REVIEW ARTICLE

Immunologic Basis and Immunoprophylaxis of RhD Induced Hemolytic Disease of the Newborn (HDN)

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ABSTRACT

RhD antigen is the most immunogenic and clinically significant antigen of red blood cells after ABO system. It has historically been associated with hemolytic disease of the newborn (HDN) which is now routinely prevented by the administration of polyclonal anti-D immunoglobulin. This management of HDN has proven to be one of the most successful cases of prophylactic treatment based on antibody mediated immune suppression (AMIS). Despite the increasing efficiency of treatment, the mechanism of action of anti-D is not completely defined. There is a widespread interest in obtaining a reliable therapeutic monoclonal anti-D, due to difficulty of maintaining a pool of high titer volunteer donors for plasma collection and also increasing demand for antenatal prophylaxis and safety issues with plasma derived products. Candidate monoclonal anti-D preparations should demonstrate appropriate functionality in both in vitro and in vivo assays comparable to polyclonal anti-D immunoglobulin. These criteria are reviewed in addition to the factors regulating development of D specific immune response in D negative individuals and its suppression in HDN prophylaxis.

Keywords: Anti-D immunoglobulin, D antigen, Hemolytic Disease of the Newborn (HDN), Monoclonal anti-D, Rh Blood Group System

INTRODUCTION

The D antigen is an extremely potent immunogen of the Rh blood group system present on the surface of human red blood cells (RBCs). It is a non-glycosylated integral membrane protein of approximately 30 kDa, encoded by the RHD gene in the RH locus of D

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positive individuals (1). Most D negative individuals have complete deletion of this gene and the frequency of this status is estimated to be 15-17% among Caucasians (2). These individuals could develop specific antibody against the D antigen (anti-D) if transfused with D positive blood. Similarly, D negative women carrying a D positive fetus may become immunized by fetal D^+ RBC at parturition. Anti-D immunoglobulin G (IgG) developed by mother can be transported across the placenta in subsequent pregnancies and lead to fetal RBC hemolysis creating a pathological condition known as hemolytic disease of the newborn (HDN) (3). Since the introduction of anti-D immunoglobulin prepared from the plasma of immunized donors 40 years ago, and its routine administration after parturition to RhD negative women delivering an RhD positive infant, rate of maternal alloimmunization has reduced dramatically (4). However, alloimmunization still occurs in some cases mainly due to exposure to fetal RBC during pregnancy (5). To prevent this, anti-D should be given antenatally; nevertheless, due to insufficient supply of anti-D, this treatment is not routinely performed universally. Monoclonal anti-D is an attractive substitute as it will provide unlimited supplies of a standardized and safe product. A number of monoclonal candidates are currently being evaluated in clinical studies for their potential to substitute polyclonal anti-D immunoglobulin. Other immunotherapeutic modalities are also being studied which need further investigation.

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RH BLOOD GROUP SYSTEM

Rh blood group system is the most immunogenic and the most polymorphic blood group system in man and currently 48 serologically defined antigens have been identified which belong to this system (2, 6). It is also the most clinically significant blood group system after ABO in transfusion medicine (7). The most important components of the Rh system are D and CcEe proteins encoded by two highly related genes RHD and RHCE, respectively. These proteins are believed to form tetrameric structures known as Rh complexes in the red cell membrane which may contain one D subunit, one CE subunit and two subunits of another molecule called Rh-associated glycoprotein (RhAG) (8). Although recently it has been proposed that Rh protein oligomeric complexes may indeed be trimolecular (9). Physiologically, it is suggested that Rh proteins could play a role in maintaining the cell membrane stability and the regulation of red cell shape (10), based on the observations that individuals lacking all the Rh proteins (Rh null phenotype) suffer from clinical syndromes characterized by a chronic hemolytic anemia of varying severity, an increased osmotic fragility and abnormalities of red cell morphology (known as sphero-stomatocyte), cation transport and membrane phospholipids organization (11, 12).
RHD and RHCE genes (96% identity) are located in the RH locus on chromosome 1p34-p36 in tandem organization and opposite orientation interspersed by a third gene, SMP1, whose function is currently not defined (6). The most common Rh polymorphism (RhD positive/RhD negative) is associated in most cases with the presence or absence of the RHD gene caused by deletion (13). With respect to Rh CcEe proteins, it has been proposed that codominant expression of Cc and Ee polypeptides is regulated by the single RHCE gene through alternative splicing events giving rise to multiple Rh isoforms (14), though distinct transcript for each polypeptide has also been isolated (15). Both RhD and RhCcEe proteins are composed of 417 amino acids and their structural

