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Current problems and future directions of transfusion-induced alloimmunization: summary of an NHLBI working group

James C. Zimring¹, Lis Welniak, John W. Semple, Paul M. Ness, Sherrill J. Slichter, Steven L. Spitalnik, NHLBI Alloimmunization Working Group

¹Center for Transfusion and Cellular Therapies, Department of Pathology and Laboratory Medicine, Children's Healthcare of Atlanta, Division of Pediatric Hematology/Oncology, Emory University School of Medicine, Atlanta, Georgia, USA.

Abstract

In April 2010, a working group sponsored by the National Heart, Lung, and Blood Institute was assembled to identify research strategies to improve our understanding of alloimmunization caused by the transfusion of allogeneic blood components and to evaluate potential approaches to both reduce its occurrence and manage its effects. Significant sequelae of alloimmunization were discussed and identified, including difficulties in maintaining chronic transfusion of red blood cells and platelets, hemolytic disease of the newborn, neonatal alloimmune thrombocytopenia, and rejection of transplanted cells and tissues. The discussions resulted in a consensus that identified key areas of future research and developmental areas, including genetic and epigenetic recipient factors that regulate alloimmunization, biochemical specifics of transfused products that affect alloimmunization, and novel technologies for high-throughput genotyping to facilitate extensive and efficient antigen matching between donor and recipient. Additional areas of importance included analysis of unappreciated medical sequelae of alloimmunization, such as cellular immunity and its effect upon transplant and autoimmunity. In addition, support for research infrastructure was discussed, with an emphasis on encouraging collaboration and synergy of animal models biology and human clinical research. Finally, training future investigators was identified as an area of importance. In aggregate, this communication provides a synopsis of the opinions of the working group on the above issues and presents both a list of suggested priorities and the rationale for the topics of focus. The areas of research identified in this report represent potential fertile ground for the medical advancement of preventing and managing alloimmunization in its different forms and mitigating the clinical problems it presents to multiple patient populations.

Address reprint requests to: James C. Zimring, MD, PhD, Emory University School of Medicine, Woodruff Memorial Building Suite 7301, 101 Woodruff Circle, Atlanta, GA 30322; jzimrin@emory.edu.
From the Center for Transfusion and Cellular Therapies, Department of Pathology and Laboratory Medicine and the Aflac Cancer Center and Blood Disorders Service, Children's Healthcare of Atlanta, Division of Pediatric Hematology/Oncology, Emory University School of Medicine, Atlanta, Georgia; the National Heart, Lung, and Blood Institute, Bethesda, Maryland; the Keenan Research Centre in the Li Ka Shing Knowledge Institute of St. Michael's Hospital, Canadian Blood Services, Departments of Pharmacology, University of Toronto, Toronto, Ontario, Canada; the Transfusion Medicine Division, Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, Maryland; the Puget Sound Blood Center and University of Washington School of Medicine, Seattle, Washington; and the Department of Pathology and Cell Biology, Columbia University, New York, New York.

CONFLICT OF INTEREST

None of the authors has a conflict of interest to declare.

Alloimmunization consists of the induction of immunity in response to foreign antigen(s) encountered through exposure to cells or tissues from a genetically different member of the same species. Although alloimmunization can occur naturally in the context of pregnancy, alloimmunization is most frequently an undesired iatrogenic result of transfusion and/or transplantation. While pregnancy leads to high levels of humoral alloimmunization to HLA antigens,^{1,2} the absolute number of alloimmunized patients is greater as a result of transfusion due to the sheer volume; in particular, approximately 5 million Americans receive transfusions each year ($>1/100$).³ Moreover, unlike transplantation, in which recipients are treated with pharmacologic immunosuppressants to prevent or mitigate rejection, transfusions are typically given without immune intervention. Thus, a large number of patients are exposed to transfused alloantigens each year without therapeutic alteration of immunity. It is worth noting that many transfusion recipients have one or more substantial underlying diseases, or they are receiving therapy that may affect their immune system such that their responses to alloantigens may not be what would be expected in a normal individual. Nevertheless, many patients receiving transfusions are not “immunosuppressed” per se.

