Nonhemolytic antibody-induced loss of erythrocyte surface antigen

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Transfusion of red blood cells (RBCs) into patients with anti-donor RBC antibodies (crossmatch-incompatible transfusion) can result in lethal antibody-mediated hemolysis. Less well appreciated is the ability of anti-RBC antibodies to specifically remove their target antigen from donor RBCs without compromising cell survival or adversely affecting the transfusion recipient. In an effort to elucidate the mechanistic details of this process, we describe the first animal model of nonhemolytic antibody-induced RBC antigen loss. RBCs from transgenic mHEL mice express surface hen egg lysozyme (HEL) as a transmembrane protein. Transfusion of mHEL RBCs into mice immunized with HEL results in selective loss of HEL antigen from donor RBCs without affecting other blood group antigens or reducing the circulatory life span of the transfused RBCs. While this process does not require the presence of a spleen, it requires both anti-RBC immunoglobulin G (IgG) antibodies and the FcγRII receptor. These studies provide mechanistic insight into the phenomenon of antigen loss during incompatible transfusion in humans.

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Introduction

It has long been appreciated that antibodies against red blood cell (RBC) antigens are a major cause of both hemolytic transfusion reactions in the case of alloantibodies and autoimmune hemolytic anemia (AIHA) in the case of autoantibodies. Most acquired anti-RBC antibodies promote phagocytosis of RBCs by cells of the reticular endothelial system (RES) leading to a delayed extravascular hemolysis that occurs over the course of days to weeks. Patients with AIHA exhibit significant and potentially life-threatening hemolysis that can involve the destruction of the majority of their own RBCs. However, some RBCs can escape antibody-mediated hemolysis through selective loss of the antigen recognized by alloantibodies or autoantibodies. “Antigen suppression” of this type has most often been observed with Kell blood group antigens,1-8 but the phenomenon has also been reported with Rh type has most often been observed with Kell blood group anti-

Materials and methods

Mice

C57BL/6 mice, RAG knock-out +/- and FcγRII knock-out (KO) mice were obtained from Jackson Laboratories (Bar Harbor, ME). FcγR2B KO...
mice were obtained from Taconic Labs (Germantown, NY). mHEL mice (available from Jackson Laboratories) were bred by the Emory Division of Animal Resources Animal Husbandry service. All knock-out mice and the mHEL mice are on a C57BL/6 background. All mice were male, aged 8 to 12 weeks.

Leukoreduction of blood
Peripheral blood was obtained from donor mice by retro-orbital bleed and was passed over a sterile pediatric leukoreduction filter (Puracell Neo Neonatal High Efficiency Leukocyte Reduction Filter; PALL Biomedical Products, East Hills, NY) preequilibrated with phosphate-buffered saline (PBS), adjusted to 340 Osm with NaCl (modified PBS [MPBS]).

Fluorescent labeling of RBCs and transfusion
Leukoreduced RBCs were labeled with chloromethylbenzamido 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate (CM-DiI) or 3,3’-dihexadecyloxacarbocyanine perchlorate (DiO) according to the manufacturer’s instructions (Molecular Probes, Eugene OR). All transfusions were carried out by left lateral tail vein injection.

Immunization with HEL or OVA
HEL and chicken ovalbumin (OVA; Sigma, St Louis MO) were emulsified in complete Freund adjuvant (CFA) at a concentration of 2 µg/µL. One hundred micrograms was injected into the left hind footpad and an additional 100 µg was injected into the left flank. All animals were used 2 to 3 weeks after immunization. After confirmation of adequate immunoreactivity with anti-HEL antisera compared with RBCs from wild-type C57BL/6 mice (Figure 1A), this interaction was specific to the anti-HEL antisera, since mHEL cells were negative using antisera to a separate antigen, OVA. Preincubation of anti-HEL antisera with soluble HEL, but not OVA, inhibited anti-HEL binding (Figure 1B-C), confirming that anti-HEL was recognizing HEL epitopes on the surface of mHEL RBCs. The mHEL-RBC anti-HEL specimen in Figure 1A-C (solid line) represents the same sample displayed in 3 panels for clarity.