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models are predicted to consist of 6 extracellular loops, 12 transmembrane and 7 intracellular protein segments with both C and N terminal protein ends residing in the cell (16, 17). D antigen differs from CcEe proteins in 32-35 amino acids, 9-10 of which are located on extracellular loops and the rest are present in the transmembrane and cytoplasmic regions but can still affect the topology of the protein in the membrane (17, 18). There is far more homogeneity between CcEe proteins such that C and c polypeptides differ in 4 (19) and E and e polypeptides only in one amino acid residue (20). The close proximity of RHD and RHCE genes on the same chromosome and the fact that they have mainly identical regions allows for numerous exchange events between them mostly by a process called gene conversion resulting in hybrid proteins accounting for "D variant" or "partial D" categories defined as individuals who lack parts of the normal D antigen. In addition, there are other RhD polymorphisms such as D_{el} and weak D (D^u) phenotypes characterized by reduced D antigen level on the surface of RBCs resulting from various mutations in the RHD gene. A review by Westhoff (21) presents an overview of the molecular complexity of the Rh D protein and its diverse phenotypes.

IMMUNE RESPONSE TO RHD ANTIGEN

Alloimmunity to D antigen in D negative individuals develops in the context of exposure to D^+ blood, which occurs either through incompatible transfusion or pregnancy. D antigen, being a protein, elicits a T cell dependent immune response (22, 23) characterized by the uptake of the antigen into antigen presenting cells (APCs) of the spleen and lymph nodes, followed by its processing into short peptides in the phagolysosome and the presentation of these peptides on the surface of APCs in association with the major histocompatibility complex (MHC) class II molecules, where they can interact with the corresponding T cell receptors on the surface of $CD4^+$ helper T cells. This interaction and further costimulation by the APCs will lead to proliferation and generation of B cell stimulatory cytokines that induce activation and proliferation of B cell clones expressing the antigen specific surface immunoglobulin and their differentiation into antibody secreting plasma cells. Upon first exposure to D antigen, the immune response is relatively slow and may not be detectable for at least 4 weeks. It might result in formation of immunoglobulin M (IgM) alloantbodies. It also produces memory B and T cells that are long-lived and upon subsequent exposure create a rapid response by production of high affinity IgG antibodies that are detectable as early as 48 h post exposure. The high immunogenicity of D antigen is due to the number of antigen specific peptides that can be generated in vivo in D negative individuals expressing only CcEe proteins that as mentioned earlier differ from D antigen in 32-35 amino acid residues. Whereas, C and c which differ by four amino acids and E and e which differ by one amino acid are potentially less immunogenic as fewer specific peptides will be generated when C/c, E/e incompatible transfusions are performed. Using synthetic linear peptides derived from the known amino acid sequence of D protein, it was shown that the level of anti-D response in D negative individuals was directly proportional to the number of peptides that could stimulate antigen specific memory T cells of these individuals to proliferate in vitro (24). It was further demonstrated in this study that these T cells were restricted primarily to human leukocyte antigen (HLA) class II-DR15. A high anti-D titer, and more severe HDN, has also been associated with HLA-DRQB*0201 (25). Similarly, a separate study showed significant overrepresentation of HLA-DRB1*1501 allele in RhD negative donors who had produced anti-D antibodies in response to exposure to RhD posi-