The initial observations of ABO blood group serology by Landsteiner remain the most well-known example of immunity to allogeneic red blood cells (RBCs); however, the biology of the ABO system does not reflect the vast majority of alloantigens on RBCs or platelets (PLTs). ABO antigens are defined by carbohydrate moieties, present on proteins and lipids in the RBC membrane. Moreover, anti-A and anti-B are “naturally occurring” antibodies, which are formed without any prior exposure to foreign RBCs (i.e., no history of transfusion or pregnancy). Finally, most anti-A and anti-B are immunoglobulin (Ig)M, suggesting that much of the anti-A and anti-B response is T-cell independent. The fame (or in some cases infamy) of the ABO system has led to some confusion regarding the general nature of most blood group antigens, the majority of which have properties quite distinct from ABO. Indeed, during the past century several hundred different human RBC transfusion-related antigens were identified.^{4,5} With only a few notable exceptions, these antigens do not consist of carbohydrates like ABO, but rather are protein antigens. In addition, with the exception of RhD, these antigens do not consist of a whole gene product that is present on donor, but absent on recipient RBCs; in contrast, most RBC antigens are single-amino-acid polymorphisms between donor and recipient (e.g., RhCE, Kell, Kidd, Duffy, Ss).^{4,5} Antibodies against these antigens require exposure to the alloantigen through transfusion or pregnancy and are typically IgG.⁶ Thus, antibody responses to most non-ABO alloantigens have the more typical characteristics of adaptive humoral immunity to foreign proteins.

Because generation of antibodies to most RBC alloantigens requires prior exposure, such antigens are not problematic the first time an individual is in need of therapeutic transfusion or is pregnant. However, once formed, the IgG molecules may have the capacity to destroy RBCs to which they bind (most commonly through opsonization and extravascular clearance, but, in some cases, through complement-mediated intravascular destruction). Thus, once transfused, if an individual mounts an antibody response against an RBC antigen, then subsequent transfusions of RBCs expressing that antigen may be associated with a significant number of adverse events. The difficulty lies not only in clearance of transfused RBCs, which obviates any therapeutic benefit of the transfusion, but also that the

downstream effects resulting from RBC clearance can lead to multiple organ failure, electrolyte perturbations, coagulopathy, and in some cases, death. In addition, with multiple transfusions, patients may generate numerous antibodies, making the identification of compatible RBCs increasingly difficult and, in some cases, impossible. During pregnancy, maternal antibodies that recognize paternally inherited antigens on fetal RBCs can produce hemolytic disease of the fetus and newborn, resulting in substantial pathology and/or death in utero.⁷ For the above reasons, humoral alloimmunization to RBC antigens is a significant clinical problem.

Alloimmunization to transfused PLTs is a slightly different scenario. Multiple human PLT antigens have been described (i.e., HPA1–15),⁸ which, like many RBC antigens, represent single-amino-acid polymorphisms between donor and recipient. In addition, unlike RBCs, PLTs express significant levels of antigens encoded by the major histocompatibility complex (MHC), particularly, MHC Class I. PLT transfusions can induce antibodies to either HPA or MHC antigens, the latter being classified as HLA antibodies. Unlike an incompatible RBC transfusion, the clearance of incompatible PLTs is not considered to be dangerous; however, PLT antibodies can cause rapid clearance of transfused PLTs, obviating the therapeutic benefit and leading to patients who are refractory to future PLT transfusion therapy. When severe, the latter can lead to a situation in which PLT therapy cannot be used to maintain hemostasis in a patient with thrombocytopenia (e.g., a patient with leukemia receiving chemotherapy). Similar to hemolytic disease of the fetus and newborn, maternal alloantibodies against paternal PLT antigens can result in neonatal alloimmune thrombocytopenia.⁹