To determine whether the presence of HEL on the surface of mHEL RBCs altered any biologic properties of RBCs, we used 2 lipophilic tracking dyes that have long half-lives and typically do not alter membrane characteristics of the labeled cells. RBCs from C57BL/6 mice were labeled with DiO, and RBCs from mHEL mice were labeled with CM-DiI. DiO and CM-DiI fluorescence at different wavelengths, which allows simultaneous tracking of both populations by flow cytometry. Initial flow cytometry studies established appropriate fluorescence compensation parameters such that no crossover in fluorescence was observed with DiO- or CM-DiI-labeled cells (data not shown).

To prevent contaminating leukocytes from interfering with interpretation, blood was stringently leukoreduced prior to labeling with DiO or CM-DiI by using commercially available RBC leukoreduction filters. Leukoreduction resulted in an approximate 4-log10 reduction in contaminating leukocytes (data not shown). Leukoreduced C57BL/6 and mHEL RBCs labeled with DiO or CM-DiI were mixed and transfused into RAG knock-out recipients on a C57BL/6 background. Using RAG knock-out mice ensured that interference by potential anti-HEL humoral immune responses to transfused mHEL RBCs would not occur. Blood from mice that had not received transfusions was used to establish flow cytometry gates such that there were no cells in the DiO or CM-DiI gates (data not shown).

Blood was collected at periodic points over a 5-week time course, and the rate of clearance of C57BL/6 or mHEL RBCs was calculated by determining the percentage of remaining cells by flow cytometry, an example of which is shown (Figure 1D). RBCs from C57BL/6 and mHEL RBCs had a similar half-life of approximately 11 days (Figure 1E). Relative rates of clearance were analyzed by dividing the percentage of remaining mHEL RBCs by the percentage of remaining C57BL/6 RBCs at each time point. This calculated percentage was constant over 5 weeks, indicating that the circulatory half-life of mHEL RBCs was not altered compared with wild-type RBCs (Figure 1F).

To determine the persistence of the mHEL antigen over the life span of transfused mHEL RBCs, blood obtained at each time point was stained with anti-HEL followed by a secondary antibody conjugated to APC. The fluorescence of APC at a wavelength distinct from either DiO or CM-DiI allowed analysis of HEL antigen on the transfused cells. Transfused RBCs from mHEL mice
CM-DiI stained positive with anti-HEL compared with transfused RBCs from C57BL/6 mice (DiO) at each of the tested time points over a 5-week period (Figure 1G). Since no significant decrease in mean fluorescence of anti-HEL staining was observed, we conclude that the mHEL antigen is stable on transfused mHEL RBCs. The staining with anti-HEL was specific, since no shift was seen in either CM-DiI or DiO populations stained with anti-OVA (data not shown). Together, the above-mentioned data demonstrate that mHEL RBCs have a normal circulatory half-life and that the mHEL antigen is stable in vivo on the RBC surface.

Immunization of recipient mice with HEL/CFA results in high-titer IgG1 anti-HEL that binds efficiently to mHEL RBCs

To generate transfusion recipients that were crossmatch incompatible with mHEL RBCs, wild-type C57BL/6 mice were immunized with HEL/CFA. Two weeks after immunization, serum was isolated from peripheral blood, and the presence of anti-HEL antibodies was assayed using a HEL-specific ELISA.