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tive RBCs (26). Interestingly, it was demonstrated that expression of human HLA-DR15 transgene in mice which normally do not react to D antigen confers on them the ability to respond to immunization with purified RhD protein and make specific IgG antibodies (27). On the other hand, there are contrasting reports defying the existence of any meaningful association between a specific HLA allele and induction of anti-D response (28, 29). Possibility of HLA restriction in T cell response to D antigen might partly explain why some D negative individuals (5-15%) remain "non-responders" to D antigen despite repeated exposures. These individuals are usually poor responders to other red cell antigens as well (23). However, those who believe the response to RhD is not HLA restricted consider the status of "non-responder" as being not absolute as it depends strongly on the volume and genotype of transfused RBC, ABO antigens incompatibility between fetus and mother, the number of injections and any history of previous administration of passive anti-D (30). Using the technique of EBV transformation in combination with limiting dilution assay (LDA) and Poisson statistical analysis, we have already demonstrated that the variation in degree of "responsiveness" to D antigen in a group of naturally immunized Rh negative women partly reflects the differences in the frequency of D specific B-lymphocyte precursors in each donor (31).

HEMOLYTIC DISEASE OF THE NEWBORN

Hemolytic disease of the fetus and newborn (HDFN) results from maternal IgG antibodies against fetal RBC antigens that cross the placenta to the fetal circulation during gestation and cause RBC destruction and complications before birth (HDF), or anemia and hyperbilirubinemia after birth (HDN) or both. In most severe cases, it causes hydrops fetalis characterized by total body edema, hepatosplenomegaly, and heart failure which usually lead to intrauterine death (32). D antigen being highly immunogenic is the most important cause of HDN (85%) followed by Kell (10%) and c (3.5%) antigens (33). Rarely, cases of HDN have been documented to be associated with immunization against other red cell antigens for instance Rh C, E/e and G antigens (34-37) and antigens of other blood group systems (38, 39). In some HDN cases a combination of antibodies to D, C and G have been identified, accurate determination of which forms an essential aspect of the management of such affected cases (40). There are also numerous reports on the contribution of each of anti-D IgG subclasses, specifically IgG1 and IgG3 antibodies to the severity of HDN (40-43). Some have suggested that HDN occurs most often when IgG1 anti-D and IgG3 anti-D are both present in the maternal serum (40, 41); whereas, others (42, 43) have shown that IgG1 anti-D alone may cause severe HDN. This isotype restriction reflects the efficiency of interaction between IgG1 and IgG3 antibodies and cell surface receptors belonging to Fcγ receptor I and III classes present on effector cells especially macrophages engaged in immune destruction of antibody sensitized RBCs. Studying the sera and clonal B cell lines obtained from a small group of women alloimmunized through pregnancy, we demonstrated that all the subjects had produced specific antibody of IgG1 subclass, while IgG3 and IgM antibodies were also generated in some donors. Production of IgG2 was identified to a lesser extent while none of the donors were shown to have produced specific antibody of IgG4 subclass. All the donors' fetuses were affected with HDN; some were saved by exchange transfusion while some either gave still birth or their fetuses were fatally affected with hydrops fetalis and were lost. However, number of donors in our study was not suffi-

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cient enough to illustrate a relationship between the isotype of specific antibodies and severity of HDN (44).

