–72.
[64] Ackerman ME, Crispin M, Yu X, Baruah K, Boesch AW, Harvey DJ, et al. Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. JCI 2013;123:2183–92.
[65] Rook GAW, Steele J, Brealey R, Whyte A, Isenberg D, Sumar N, et al. Changes in IgG glycoform levels are associated with remission of arthritis during pregnancy. J Autoimmun 1991;4:779–94.
[66] Kumpel BM, Saldova R, Koeleman CAM, Abrahams JL, Ederveen AH, Armour KL, et al. Anti-D monoclonal antibodies from 23 human and rodent cell lines display diverse IgG Fc-glycosylation profiles that determine their clinical efficacy. Sci Rep 2020;10:1464.
[67] Winkler A, Berger M, Ehlers M. Anti-rhesus D prophylaxis in pregnant women is based on sialylated IgG antibodies. F1000Res 2013;2:169.
[68] Kapur R, Della Valle L, Verhagen OJHM, Hipgrave Ederveen A, Ligthart P, de Haas M, et al. Prophylactic anti-D preparations display variable decreases in Fc-fucosylation of anti-D. Transfusion 2015;55:553–62.
[69] Sonneveld ME, Koeleman CAM, Plomp HR, Wuhrer M, van der Schoot CE, Vidarsson G. Fc-Glycosylation in human IgG1 and IgG3 Is Similar for both total and anti-red-blood cell anti-K antibodies. Front Immunol. 2018;9:129.
[70] Sonneveld ME, Koelewijn J, de Haas M, Admiraal J, Plomp R, Koeleman CA, et al. Antigen specificity determines anti-red blood cell IgG-Fc alloantibody glycosylation and thereby severity of haemolytic disease of the fetus and newborn. Br J Haematol 2017;176:651–60.
[71] Kapur R, Della Valle L, Sonneveld M, Hipgrave Ederveen A, Visser R, Ligthart P, et al. Low anti-R h DI g G-F c-fucosylation in pregnancy: a new variable predicting severity in haemolytic disease of the fetus and newborn. Br J Haematol 2014;166:936–45.
[72] Sonneveld ME, Natunen S, Sainio S, Koeleman CAM, Holst S, Dekkers G, et al. Glycosylation pattern of anti-platelet IgG is stable during pregnancy and predicts clinical outcome in alloimmune thrombocytopenia. Br J Haematol 2016;174:310–20.
[73] Shinde P, Howie HL, Stegmann TC, Hay AM, Waterman HR, Szittner Z, et al. IgG subclass determines suppression versus enhancement of humoral alloimmunity to kell RBC antigens in mice. Front Immunol 2020;11:1516.
[74] Gruber DR, Richards AL, Howie HL, Hay AM, Lebedev JN, Wang X, et al. Passively transferred IgG enhances humoral immunity to a red blood cell alloantigen in mice. Blood Adv 2020;4:1526–37.
[75] Qiu A, Miller A, Zotti FD, Santhanakrishnan M, Hendrickson JE, Tredicine M, et al. FcγRIV is required for IgG2c mediated enhancement of RBC alloimmunization. Front Immunol 2022;13:972723. doi:10.3389/fimmu.2022.972723.
[76] Junker F, Gordon J, Qureshi O. Fc gamma receptors and their role in antigen uptake, presentation, and T cell activation. Front Immunol 2020;11:1393. doi:10.3389/fimmu.2020.01393.
[77] Jajosky RP, Patel KR, Allen JWL, Zerra PE, Chonat S, Ayona D, et al. Antibody–mediated antigen loss switches augmented immunity to antibody-mediated immunosuppression. Blood 2023;142:1082–98.
[78] Hyland CA, O'Brien H, Flower RL, Gardener GJ. Non-invasive prenatal testing for management of haemolytic disease of the fetus and newborn induced by maternal alloimmunisation. Transfus Apher Sci 2020;59:102947.
