Review: red cell alloantibody formation in the neonate and infant: considerations for current immunohematologic practice

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The most important function of a hospital blood transfusion service is to ensure that blood component therapy for the transfusion recipient is the safest possible. Historically, "safe" has meant that the donor and the recipient are ABO compatible. In addition, both screening of the recipient serum with reagent red cells and the crossmatch between recipient serum and prospective blood donor red cells have been used to detect clinically significant recipient red cell alloantibodies. In this manner hemolytic transfusion reactions, which may be associated with significant patient morbidity and mortality, are largely avoided.

Although red cell alloantibodies are not uncommonly detected in the general patient population (see below), they are rare in neonates (less than 1 month old) and infants less than 4 months of age. In fact, crossmatching is not performed in this patient population unless there is evidence of passively acquired maternal red cell alloantibodies. The neonatal immune response to an antigenic challenge such as blood transfusion is qualitatively and quantitatively different from that of older infants, children, and adults. Recently, great strides have been made in the elucidation of neonatal immunobiology, and we are beginning to understand the biologic basis for this lack of alloantibody formation. Indeed, a complex array of cellular as well as extracellular antigenic exposure, such as blood transfusion, serves as an important experimental model from which to gain further insights into the nature of the neonatal immune system.

This article will briefly summarize selected aspects of the neonatal immune response as well as current studies from my laboratory describing interactions between neonatal lymphocytes and transfused allogeneic red cells. In addition, a case report on infant red cell alloantibody formation will be discussed, together with recommendations regarding changes in current blood bank standards relating to neonatal and infant transfusions.

Neonatal Immunobiology

B-cell mediated immunity

Immunoglobulin production by neonatal B cells is mainly restricted to the IgM isotype. There is a relatively slow progression from IgM to IgG production, the last being IgA responses. This delay does not appear to be due to a lack of precursor B cells, since the peripheral blood of the newborn has large numbers of circulating B cells expressing surface IgM, IgG, and IgA. The mechanisms accounting for the failure of neonatal B cells to differentiate normally are not understood. Various aspects of the neonatal B cell response may be involved. Early B cell activation, including the capability of cooperating effectively with neonatal T cells as well as surface membrane signal transduction, is suspected. In fact, regulatory imbalances between T cell–mediated helper and suppressor function, as well as intrinsic B cell immaturity and isotype restriction, may be responsible for poor antibody production.

Mixing experiments have provided additional interesting clues to neonatal B cell immaturity.

Studies performed with pokeweed mitogen (PWM)–stimulated cord mononuclear cells have shown minimal Ig production, despite a strong proliferative response. In vitro Ig production is principally IgM and is approximately 25 percent of that seen in cultures from adult peripheral blood
mononuclear cells. The addition, or mixing, of adult T cells to the IgM PWM-stimulated neonatal B cells enhances IgM production considerably. Interestingly, IgG and IgA plasma cell responses remain deficient. Further evidence of deficient neonatal T helper function is that less than 5 percent of cord blood T cells express HLA-D antigens as an activation marker after PWM culture. Additional data suggest that enhanced neonatal T cell suppressor activity in addition to deficient T helper cell function may be responsible for poor B cell responses.

**T cell-mediated immunity**

The enumeration of neonatal T cell subsets on the basis of surface marker antigens has disclosed some differences from cells of older individuals. While the percentage of CD4-positive cells (T helper cells) is similar in neonates and adults, the proportion of CD8-positive cells (T suppressor cells) is significantly higher in neonates. In fact, a peculiar subset of CD8-positive circulating lymphocytes has been described that are negative for CD3 (a general T cell marker) and CD4. Other investigators have observed cord blood lymphocytes that co-express both CD4 and CD8; some believe that this population may represent a unique activation state due to premature exit from the thymus. Immunosuppression is very active in the neonate. This may have important implications for in vivo inhibition of maternal cell proliferation and fetal rejection.

The subsets of T cells identified by CD45 RA (2H4), CDw29 (4B4), and UCHL-1 represent naive and memory (previously activated) T lymphocytes. CD45 RA expression on CD4 lymphocytes (suppressor-inducer) undergoes down-regulation shortly after antigenic stimulation, while 4B4 (helper-inducer) and UCHL-1 increase in cell surface expression. Reference levels of these subpopulations in the neonate indicate that, contrary to what is seen in the adult, there is a predominance of suppressor-inducer lymphocytes and a decrease in the number of helper-inducer cells.

Some investigators have associated the lack of neonatal helper cell function to the presence of CD4-positive suppressor cells in cord blood. One group recently showed that the dominant immunoregulatory function of cord blood CD4-positive cells is suppression mediated by CD4-positive, CD45 RA-positive cells. The mechanism by which cord blood CD4-positive, CD45 RA-positive cells suppress Ig production is unknown.