IMMUNOPROPHYLAXIS OF HDN

HDN due to D immunization is prevented in the vast majority of cases by passive administration of anti-D immunoglobulin, prepared from plasma of D negative hyperimmunized donors, to RhD negative women within 72 hours of each delivery of an RhD positive fetus. A dose of 300 µg is commonly used in North America, 100 µg in the UK and 200-250 µg in Europe and elsewhere which will protect against 10-15 ml of fetal RhD positive red cells (45). In Iran a dose of 300 µg is routinely used for both postnatal and antenatal intervals. Fetomaternal haemorrhage (FMH) at parturition in 99.3% of women leads to transfer of less than 4 ml fetal red cells which will be effectively removed from maternal circulation by one injection of anti-D immunoglobulin; however, 0.3% of women have more than 15 ml FMH at delivery hence should be given an additional dose of anti-D (46). In some countries where FMH is monitored, patients with large FMH are followed up for 1 to 2 days after injection to check whether fetal red cells have been cleared and if not, additional anti-D is given (47). A high titer intravenous anti-D preparations (1500 IU) are also employed to prevent sensitization of RhD negative mothers receiving large volumes of fetal RhD+ blood. Anti-D is prescribed to all RhD negative and D variant women following a potentially sensitizing event such as delivery of an RhD positive infant. The incidence of D alloimmunization in pregnancy has decreased from 14% to between 1 and 2% following the introduction of postnatal prophylaxis with passive anti-D. The addition of routine antenatal anti-D prophylaxis at weeks 28 and 34 of gestation in some countries has reduced the immunization cases to 0.1% (32). Nevertheless, antenatal prophylaxis is not universally applied due to a variety of factors one of which is availability and cost of anti-D. Many of women who do receive antenatal anti-D treatment might eventually give birth to a D negative fetus; therefore, identification of fetal blood group status is important in order to be able to restrict antenatal anti-D immunoglobulin to mothers carrying an RhD positive fetus. Although genotyping can be performed on fetal DNA obtained through chorionic villus sampling or from amniotic fluid, current research is focused on identifying fetal genotype from maternal plasma as a source of fetal DNA as a far less invasive procedure (45). This technique is being developed as a routine diagnostic test.

IMMUNOLOGIC BASIS OF ANTI-D PROPHYLAXIS

Despite the highly effective clinical use of anti-D in preventing HDN, the mechanism of treatment remains poorly understood. It is proposed to be mainly dependent on the rapid clearance of anti-D coated D^+ RBC from maternal circulation by the spleen following the interaction of IgG anti-D with macrophages expressing three classes of IgG receptors, FcγRI, FcγRII and FcγRIIIa. This will lead to early destruction of D antigen before it is recognized by the immune system (48). Nevertheless, in some cases when more than 10 ml FMH occurs following delivery, anti-D coated D^+ RBC are not cleared from the circulation at the same rate and yet passive anti-D still protects against a specific immune response (49). This observation suggests that antibody clearance theory cannot be the sole mechanism in suppressing the humoral immune response against D antigen. Further support has come from studies of sheep red blood cell clearance in a mice

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model of AMIS (antibody mediated immune suppression), showing that T cell priming was not prevented after transfusion of IgG-opsonized RBCs, indicating that the antigen was visible to the immune system. Moreover, delivery of IgG-opsonized RBCs to phagocytic cells was insufficient to attenuate the B-cell response to RBCs. Finally, it was demonstrated that upon challenge with a mixture of opsonized and untreated RBCs, there was a dose dependent reduction of the antibody response. The authors suggest that the attenuation of the antibody response by anti-RBC IgG is not due to active immune suppression or clonal deletion at T-cell or B-cell level, but rather it is the result of B cell unresponsiveness to IgG-opsonized RBCs (50, 51). Another hypothesis is dependent on the role of immunomodulatory cytokines in explaining anti-D mediated immune suppression as demonstrated by Branch et al. (52) showing that the plasma levels of transforming growth factor β (TGF-β) and prostaglandin E2 were significantly increased 48 hours after administration of antenatal anti-D immunoglobulin. TGF-β may bind to aggregated IgG on coated red cells and be localized to antigen-specific B cells and exert its inhibitory effect. In addition to B cells, TGF-β has a profound long-lasting inhibitory effect on T cell priming (53).