Serum from HEL/CFA-immunized mice contained high levels of anti-HEL IgG antibodies compared with serum from unimmunized mice (Figure 2A). No significant anti-HEL of the IgA, IgE, or IgM type was detected. Each of the secondary antibodies was reactive with wells coated with whole mouse serum (data not shown), indicating that the lack of IgA, IgE, or IgM was not an artifact of nonreactive secondary antibodies. To determine which subtypes of IgG were involved in the anti-HEL response, additional ELISA assays were carried out, using IgG subtype-specific antibodies (Figure 2B). IgG1 was the most abundant anti-HEL antibody, detectable at a titer of at least 1:20 000. Considerable amounts of IgG2b and lesser but significant amounts of IgG2c and IgG3 were also observed. No anti-HEL IgG2a was detectable in any specimens from HEL/CFA-immunized mice. Since IgG2a is not expressed by C57BL/6 mice, the absence of detectable IgG2a is a predicted finding that serves as a negative control for the specificity of the anti-IgG reagents.

To test the capacity of the different subtypes of IgG to bind to mHEL RBCs, serum from HEL/CFA-immunized mice was incubated with RBCs from either mHEL or C57BL/6 mice, followed by staining with secondary antibodies specific for IgG subtypes (Figure 2C). On the basis of this flow cytometric crossmatch procedure, IgG1 was the predominant isotype that bound to mHEL RBCs. Lesser amounts of IgG2b and IgG2c were detected, whereas no significant IgG3 or IgG2a was observed binding to mHEL RBCs. Thus, HEL/CFA-immunized mice were crossmatch incompatible with mHEL RBCs, and IgG1 was the predominant subtype of antibody that bound mHEL RBCs.

Transfusion of mHEL RBCs into HEL-immune mice

We hypothesized that transfusion of mHEL RBCs into HEL/CFA-immunized mice would result in rapid removal of mHEL RBCs. To test this hypothesis, leukoreduced mHEL RBCs were transfused into HEL/CFA-immunized or unimmunized mice (Figure 3). At 6 hours after transfusion, the amount of antibody bound to mHEL RBCs was visualized by staining with anti–mouse Ig. This assay is...
with anti-HEL antibodies demonstrated a population of mHEL RBCs (Figure 3B-C). Staining of blood specimens C57BL/6 RBCs or from unimmunized mice that received transfusion, which constituted 2.3% of RBCs, was absent from either received transfusions of mHEL RBCs (Figure 3A). This population are representative results. O.D. indicates optical density.

Figure 2. IgG1 is the predominant antibody that binds to mHEL RBCs in HEL/CFA-immunized mice. C57BL/6 mice were immunized with HEL/CFA. Two weeks after immunization, serum was harvested. The titer, isotype, and subtype of anti-HEL antibodies were determined by HEL-specific ELISA (A-B). The capacity of each subtype to bind to mHEL RBCs was determined by incubating serum from HEL/CFA-immunized mice, at a dilution of 1:50, with RBCs from either mHEL or C57BL/6 mice followed by IgG subtype-specific antibodies. Binding was measured by flow cytometry (C). Solid line indicates mHEL RBCs; and dotted line, B6 RBCs. Analysis of this type was performed on all groups of immunized mice in all subsequent experiments to confirm similar patterns of immunization. Essentially identical results were obtained in all animals. The experiments shown in this figure have been reproduced in at least 3 separate experiments. The data presented in this figure are representative results. O.D. indicates optical density.

Figure 3. Transfusion of mHEL RBCs into HEL/CFA-immunized mice results in rapid removal of HEL + RBCs in HEL/CFA-immunized mice and is consistent with what is seen in the early stages of a delayed hemolytic transfusion reaction in humans. However, the ratios of mHEL to B6 RBCs then stabilized and remained constant until at least 72 hours (Figure 4A).