Because transfused patients are screened for alloantibodies before any RBC transfusion event, the relative rates of immunization to RBC antigens are well described. Likewise, rates of HLA and HPA alloimmunization to transfused PLTs have been well studied. In the general population, the rate of RBC alloimmunization detected by the clinical laboratory is quite low, approximately 2%, even in multiply transfused patients.^{10,11} Similarly, approximately 8% of recipients mount detectable responses to HPA antigens after PLT transfusions.¹² HLA antigens are more immunogenic, with a 45% rate of alloimmunization even in acute myelogenous leukemia patients undergoing induction chemotherapy.¹³ The increased anti-HLA responses may be due to the immunogenic nature of the white blood cells (WBCs) that contaminate PLT units; however, even leukoreduced units induce an HLA alloimmunization rate of approximately 20%.¹³ Whether this residual HLA immunogenicity is due to the remaining WBCs, or to the immunogenicity of MHC I on the PLTs, is a matter of debate. Although alloimmunization rates may appear low at first glance, the prevalence of alloimmunized patients is still considerable because of the enormous number of transfusions.³

The relatively low rates of immunization to alloantigens on transfused blood products is in stark contrast to what is observed for immune responses to infectious agents. With the exception of severe immunosuppression, close to 100% of individuals infected with pathogens make a detectable antibody response (e.g., human immunodeficiency virus, hepatitis B, hepatitis C). Indeed, before nucleic acid amplification technologies, serologic screening was the method of choice for diagnosing and monitoring infection and remains the

method for assessing prior infection. Similarly, efficient vaccines induce a detectable antibody response in the vast majority of individuals. Importantly, the low alloimmunization rates to transfused blood products are not because alloantigens are weakly immunogenic by nature, as essentially 100% of solid organ transplants are rejected in the absence of potent pharmacologic suppression. Upon initial consideration, the lower-than-expected rate of alloimmunization to blood transfusion appears to be an unexplained anomaly within the current paradigms of adaptive immunity. Hence, a detailed mechanistic analysis of the biologic particulars of transfusion-induced alloimmunization would both generate new basic understanding of immunology in general, as well as provide a basis for rational interventions to circumvent alloimmunization. However, as above, it must also be considered that some of these low rates of alloimmunization may be due to the recipient's immune status, for example, immunosuppression due to either medication and/or disease.

Chronically transfused patients represent a special class of alloimmunized individuals, for whom the development of multiple alloantibodies may ultimately lead to death. For example, patients with sickle cell disease often require multiple transfusions and some of these patients are placed on chronic prophylactic transfusion protocols. In this population, mean alloimmunization rates are approximately 25%, with some estimates as high as 47%.¹⁴ In addition, these alloimmunized patients have a tendency to produce alloantibodies against multiple clinically significant blood group antigens. Some patients synthesize so many different alloantibodies, that units of compatible RBCs become difficult, or impossible, to obtain. Substantial fiscal resources are required to manage patients in this state; however, more importantly, multiply alloimmunized individuals can die for want of a transfusion, because compatible blood donors cannot be identified. Thus, for chronically transfused patients, alloimmunization is of considerable medical importance.

In recognition of both the scientific and the medical importance of the study of transfusion-induced alloimmunization, the National Heart, Lung, and Blood Institute (NHLBI) convened a working group on April 7, 2010, in Bethesda, Maryland, to generate a dialogue between both basic and clinical researchers in the field. The purpose of this article is to provide a synopsis of the discussions that occurred and to present a consensus list of questions and specific areas of investigation that the working group identified as having high priorities for advancing our understanding of the mechanisms and management of alloimmunization to both RBCs and PLTs.

COMPOSITION OF THE WORKING GROUP AND SUMMARY OF THE PRESENTATIONS

The NHLBI convened a working group to identify research strategies to improve our understanding of alloimmunization caused by the transfusion of allogeneic blood components and to evaluate potential approaches to reduce its occurrence. The group consisted of five presenters and 18 other participants representing academia and blood bank centers, as well as clinical and basic research scientists. Both senior and junior investigators in the field of transfusion medicine were present.