T cells invariably recognize antigens on the surface of other cells and carry out many of their functions by interacting with antigen-presenting cells (APCs). Although B cells may act as APCs, numerous investigations have focused on a family of bone marrow-derived cells with prominent antigen-presenting function. Until recently it was believed that the principal APCs among peripheral blood mononuclear cells were monocytes and that tissue-based macrophages were critical for antigen presentation occurring in secondary lymphoid organs. We now know that APC function is due mainly to a heterogeneous family of cells known collectively as dendritic cells. In fact, cells with dendritic morphology provide important and critical functions such as antigen processing, antigen retention on the cell surface, antigen transport to lymphatic tissues, and presentation to T lymphocytes. In addition, they are important to the induction of T cells to secrete lymphokines such as those involved in B cell activation and antibody secretion.

Recently, the function of neonatal and infant APCs was evaluated by our group. A specific maturative sequence was noted in the ability of neonatal and infant APCs to present alloantigen to self T helper cells. In fact, subjects less than 2 years of age were incapable of responding in vitro to irradiated stimulator, APC-depleted allogeneic adult lymphocytes, as measured by the ability of mixed lymphocyte culture (MLC) supernatants to support an IL-2 dependent cytotoxic T-lymphocyte (CTL) line. At this time, the specific cause of such poor function is not clearly known. It is already known that neonates synthesize little specific mRNA gamma-interferon, a powerful inducer of class II expression on APCs. Additional co-stimulatory, accessory molecules are also essential in optimal APC function and may be defective in the neonate and infant. Independent of the cause, the finding of poor APC function in this age group helps explain the previously described poor B cell and T cell responses to various allogeneic challenges. The following section explains the relevance of neonatal APC function and the ability of the neonatal transfusion recipient to utilize donor APC for alloantibody formation in response to allogeneic blood transfusion.

**The Neonatal Immune Response to Blood Transfusion**

Repeat red cell compatibility testing for infants younger than 4 months is routinely omitted, provided
that initial antibody screening methods reveal no alloantibodies. In 1983 one group of investigators proposed that because neonates' immune systems are immature, they do not form red cell alloantibodies, while another investigator suggested in 1961 that transfusion early in life prevents alloimmunization through an acquired tolerance mechanism. Much has been learned about neonatal immunobiology since the appearance of these reports; clearly, as discussed in the preceding section, numerous factors may be responsible for this poor alloantibody response.

Two studies using conventional immunohematologic serology documented the lack of alloantibody formation against red cell antigens in neonates and infants. A combined total of 143 infants had received numerous donor exposures and red cell transfusions; some were followed up to 30 months of age. In no case was red cell alloantibody detected. These studies, however, were not designed to investigate mechanisms responsible for the lack of alloantibody formation. One additional report documented long-term transfusion effects on the CD4/CD8 ratio in infants between 2 and 12 weeks of age. Although a significant inverse relationship between the number of blood transfusions and the CD4/CD8 ratio was documented, no insight was derived into the mechanisms responsible for this influence on the T cell subset distribution.

A series of studies, principally from our laboratory, have delineated several important characteristics of the early neonatal immune response to blood transfusion. We evaluated neonates undergoing extracorporeal membrane oxygenation (ECMO), a modified cardiopulmonary bypass procedure for patients requiring cardiac and respiratory support, sometimes for many days. All neonates undergoing ECMO received a priming volume of two units of packed red blood cells on the day of the procedure, equivalent to at least a two-volume exchange transfusion. Blood samples obtained on day 1 after the procedure, and those obtained up to 7 days after the first blood transfusion, failed to show an increase in either IL-2R or HLA-DR expressing T cells (activation markers) or an increase in B cells. A control group of neonates receiving 10 mL/kg aliquot blood transfusions of washed, irradiated red cells also failed to show any increase in lymphocyte activation markers based on flow cytometric analysis. In fact, no changes were seen in CD3, CD4, CD8, CD19 (a B cell marker), or natural killer cell numbers.

In a follow-up study, we evaluated an additional cohort of neonates using flow cytometric analysis of additional lymphocyte subpopulations and serum cytokine determinations. These neonates also received irradiated, washed red blood cell transfusions at a dose of 10 mL/kg with an average white cell concentration of $2 \times 10^7$ cells per transfusion. The blood was irradiated with 2,800 rads of gamma radiation, and the mean donor exposure was $2 \pm 1.5$. No changes in T helper cells expressing CD45-RA, CDw29, or UCHL-1 were noted following transfusion. These antigens are indicative of a naive to memory shift in the lymphocytes that express them. No shift in fluorescence signal was seen, also indicative of a lack of cellular activation. Posttransfusion levels of gamma interferon and neopterin (a cytokine and a metabolite that increase following lymphocyte activation) were essentially unchanged compared with pretransfusion concentration in these neonates. The failure to observe lymphocyte activation, although possibly due to the low number of donor white blood cells, was in our opinion due to a lack of recipient recognition of the transfused donor allogeneic blood cell antigens.