To assess the extent to which the transfused RBCs were being coated with antibodies, DAT testing was performed by staining blood with anti–mouse Ig. Antibody coating of RBCs was assessed by gating on transfused mHEL RBCs (CM-DiD) and comparing their anti–mouse Ig staining with transfused C57BL/6 RBCs (DiO) (Figure 4B). At 6 hours after transfusion, many of the transfused mHEL RBCs were positive compared with transfused C57BL/6 RBCs. At 17 hours after transfusion, the transfused mHEL RBCs remained positive compared with transfused C57BL/6 RBCs, but the binding of anti–mouse Ig was decreased from the 6-hour determination. By 72 hours, no detectable IgG was on the surface of transfused mHEL RBCs compared with transfused C57BL/6 RBCs. No IgG was detected on mHEL RBCs that were transfused into control OVA/CFA-immunized mice at any time point. The progressive decline in DAT + cells was not an artifact of exhausting the anti-HEL, as anti-HEL titers remained high in HEL/CFA after transfusion (data not shown).

While the above-mentioned data suggest limited hemolysis of transfused mHEL RBCs at early time points, a second population containing the majority of transfused mHEL RBCs continue to circulate. On the basis of these data, we rejected the hypothesis that the disappearance of DAT + cells from this second population was due to hemolysis. Since the transfused mHEL cells progressed from a DAT + state to a DAT - state from 6 to 72 hours without a concomitant decrease in the number of circulating RBCs, we hypothesized that the mHEL cells were progressively losing mHEL antigen. To test this possibility, blood from the 72-hour time point was stained with anti-HEL (Figure 4C). No HEL antigen was detected on mHEL RBCs that were transfused into HEL/CFA-immunized mice, but high levels of HEL antigen were present on mHEL RBCs that had been transfused into control OVA/CFA-immunized mice. The lack of detectable HEL on transfused mHEL RBCs from HEL/CFA-immunized mice was not an artifact of existing antibodies masking the HEL epitope, since the DAT (Figure 4B), which used a pan–mouse globulin reagent that
reacts with IgG, IgA, and IgM, was negative. Loss of HEL from the RBC surface was an antigen-specific event, as no decrease in staining with an antibody against a separate blood group antigen (TER-119) was observed (data not shown).

Transfused mHEL RBCs that have lost their antigen have a normal circulatory life span

In the setting of human transfusions, RBCs undergoing an antibody-mediated hemolysis can have a portion of their membrane removed by cells of the RES. In this scenario, the remaining cell portion mediated hemolysis can have a portion of their membrane removed in the setting of human transfusions, RBCs undergoing an antibody-mediated hemolysis can have a portion of their membrane removed by cells of the RES.

Figure 4. Transfusion of mHEL RBCs into HEL/CFA-immunized mice results in loss of HEL antigen without clearance of mHEL RBCs. C57BL/6 mice were immunized with HEL/CFA or OVA/CFA. Immunized mice then received transfusions of a mixture of leukoreduced CM-Dil-labeled mHEL RBCs and DiO-labeled C57BL/6 RBCs. Peripheral blood was obtained at the indicated time points. The remaining percentages of transfused mHEL or C57BL/6 RBCs (given in graphs) were determined by detecting residual-labeled RBCs by flow cytometry (A). At the indicated time points, peripheral blood was stained with anti–mouse Ig and the amount of immunoglobulin coating RBCs was determined by gating on transfused mHEL or C57BL/6 RBCs and measuring bound anti–mouse Ig by flow cytometry (B). Levels of HEL antigen were measured by staining with anti-HEL followed by anti–mouse Ig and comparing staining on transfused mHEL or C57BL/6 RBCs (C). The experiments shown in this figure have been reproduced in at least 3 separate experiments. The data presented in this figure are representative results.

Passive transfer of anti-HEL serum results in antigen loss in vivo but not in vitro

Cell-free serum was isolated from HEL/CFA- or OVA/CFA-immunized mice and was passively transferred to unimmunized recipients. The level of anti-OVA in serum from OVA/CFA-immunized mice was equivalent to anti-HEL in serum from HEL/CFA-immunized mice (data not shown). Several hours after transfer of serum, recipient mice received transfusions of a mixture of mHEL RBCs (CM-Dil) and B6 RBCs (DiO). At the same time that mice received transfusions, a portion of the transfusion mixture was placed in tissue culture with serum from HEL/CFA- or OVA/CFA-immunized mice. Since HEL antigen is generally undetectable on mHEL RBCs after 3 days of circulation in HEL/CFA-immunized mice, blood was harvested 2 days after transfusion to allow analysis of samples at a time point when antigen loss should be ongoing. All specimens were stained with anti–mouse Ig alone (DAT) or with anti-HEL.