[79] Castleman JS, Moise KJ Jr, Kilby MD. Medical therapy to attenuate fetal anaemia in severe maternal red cell alloimmunisation. Br J Haematol 2021;192:425–32.
[80] Khan R, Vandelaer J, Yakubu A, Raza AA, Zulu F. Maternal and neonatal tetanus elimination: from protecting women and newborns to protecting all. Int J Womens Health 2015;7:171.
[81] Adler C, Haelterman E, Barlow P, Marchant A, Levy J, Goetghebuer T. Severe Infections in HIV-Exposed Uninfected Infants Born in a European Country. PLoS One 2015;10:e0135375.
[82] Antohe F, Rădulescu L, Gafencu A, Gheție V, Simionescu M. Expression of functionally active FcRn and the differentiated bidirectional transport of IgG in human placental endothelial cells. Human Immunol 2001;62:93–105.
[83] Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol 2007;7:715–25.
[84] Clements T, Rice TF, Vamvakas G, Barnett S, Barnes M, Donaldson B, et al. Update on transplacental transfer of IgG subclasses: impact of maternal and fetal factors. Front Immunol 2020;11:1920.
[85] Wyckoff SL, Hudson KE. Targeting the neonatal Fc receptor (FcRn) to treat autoimmune diseases and maternal-fetal immune cytopenias. Transfusion 2021;61:1350–4. doi:10.1111/trf.16341.
[86] Chen P, Li C, Lang S, Zhu G, Reheman A, Spring CM, et al. Animal model of fetal and neonatal immune thrombocytopenia: role of neonatal Fc receptor in the pathogenesis and therapy. Blood 2010;116:3660–8.
[87] Roy S, Nanovskaya T, Patrikeeva S, Cochran E, Parge V, Guess J, et al. M281, an anti-FcRn antibody, inhibits IgG transfer in a human ex vivo placental perfusion model. AJOG 2019;220:498.e1–498.e9.
[88] Li C, Piran S, Chen P, Lang S, Zarpellon A, Jin JW, et al. The maternal immune response to fetal platelet GPIbα causes frequent miscarriage in mice that can be prevented by intravenous IgG and anti-FcRn therapies. J of Clinic Invest 2011;121:4537–47.
[89] Ling LE, Roy S, Daly T, Cochran E, Tyler S, Markowitz L, et al. M281: a therapeutic Anti-FcRn blocking antibody for rapid clearance of IgG and IgG autoantibodies in immune cytopenias and other auto/allo-immune disease. Blood 2015;126:3472.
[90] Gable KL, Guptill JT. Antagonism of the neonatal Fc receptor as an emerging treatment for myasthenia gravis. Front Immunol 2020;10:3052.
[91] Johnson & Johnson. New Phase 2 data demonstrate potential benefit of Nipocalimab for pregnant individuals at high risk of early-onset severe hemolytic disease of the fetus and newborn (HDFN). PRNewswire: Cision, 2023.
[92] Ulrichts P, Guglietta A, Dreier T, van Bragt T, Hanssens V, Hofman E, et al. Neonatal Fc receptor antagonist efgartigimod safely and sustainably reduces IgGs in humans. J Clinic Invest 2018;128:4372–86.
[93] Kiessling P, Lledo-Garcia R, Watanabe S, Langdon G, Tran D, Bari M, et al. The FcRn inhibitor rozanolixizumab reduces human serum IgG concentration: a randomized phase 1 study. Science Transl Med 2017;9:eaan1208.
[94] Howard JF Jr, Bril V, Vu T, Karam C, Peric S, Margania T, et al. Safety, efficacy, and tolerability of efgartigimod in patients with generalised myasthenia gravis (ADAPT): a multicentre, randomised, placebo-controlled, phase 3 trial. Lancet Neurol 2021;20:526–36.