The half-day working group was structured into two sections. The first section consisted of five presentations, initiated with a historical perspective on human and animal studies in the area of RBC and PLT alloimmunization, delayed hemolytic transfusion reactions, and both the medical and the economic consequences of these problems. Two presentations focused on recent progress and also identified barriers to future advances in the areas of basic studies of alloimmunization to RBCs and PLTs, respectively. Finally, two presentations focused on recent progress and also identified problems for future study in the areas of clinical research on alloimmunization to RBCs and PLTs, respectively. After the presentations, all workshop attendees participated in a discussion of the outstanding basic and clinical research needs and opportunities to further our understanding of the determinants of alloimmunization and/or immunotolerance and approaches required to reduce associated clinical complications. This discussion provided recommendations to the NHLBI for optimal areas of future support.

The presenters' overviews of basic research emphasized much recent progress in the general understanding of innate immunity and its central role in regulating the ultimate outcome and nature of adaptive responses. Examples of studies on how innate immune activation and inflammation affect transfusion-induced alloimmunization, and recent progress in animal models, were emphasized by the speakers as areas of high interest. The speakers also outlined technical limitations with current animal model systems and the lack of certain analytic tools within such constructs. The overviews of clinical research described the abundant information that can be generated by large multicenter clinical studies; however, they also emphasized the considerable logistical and financial support required to perform such studies.

WORKSHOP DISCUSSION AND RECOMMENDATIONS REGARDING RESEARCH IN TRANSFUSION-INDUCED ALLOIMMUNIZATION

The second section was an open discussion, using the prior presentations as a starting point for a dialogue among the participants. Substantial discussion ensued, leading to a general consensus in several areas, including specific recommendations. Areas of dispute also were identified. The results of these discussions are presented below.

Summary of the working group recommendations

The working group made a series of recommendations to NHLBI, which can be broadly categorized into 1) research systems, 2) research infrastructure, and 3) research topics of focus.

Research systems

Promoting development of experimental and/or analytic systems to investigate immunologic mechanisms of alloimmunization to blood cells.: The Workshop acknowledged that great progress has been made over the past several decades in defining and characterizing human RBC and PLT antigens, at both the protein and the nucleic acid levels, through analysis of human blood and blood products. In addition, great understanding has been generated regarding the frequency, immunogenicity, and clinical significance of the

alloantigens, through epidemiologic population analysis of cohorts of transfused patients. Because these studies focused on defining the nature and characteristics of particular human alloantigens, by definition, human blood products and human subjects were required. However, these analyses focused almost exclusively on the molecular nature of the donor alloantigens and their cognate antibodies. Substantially less progress has been made on studying the recipient's cellular immunology that is responsible for antibody production or immunoregulatory mechanisms that determine whether patients become alloimmunized. In large part, lack of progress in the latter areas is due to limitations of current experimental technologies. Two specific areas were identified.

1. Analysis of cellular immunology in transfused humans.

In the human setting, few, if any, tools have been used by transfusion medicine investigators to analyze the biology of antigen-presenting cells, T cells, or B cells *in vivo*. Fortuitously, while the transfusion community has been performing the essential first step(s) of defining the antigens, immunologists interested in infectious disease made great progress in developing sophisticated tools to study cellular immunology, including highly specific visualization of cell subsets (through flow cytometry and fluorescent microscopy), visualization of T cells specific for a given antigen (through tetramer technology), enumeration of antigen-specific T-cell phenotype and function (through intracellular cytokine staining), and enumeration of antigen-specific plasma cells (through ELISpot). Moreover, sophisticated platforms now exist for analyzing protein arrays and cytokine panels. These techniques have not yet been adapted to study the cellular immunology of transfusion-induced alloimmunization. Therefore, the panel recommended that NHLBI support efforts to adapt these technologies to the field of transfusion medicine.

Of note, the utility of such tools is not limited to understanding the biology of alloimmunization; rather, such tools would also allow analysis of multiple additional medical questions relevant to transfusion, for example, the possible role of alloimmunization, particularly cellular immunity, on the development of other immune disorders later in life. Adverse or protective effects of transfusion against neoplasia, infections, and solid organ transplantation are currently open questions. If such constitute long-term adverse effects of transfusion, it will be important to assess their relationship to alloimmunization.