Staining of a pretransfusion specimen demonstrated that the mHEL RBCs were strongly immunoreactive with anti-HEL compared with C57BL/6 RBCs (Figure 6A). After 2 days, mHEL RBCs transfused into mice that received anti-HEL serum showed a low level of antibody coating as measured by DAT using anti–mouse Ig (Figure 6B), while no DAT+ cells were detected in transfusion recipients that received either anti-OVA serum or no serum (Figure 6C-D). Staining with anti-HEL revealed high levels of HEL antigen on mHEL RBCs transfused into control mice (Figure 6H-I) that were comparable to the pretransfusion sample. However, the level of anti-HEL staining of mHEL RBCs transfused into mice given anti-HEL serum was significantly reduced and at a level equivalent to the DAT (Figure 6G). These findings demonstrate that similar to HEL/CFA-immunized mice, mHEL RBCs become DAT+ with loss...
of detectable HEL antigen when transfused into unimmunized animals that received a passive transfer of anti-HEL serum. In contrast to these in vivo findings, cells incubated for the same period of time in the presence of anti-HEL serum in vitro remained DAT+ and displayed the same level of HEL antigen as the pretransfusion specimen (Figure 6E, J). In vitro incubation of control anti-OVA serum had no positive signal on DAT and did not alter levels of HEL antigen (Figure 6F, K).

Together, these data indicate that passive transfer of cell-free immune serum was sufficient to induce loss of mHEL from RBCs. This was not an effect of cytokines or other nonspecific inflammatory molecules associated with immunization, as serum from OVA/CFA-immunized mice did not induce antigen loss. These findings suggest that anti-HEL antibodies induced by HEL/CFA immunization are responsible for HEL antigen loss and that cellular immunity is not required. In addition, the process of antigen loss does not appear to be an intrinsic property of the antibody-RBC interaction, as no antigen loss was observed with RBCs that were incubated with immune serum in vitro. Thus, the process of antigen loss appears to require interactions between antibodies and other in vivo host factors.

Role of Fcγ receptors in antibody-induced antigen loss from transfused RBCs

Since mHEL RBCs must be both exposed to antibody and circulating in vivo for antigen loss to occur, and anti-HEL in HEL/CFA-immunized mice is almost exclusively of the IgG isotype, we hypothesized that FcγRs would be involved in antibody-induced antigen loss from mHEL RBCs. Three FcγRs have been described in mice.18,19 FcγRI is a high-affinity receptor and has the ability to bind uncomplexed monomeric IgG.18,19 FcγRII and FcγRIII have a lower affinity than FcγRI.18,19 Due to decreased affinity, FcγRII and FcγRIII only bind to IgG that is involved in either an immune complex or bound to a cell surface, such as antibody-coated RBCs.18,19 FcγRIII is a stimulatory receptor, the ligation of which leads to increased phagocytosis and cellular activation, while FcγRII is inhibitory to these processes.18 It has been reported that FcγRIII is required for phagocytosis of RBCs coated with IgG1 in mice and that FcγRII plays a central role in antibody-induced AIHA.20 While FcγRI can also be involved in phagocytosis of RBCs coated in antibody, it preferentially binds to RBCs coated with IgG2a antibodies.20 Since FcγRIII is required for phagocytosis of IgG1-coated RBCs by macrophages, and the main anti-HEL antibody coating mHEL RBCs transfused into HEL/CFA-immunized mice is IgG1 (see Figure 2), we hypothesized that FcγRIII would be required for antibody-induced antigen loss.