Concurrent engagement of the immunoglobulin molecules on surface membrane of antigen-specific B-cells and the FcγRIIb is known to induce inhibitory signals leading to suppression of antibody production to the corresponding antigen (54). This mechanism has been proposed to be effective in anti-D immunoprophylaxis (55, 56). Infusion of intravenous immunoglobulin (IVIG) collected from plasma of normal individuals with no previous history of RhD immunization has also been shown to downregulate the immune response in some clinical conditions such as recurrent spontaneous abortion and autoimmune thrombocytopenic purpura (57). These immunological effects have been attributed to signaling through both activating FcγR (FcγRI and FcγRIII) on dendritic cells and the inhibitory FcγRIIb on B-cells (58).

MONOCLONAL ANTI-D FOR PROPHYLACTIC TREATMENT

With increasing success of passive anti-D prophylactic program in treatment of HDN, very few women are now immunized and able to donate plasma. Immunization of D negative male volunteers hence serves to make up for the shortage. On the other hand, despite stringent safety guidelines, there are concerns over the inherent risk of transmission of agents such as variant-Creutzfeldt-Jakob disease (vCJD) through administration of human plasma derived products (59). An effective monoclonal anti-D antibody would eliminate the need for preparation and use of plasma derived anti-D.

Over the years, several monoclonal anti-D antibodies have been produced in different laboratories through Epstein-Barr virus (EBV) immortalization and generation of B-lymphoblastoid cell lines (LCLs) derived from immunized D negative donors (60, 61). Since mice do not recognize the D antigen, thus no murine monoclonal anti-D antibodies have ever been produced. In order to enrich for D specific LCLs, some laboratories have utilized rosetting of D-specific B-LCLs with D^+ RBC before cloning (60, 62). In an attempt to increase the antibody production of these cell lines, some have been fused with mouse myeloma cells to produce heterohybridomas (62-64). Monoclonal anti-D antibodies of IgM isotype derived by similar techniques in our laboratory (65) and by other workers (66, 67) serve as efficient D typing serological reagents; however, in order to be effective as a replacement for plasma derived anti-D immunoglobulin, the monoclonal anti-D should be of IgG isotype.

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Aside from traditional techniques of human monoclonal antibody production, more recently human RhD specific monoclonal antibodies have also been generated by various approaches using recombinant DNA technology. These include production of anti-D recombinant antibodies by panning phage display libraries of Fab fragments from a hyperimmune donor and the expression of a complete immunoglobulin construct in baculovirus-inscet cell expression system (68) or Chinese hamster ovary (CHO) cells (69) and by antibody engineering technique from the DNA coding for anti-D in lymphoblastoid cell lines and heterohybridomas and its expression in rodent myeloma cell lines (55).
In order to be considered for prophylactic treatment, the monoclonal anti-D preparations must fulfill certain criteria and their performance evaluated in a number of in vitro and in vivo functional assays. For the rest of this review, we will try to present an overview of these assays in relation to their use in estimating the efficacy of a number of monoclonal anti-Ds in quest for a suitable and efficient substitute for polyclonal anti-D.
Primarily, the antibodies should be serologically determined to be specific for the D antigen and bind only to D positive but not D negative red cells. They should also demonstrate a high affinity constant for their antigen comparable to polyclonal anti-D preparations for which the functional affinity constants varied between 3.1 x 10^8 to 4.2 x 10^8 mol/l, as determined by an enzyme linked immunosorbent assay (ELISA) of solubilized anti-D bound to sensitized RBCs (70). Monoclonal anti-Ds with lower affinity constants show greater dissociation from the fetal red cells, a condition not exactly desirable for generation of monoclonal substitutes with prophylactic value. Epitope specificity of the candidate monoclonal anti-D antibodies is also a determining factor in their selection for immunoprophylaxis. The RhD antigen is composed of different epitopes and a polyclonal anti-D preparation contains a mixture of immunoglobulins directed against these various epitopes. Individuals known as "D variants" or "partial D" lack parts of the normal D antigen and if challenged with normal D positive blood can produce antibody to those epitopes of the antigen which they lack (71). The last international workshop on Rh serology summarized the agglutination pattern of 142 Rh specific monoclonal antibodies with D variant red cells and concluded that there are 30 different reaction patterns or epitopes for D antigen (72). No single anti-D monoclonal antibody is capable of reacting with all the partial D types implication of which is that in order to ensure that a monoclonal anti-D for immunoprophylaxis will be efficient in clearing fetal cells with partial D antigens that could potentially induce an anti-D response in a D negative mother, it might be necessary to blend a mixture of monoclonal anti-D antibodies with different specificities into a functional cocktail (73). Despite the apparently diverse epitope specificity, anti-D antibodies seem to recognize limited antigenic determinants of the RhD molecule. This is supported by the fact that of the 35 D-specific amino acids only seven residues are located in the extracellular region of the D molecule. Thus, only these residues are available for binding of anti-D on RhD+ RBC (23, 74). Restricted epitope specificity of the anti-D antibodies is also indicated by the restricted immunoglobulin variable region heavy (VH) and light (VL) chain genes repertoire. More than 70% of the VH genes rearranged in anti-D antibodies are selected from the VH3.33 superspecies genes (75, 76) and almost 10% of the anti-D VH genes belong to the VH4.34 member of the VH4 gene family (75). Our recently established IgM anti-D monoclonal antibody (MG-1G7) was also found to express the VH4.34 germline gene (unpublished observation). Altogether, these findings imply involvement of a focused antibody response to the RhD antigen.