2. Animal models of transfusion-induced alloimmunization.

The Workshop highlighted the paucity of tractable animal models with sufficient analytic power to dissect the questions related to immunologic responses to foreign antigens on blood cells. Although several animal models have been used to study alloimmunity to transfused cells, these have either lacked the full complement of analytic tools (i.e., the identity of the relevant antigens were not known and cellular responses could, thus, not be identified) or they were too artificial to reflect the normal biology of alloimmunization. For example, although transfusion of sheep RBCs into mice is interesting as a general model of humoral immunization, it has many biologic differences because of its

xenogeneic nature, such that profound differences exist compared to the known biology of human transfusion alloimmunization. Recently, several investigators have begun efforts to engineer animal models of transfusion-induced alloimmunization that both mimic the human scenario (i.e., transfusion within the same species) and also focus on defined alloantigens that have sufficient tools available to perform sophisticated immune studies.¹⁵ However, the panel concluded that the new models still require additional development and refinement, so as to parallel human transfusion biology as much as possible. Thus, the panel recommended that NHLBI support the development of animal models of transfusion-induced alloimmunization.

Integration of human and animals studies as a research field.: The panel confirmed the advantage of animal models in that they allow rapid mechanistic dissection of a nature and magnitude that is neither ethically permissible nor technically feasible in humans. The panel also confirmed the weakness of animal models in that their relevance to human alloimmunization is always subject to the assumption that the animal biology reflects human biology, an assumption that has held up in many previous situations, but has also broken down in others. Accordingly, the most rapid progress is to be made by aggressively utilizing animal models to generate evidence-based hypotheses, but once new understanding is generated, testing it in ethically rigorous human studies. The panel recommended support for simultaneously developing animal models and human analytic systems, as outlined in the discussion summary above.

Research infrastructure

Development and support of the community of transfusion researchers.: The panel acknowledged previous NHLBI support of this field, but also identified a lack of sufficient numbers of investigators in the field of transfusion medicine with specific expertise to take full advantage of current and developing investigative technologies. The panel thus strongly recommended ongoing investment by NHLBI in support of young investigators and training mechanisms to encourage students, thereby developing the next generation of transfusion medicine investigators. This includes supporting clinically trained physicians (e.g., transfusion medicine physicians and hematologists) to obtain appropriate training for translational research and also developing mechanisms to encourage individuals with basic science backgrounds (e.g., PhDs and research track MDs) to focus their interests on transfusion medicine and alloimmunization.

The panel also emphasized the importance of collaboration and information exchange to increase the value of independent studies. This is especially relevant when studies cross specialty areas (e.g., clinical epidemiology studies and mechanistic animal studies) and/or in studies in which sufficient numbers of patients cannot be enrolled in a single center. Thus, the panel recommended support for mechanisms that encourage synergistic crossgermination across subspecialties and foster collaboration between investigators from various disciplines.

Research topics of highest priority—In the context of this discussion, the Workshop identified specific recommendations regarding scientific areas of highest priority.

1. Promoting studies that help identify the host characteristics and the clinical situations that result in the development of a clinically relevant alloresponse to transfused blood cells.

The participants extensively discussed many of the issues related to identifying the factors that determine if clinically relevant alloantibodies develop in a given patient. They acknowledged both historical and recent data to support a scenario in which transfusion recipients are categorized into two groups: responders and nonresponders.¹⁶ Probabilistically, both groups are exposed to multiple allogeneic determinants on blood cells, against which they have the potential to develop alloantibodies. However, many patients do not develop antibodies (i.e., nonresponders). Patients who develop at least one alloantibody (i.e., responders) are more likely to develop additional antibodies, but this does not appear to be a linear relationship.¹⁶

The extent to which the definition of responders and nonresponders reflects the sensitivity of current assays for alloantibody detection was also debated. It was a matter of dispute as to whether all recipients make alloantibodies, but with a wide range of titers, and that “nonresponders” simply represent those with levels below the detection limits of current serologic techniques. It has recently become clear that decreasing titers over time is an important factor that may not have been fully appreciated in the past.^{17,18} Such a distinction is of high importance in mechanistic understanding of alloimmunization and should be investigated at a basic level. However, it was also acknowledged that responder and nonresponder status is a medically meaningful distinction. Even if all members of the nonresponder group produce low-titer antibodies, the absence of hemolytic transfusion reactions or development of PLT refractoriness indicates that, if low levels of antibodies exist, they do not appear to be clinically significant.