To test this hypothesis, anti-HEL serum was passively transferred into mice with a targeted deletion of the FcγRIII (FcγRIII KO) or the FcγRII (FcγRII KO) genes. As a positive control, antiserum was also transfused into wild-type C57BL/6 mice. Six hours after transfer of antibody, mice received transfusions of a mixture of mHEL RBCs and C57BL/6 RBCs labeled with CM-DiI or DiO, respectively. As a negative control, animals from each group that had received no antiserum also received transfusions. Peripheral blood was collected 4 days after transfusion and analyzed by flow cytometry.

Transfusion into C57BL/6 mice that received anti-HEL resulted in a loss of HEL antigen (Figure 7A-B), while HEL antigen persisted on RBCs transfused into C57BL/6 recipients that did not receive anti-HEL antiserum (Figure 7C-D). In contrast, mHEL RBCs transfused into FcγRII KO mice were coated with immunoglobulin but did not undergo antigen loss (Figure 7E-F) compared with FcγRII KO mice that did not receive anti-HEL antiserum (Figure 7G-H). This observation was not an artifact of the anti-HEL antiserum not functioning in the FcγRIII KO mice, as the mHEL RBCs were coated with IgG (Figure 7E). This demonstrates that antigen loss does not occur in FcγRII KO mice and suggests that FcγRIII is required for antibody-induced antigen loss from mHEL RBCs. HEL antigen was lost from mHEL transfused into FcγRII KO to the same extent as wild-type C57BL/6 mice (Figure 7I-J). No antigen loss was observed in FcγRII KO mice that did not receive anti-HEL antiserum (Figure 7K-L). Since the FcγRII and FcγRII KO mice are on a C57BL/6 background, these findings are not likely the result of genetic differences other than the knocked-out gene.

We hypothesized that splenic macrophages were involved in this process. To test this hypothesis, after confirming adequate seroconversion of HEL/CFA-immunized C57BL/6 mice, surgical splenectomies were performed followed by transfusion of CM-DiI-labeled mHEL RBCs and DiO-labeled B6 RBCs. Neither the kinetics nor the extent of antigen loss was diminished in splenectomized animals (data not shown).
Together, these data indicate that antigen loss of HEL from transfused mHEL RBCs depends on recognition of antibody-coated RBCs by FcγRIII-bearing cells. Precisely which cell type is unclear, but splenic macrophages are not required.

Discussion

The above data formally demonstrate the phenomenon of alloantibody-induced nonhemolytic antigen loss in a murine model of RBC transfusion. Although the frequency of nonhemolytic antigen loss in the clinical setting is unknown, it is clear that this phenomenon does occur in humans. Suppression of blood group antigens has most often been documented in the setting of AIH.1-13 The precise frequency of antigen suppression in AIHA is uncertain, but approximately 10% of patients with AIHA have a negative DAT. Although the percentage of patients with DAT-negative AIHA who have antigen suppression is unknown, the frequency of antigen suppression in AIHA may be significant.

Speculation as to the frequency of antigen suppression due to alloantibodies after transfusion of crossmatch-incompatible blood is less straightforward. The main existing paradigm in the field of transfusion-based immunohematology dictates that a hemolytic transfusion reaction is the likely outcome of transfusing crossmatch-incompatible blood into
a patient with an alloantibody against a clinically significant antigen, such as Rh, Kell, Kidd, or Duffy. However, antigens in each of these blood group systems have also been shown to be capable of undergoing a process of antigen suppression in the setting of autoantibodies.1-13 and antigen suppression on transfused RBCs has been confirmed in the case of several alloantigens.2,3 In the rare cases that crossmatch-incompatible transfusions are given, either as a “least incompatible” unit or due to a clerical error, a progressive decrease in DAT is interpreted as antibody-mediated destruction of the transfused RBCs. Given the clinical signs and symptoms of hemolysis that can occur, some degree of antibody-mediated hemolysis is almost a certainty in at least some cases. However, since a negative DAT is equally consistent with RBC destruction and antigen loss, and because transfused RBCs are not routinely detected by means other than DAT, the extent to which conversion to a DAT-negative status reflects destruction of transfused RBCs as opposed to nonhemolytic antigen loss is unclear.