Our attention focused next on the ability of neonatal and infant antigen-presenting cells (self APCs) to effectively prime self T helper cells. This aspect of the afferent immune arm to alloantigen stimulation is essential for an immune response to T cell–dependent antigens such as those expressed both cellularly and extracellularly in transfused blood. As previously mentioned, we have observed a specific maturative sequence in APCs from birth through infancy and ultimately into childhood and adulthood. Lymphocytes from subjects 24 months of age or less were incapable of responding to irradiated APC-depleted allogeneic stimulator lymphocytes. In addition, it was noted that an infant's T helper lymphocytes could utilize allogeneic APCs (allo APCs) for an in vitro response in a one-way MLC. Therefore, a neonate or older infant may synthesize red cell alloantibodies if stimulated by a critical number of allo APCs. It has been established that T helper pathways can utilize both self and allo APCs, and therefore the presence of immature self APCs does not exclude the possibility of a T helper cell mediated response.

We recently reported the first well-documented case of alloantibody production in a massively transfused
11-week-old infant. This infant had had a significant exposure to donor allo APCs as a neonate through 31 red cell transfusions as well as additional exposures from fresh frozen plasma and platelet transfusions. Six weeks following the first blood transfusion and prior to cardiac surgery, alloanti-E was noted. The serum agglutinated an Rh R2 screening cell after 37°C incubation and produced a 1+ antiglobulin reaction with anti-IgG. Agglutination strength did not diminish following treatment with 0.01M dithiothreitol, thus suggesting an IgG alloantibody and secondary immune response. Primary sensitization most likely took place in the first month of life. This latest investigation definitively documents, with follow-up studies, the presence of the red cell alloantibody anti-E in an 11-week-old infant. All donor sera were screened and found to be nonreactive in a three-reagent screening cell panel after 37°C incubation and the antiglobulin phase using anti-IgG. The mother's antibody screen was also negative. The infant's alloanti-E persisted for 4 weeks following the last blood transfusion. It remains to be ascertained whether there is a threshold concentration of allogeneic APCs that can overcome an immature T cell priming circuit and whether additional co-stimulatory factors are responsible for alloantibody formation. Additional studies are required to more fully evaluate posttransfusion changes in the neonatal immune system.

**Recommended Changes in Neonatal and Infant Immunohematologic Practices**

The current American Association of Blood Banks (AABB) standards have an expanded section that deals with special problems of the neonatal (under 4 months) recipient. Standard G6.300 states that "An initial pretransfusion specimen must be tested for unexpected antibodies... if negative, it is unnecessary to crossmatch...". If the initial antibody screen is negative in this age group, it is common practice to eliminate crossmatching, given the clinical observation that alloantibody formation in the first 4 months of life is rare. Clearly, infants who are 4 months of age or less do not make alloantibody as frequently as older children because they have an immature immune system that makes it quite unlikely. However, in certain instances, they have made alloantibodies, as substantiated both in our report and anecdotally.

In most instances of alloantibody synthesis in infants, massive donor exposure has occurred. In the case of the 11-week-old infant reported here, approximately 80 separate donor exposures had taken place in the first 4 weeks of life. It is known that an infant's T lymphocytes can utilize a donor's APCs and therefore be able to promote B cell function and alloantibody synthesis. Perhaps a critical number of donor white cells and accompanying APCs is necessary before this effect is seen.

If whole blood or unwashed packed red cells are transfused in large numbers, especially if concomitant with platelet concentrates, which also have high numbers of donor white cells, alloantibody formation in infants may be a more frequent event. Rescreening of those infants who have received such a large number of components may identify a greater incidence of alloantibody formation than previously expected. Commonly, relatively "fresh" units of whole blood are used for exchange transfusions and for cardiopulmonary bypass procedures. Both the high white count and the greater number of viable white cells contained in these components may cause alloantibody formation in the massively transfused infant. Since the blood volume of an infant less than 4 months of age is approximately 500 mL, massive transfusions occur frequently.

At this institution, infants less than 4 months of age are routinely screened for atypical antibody prior to blood transfusions equivalent to their blood volume, because such a large volume of blood, if incompatible, would likely result in a severe hemolytic transfusion reaction. Indeed, routine antibody screening revealed the alloanti-E in the 11-week-old infant described earlier. Although additional studies may be necessary to document the incidence of such an occurrence, rescreening of previously massively transfused infants less than 4 months of age may also prevent the transfusion of incompatible red cells. In addition to exposure to a large number of donors, neonates and infants are transfused with blood that is only several days old containing relatively large numbers of viable donor APCs. This author believes that antibody screening of previously massively transfused infants should be done every 72 hours, as currently mandated by standards for all other patients. Indeed, the development of alloanti-E in the previously described infant was documented over a 3-day period using stored sera from the laboratory. Therefore, the time frame of the infant's secondary immune response to a red cell alloantigen was similar to that of an adult.
In summary, previously massively transfused neonates and infants less than 4 months of age may be at greater risk for the development of red cell alloantibodies. In vitro evidence of neonate interaction with donor APCs, as well as several case reports, indicates that red cell alloantibody synthesis in this age group is possible. Periodic posttransfusion antibody screening in this group of patients can prevent incompatible red cell transfusions.

References

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