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In addition to the above factors which can be grouped as serological criteria, selection of monoclonal anti-D for therapeutic use depends on its functional ability in interacting with effector cells mainly through binding of the Fc region of anti-D to the receptors FcγRI, FcγRIIa and FcγRIII. These receptors are present on splenic macrophages; however, normally these particular cells are not available for experimental purposes. Hence, a number of in vitro assays have developed that use $Fc\gamma R^+$ effector cells from peripheral blood. Monocytes express FcγRI and FcγRIIa and natural killer (NK) cells have FcγRIIIa (30). Adherence and formation of rosettes, and phagocytosis as measured by chemiluminescence due to oxidative burst caused by this process are in vitro measures of interaction with FcγRI. Antibody dependent cellular cytotoxicity (ADCC) as measured by radiolabeled chromium release from red cells in presence of NK cells is an indication of the interaction with FcγRIIIa (73). In general IgG1 anti-D promotes greater monocyte phagocytosis than IgG3; whereas IgG3 anti-D promotes more adherence and extracellular lysis by monocytes of sensitized red cells than IgG1 anti-D (77). With regards to anti-D mediated haemolysis via FcγRIIIa on NK cells, only some monoclonal IgG1 anti-Ds have proven effective and IgG3 anti-Ds are generally inactive in this assay (78). Two monoclonal anti-D antibodies (BRAD-3 & BRAD-5) produced by Epstein-Barr virus-transformed B-LCLs (62) are two examples of antibodies with in vitro effector functions almost comparable to polyclonal anti-D. BRAD-5 (IgG1) was found to mediate high extracellular hemolysis of antibody coated red cells by NK cells in ADCC assay; whereas BRAD-3 (IgG3) was shown to adhere to monocytes and induce FcγRI-mediated phagocytosis, chemiluminescence and extracellular lysis of antibody coated red cells more efficiently than BRAD-5 (79). Both antibodies recognize an immunodominant epitope (ep D6/7) on the RhD antigen (62) and have been adapted to large scale culture in hollow-fibre bioreactors with a similar glycosylation pattern to that of human serum IgG (80). They have both been used in clinical studies as the next step in generation of therapeutic monoclonal anti-D.