[95] Bril V, Benatar M, Andersen H, Vissing J, Brock M, Greve B, et al. Efficacy and Safety of Rozanolixizumab in moderate to severe generalized myasthenia gravis. Neurology 2021;96:e853.
[96] Capraru A, Raio L, Müller M, Daskalakis M, Rovo A, Henri HC, et al. Immunoadsorption, Intravenous Immunoglobulins and Rituximab (IIR): successful new treatment approach for severe anti K- alloimmunisation during pregnancy. Blood 2020;136:16–17.
[97] Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc Sialylation. Science 2006;313:670–3.
[98] Anthony RM, Kobayashi T, Wermeling F, Ravetch JV. Intravenous Gammaglobulin suppresses inflammation through a novel TH2 pathway. Nature 2011;475:110–13.
[99] Branch DR, Scofield TL, Moulds JJ, Swanson JL. Unexpected suppression of anti-Fya and prevention of hemolytic disease of the fetus and newborn after administration of Rh immune globulin. Transfusion 2011;51:816–19. doi:10.1111/j.1537-2995.2010.02905.x.
[100] Branch DR, Shabani F, Lund N, Denomme GA. Antenatal administration of Rh-immune globulin causes significant increases in the immunomodulatory cytokines transforming growth factor-beta and prostaglandin E2. Transfusion 2006;46:1316–22. doi:10.1111/j.1537-2995.2006.00898.x.
[101] Kaufman RM, Schlumpf KS, Wright DJ, Triulzi DJ. NHLBI retrovirus epidemiology donor study-II. Does Rh immune globulin suppress HLA sensitization in pregnancy? Transfusion 2013;53:2069–77.
[102] Temming AR, Dekkers G, van de Bovenkamp FS, Plomp HR, Bentlage AEH, et al. Human DC-SIGN and CD23 do not interact with human IgG. Sci Rep 2019;9:9995.
[103] Marco H, Smith RM, Jones RB, Guerry M-J, Catapano F, Burns S, et al. The effect of rituximab therapy on immunoglobulin levels in patients with multisystem autoimmune disease. BMC Musculoskelet Disord 2014;15:178.
[104] Nakou M, Katsikas G, Sidiropoulos P, Bertsias G, Papadimitraki E, Raptopoulou A, et al. Rituximab therapy reduces activated B cells in both the peripheral blood and bone marrow of patients with rheumatoid arthritis: depletion of memory B cells correlates with clinical response. Arthritis Res Ther 2009;11:R131.
[105] Lonze BE, Tatapudi VS, Weldon EP, Min ES, Ali NM, Deterville CL, et al. IdeS (Imlifidase): a novel agent that cleaves human igg and permits successful kidney transplantation across high-strength donor-specific antibody. Ann Surg 2018;268:488–96.
[106] Lorant T, Bengtsson M, Eich T, Eriksson B-M, Winstedt L, Järnum S, et al. Safety, immunogenicity, pharmacokinetics, and efficacy of degradation of anti-HLA antibodies by IdeS (imlifidase) in chronic kidney disease patients. Am J Transplant 2018;18:2752–62.
[107] Jordan SC, Lorant T, Choi J, Kjellman C, Winstedt L, Bengtsson M, et al. IgG Endopeptidase in highly sensitized patients undergoing transplantation. N Engl J Med 2017;377:442–53. doi:10.1056/NEJMoa1612567.
[108] Klontz EH. Chop-chop: the future of bacterial enzymes in transfusion medicine. Trans Med Rev 2022;36:246–51.
[109] Gupta A, Kao KS, Yamin R, Oren DA, Goldgur Y, Du J, et al. Mechanism of glycoform specificity and in vivo protection by an anti-afucosylated IgG nanobody. Nat Commun 2023;14:2853. doi:10.1038/s41467-023-38453-1.
[110] Cruz-Leal Y, Lazarus AH. Could antigen loss be a potential mechanism to explain antibody-mediated immune suppression? Transfusion 2021;61:1004–6.

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