To date, several hypotheses have been proposed to explain the mechanism of antibody-induced antigen suppression. One explanation suggests that the autoantibodies, or other host factors, create an environment in which synthesis of the recognized antigen is inhibited.2,6 This hypothesis was ruled out in 2 cases of AIHA-induced suppression of Kell antigens by demonstrating loss of presynthesized antigens on transfused RBCs.2,8 Consistent with the notion of loss of presynthesized antigen, several groups have proposed that antigens are degraded on mature erythrocytes by proteases, deglycosidases, or reductases from antibody, several groups have proposed that antigens are degraded on mature erythrocytes by proteases, deglycosidases, or reductases from microbial pathogens infecting the patient during the AIHA illness.2,7 This possibility cannot be excluded, as some patients with antigen suppression have documented microbial infections.8 However, reappearance of the antigen in question on the RBC surface is routinely seen after resolution of the autoantibody,2,4,8,9,11 and relapse of the antibody response is responsible for antigen loss in some settings and hemolysis in others. Ongoing investigations into the nature of antibodies capable of inducing nonhemolytic antigen loss may lead to the generation of antibody reagents that can be used to purposefully induce antigen loss. Such reagents could have considerable therapeutic potential by allowing transfusion of crossmatch-incompatible units to multiply alloimmunized patients.

In our model of nonhemolytic antibody-mediated loss of alloantigen, suppression of antigen synthesis is not a possibility since, unlike AHI A, mature antigen-positive RBCs are transfused into alloimmunized recipients. Thus, the conversion of mature HEL+ RBCs to HEL- RBCs confirms a mechanism of antigen loss rather than suppression of synthesis. In this setting, destruction of antigen by microbial infection is highly unlikely, since mice were kept in pathogen-free environments. In addition, antigen loss occurred in 100% of animals with anti-HEL antibodies and 0% of animals without anti-HEL antibodies in numerous experiments conducted over the course of 8 months, during which time multiple animals were used from different litters. On the basis of these factors, we reject the hypothesis that antigen is destroyed by microbial pathogens.

The ability to induce antigen loss can be transferred with cell-free serum from HEL/CFA-immunized mice, but not serum from OVA/CFA-immunized animals. In addition, antigen loss does not occur in mice with a targeted deletion of FcγRIII, which specifically binds to immunoglobulins. Together, these data support a model in which antigen loss is induced through the interaction of RBCs, anti-RBC antibodies, and FcγRIII-bearing cells. The exact mechanism of antigen loss remains to be determined, but it may include either shedding of the antigen by the RBC or enzymatic destruction of the antigen through release of proteases from FcγRIII-bearing macrophages that degranulate on antibody-coated RBCs.

It is unclear why some crossmatch-compatible transfusions in humans clearly lead to hemolysis, while others lead to nonhemolytic antigen loss. Differences in RBC antigens are an unlikely explanation, since multiple transfusions of Kell-positive RBC units into a patient with anti-Kell AIHA gave consistent antigen loss of Kell from a variety of different RBC donors.9 It, thus, seems more likely that a particular characteristic of a given anti-Kell antibody response is responsible for antigen loss in some settings and hemolysis in others. Ongoing investigations into the nature of antibodies capable of inducing nonhemolytic antigen loss may lead to the generation of antibody reagents that can be used to purposefully induce antigen loss. Such reagents could have considerable therapeutic potential by allowing transfusion of crossmatch-incompatible units to multiply alloimmunized patients.

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References

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