Basically a number of criteria are considered important in determining the in vivo efficacy of a monoclonal anti-D. The anti-D must demonstrate a long plasma half-life which is desirable for antenatal prophylaxis to ensure anti-D levels are enough to prevent D-immunization at the time of FMH; it must be able to efficiently clear RBCs from the circulation and must prevent D immunization. A recent insightful review by Kumpel presents an excellent overview of the use of different available monoclonal anti-Ds in clinical trials (81). To determine the plasma half life of anti-D, it is injected into D negative subjects in the absence of D positive red cells and kinetics of antibody survival is then calculated by quantitating anti-D concentrations in serum samples taken at intervals (81). BRAD-3 an BRAD-5 were determined to have plasma half lives of 22.2 and 10.2 days, respectively upon intramuscular injections of 300 μg BRAD-5 and 1500 μg BRAD-3. The plasma half life of polyclonal anti-D (100 μg) in the same study was determined to be 15.6 days (82). There are variations between such studies because of different dose of anti-D, route of injection, timing of samples and method of calculation; however in general some monoclonal anti-Ds and recombinant anti-Ds are rapidly eliminated compared to polyclonal anti-D (81).

Clearance studies are usually performed by two different approaches: 1) clearance of ex vivo labelled autologous RBCs presensitized with anti-D antibodies, 2) clearance of labelled allogeneic D positive RBCs in male D negative volunteers after intravascular or intramuscular administration of anti-D. The second approach is usually followed by multiple samplings over a period of at least 6 months to document any immunization.

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Additionally the donors are later challenged unprotected with D positive RBC to assess the nature of immune response (primary as opposed to secondary response) (83). BRAD-3 showed rapid red cell clearance of autologous D positive red cells at almost similar rates to polyclonal anti-D (84) and both BRAD-3 and BRAD-5 were efficient in removing more than 90% of allogeneic red cells from the circulation of D negative volunteers with BRAD-5 being similar to polyclonal anti-D (85); however, both antibodies were used at doses three times that of polyclonal anti-D. Challenge with D positive RBC indicated that all the responders had been protected from immunization by passive BRAD-3 or BRAD-5. Overall, the anti-D antibodies (both monoclonal and recombinant) used in clinical studies have produced variable results in terms of clearance efficiency, protection against immunization, induction of pro-inflammatory events and half lives, details of which are described elsewhere (81).
Recently a recombinant monoclonal anti-D (IgG1), R297, was tested in a clinical phase I study to assess its ability to induce the clearance of autologous antibody coated red cells in male D negative volunteers (86). It demonstrated a more rapid RBC elimination compared to that of polyclonal anti-D at similar coating levels and induced no clinical, biological or immunologic adverse effects in the subjects studied. Authors believe the high efficiency of R297 in clearance studies to be due to its higher FcγRIIIa binding and ADCC activity which is the result of its optimum glycosylation profile (55). In summary, many monoclonal anti-Ds have been developed and studied in laboratories around the world for their potential as prophylactic reagent and all of these studies have contributed a great deal to our understanding of interaction of antibodies with the effector cells of the immune system and the mechanism of anti-D mediated immune suppression.

FUTURE WORK AND RESEARCH

Basically, three different alternative strategies to prevent HDN which are independent of the classical anti-D immunoglobulin administration are currently under investigation:
1. Development of recombinant mutant IgG anti-D deficient in hemolytic activity in order to block the hemolytic activity of maternal anti-D by binding to D^+ fetal RBC (87, 88). These engineered antibodies have been generated either by deletion of the hinge region of the heavy chain and a further mutation to link the light chains by a disulfide bond for added stability, which efficiently removed the Fcγ receptor mediated hemolysis and phagocytosis of RBC (87), or by designing an IgG1 hybrid Fc region which was inactive in binding to Fcγ receptors and complement (88).
2. Investigation on the effect of regulatory $CD25^+$ T cells (Treg1) which inhibit allogeneic reactions through the production of IL-10 and serve to suppress the antibody response in vivo (89).
3. Use of immunodominant RhD peptides to switch off (tolerize) the immune response to D antigen by administration through nasal route. Proof of concept for this strategy has already been demonstrated in humanized HLA-DR15 transgenic mice in which administration of each of these peptides to the nasal mucosa before immunization with purified RhD protein led to inhibition of T-cell priming and blocked specific antibody response (27). Clinical trials for this novel, noninvasive protocol of suppressing anti-D production in women are currently under consideration (90).

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