The main medical importance of the nonresponder versus responder distinction falls into several categories. First, there is currently no known test or variables that can identify a potential responder or nonresponder other than providing transfusions and monitoring for the development of alloantibodies. It was recommended that substantial support be provided for efforts aimed at elucidating a priori predictors of responder or nonresponder status, including genetic factors, biomarkers, clinical settings, and disease associations. Such predictors would have immediate benefits in the distribution of limited blood resources in the context of a priori matching of RBC units for clinically relevant antigens in patients with a high likelihood of exhibiting a responder phenotype. In the context of PLT transfusions, benefit would be in not requiring the use of methods to reduce the immunogenicity of transfused PLTs, for example, leukoreduction. Of equal importance, identifying factors that correlate with alloimmunization, and have a potential causal role, will provide a rational basis for therapeutic intervention to prevent alloimmunization. In this context,

resources will be needed to carry out the above studies prospectively and in cohorts that involve analysis of different patient populations, such as neonates, children, pregnant women, and the elderly. In addition to the better studied antibodies to RBC antigens and PLTs, analysis of anti-HNA is also an important factor as they play roles in pathologies such as transfusion-related acute lung injury. Despite the large cohorts of patients transfused each year, most studies are performed retrospectively without attempts to complete follow-up and utilize appropriate cohorts of controls. The practice of transfusion is a de facto large-scale in vivo experiment, which could and should be much more extensively used for examining immune responses as part of routine medical care. Systematic prospective studies may reveal clinically and biologically important details of the immune response, that are impossible to obtain by any other study approach.

2. Promote studies on both prophylactic and therapeutic options for transfused patients.

The Workshop recommended support for studies focusing on new strategies to identify and minimize the development of clinically relevant alloantibodies and to determine the best strategies to manage alloimmunized patients, thereby reducing the impact on transfusion services of supporting these patients. Such support may both evaluate existing immunoregulatory approaches that have not yet been tested in the context of transfusion and also support novel approaches. Development of new interventions largely depends on novel mechanistic understanding. As outlined above, further development and utilization of tractable animal models in concert with detailed human studies is envisioned as the most promising route to identify novel therapies and management strategies.

3. Ongoing development of matching technologies and resources.

Although not a focus of the discussion, it is generally agreed upon that one of the most certain ways to avoid alloimmunization is to avoid exposure to alloantigens. The logistical and fiscal feasibility of such approaches must always be weighed against the actual medical benefit;¹⁹ nonetheless, in high-responder patients, matching of blood products becomes an unavoidable necessity whether one is avoiding future alloimmunization or responding to existing antibodies. Much progress has been made in both adapting serologic phenotyping to high-throughput platforms and also developing technologies to allow detailed genotyping for multiple alloantigens. However, given that some multiply alloimmunized patients still die from the inability to locate sufficient numbers of compatible RBC or PLT units, the current capabilities of antigen-matching are insufficient. Thus, there is a clear need for support to continue developing new tools and technologies to facilitate antigen matching. Improvements in recruiting donors of rare phenotypes, in technologies for high-throughput phenotyping and genotyping, and in engineering of blood cells (e.g., ex vivo culture of RBCs or PLTs) are important areas of development.

The possibility that sufficient matching may never be consistently achieved underscores the need for research in other approaches to avoid alloimmunization,

as detailed in this report. Nevertheless, the molecular analysis of blood group antigens has made major progress over the past 15 years.²⁰ The application to wider patient populations is becoming increasingly feasible with the development of high-throughput and affordable technologies, which have only recently become available. Development of these technologies beyond tertiary care hospitals and reference laboratories will require significant support, both to implement the approach and to gauge its successes.

Study of potential unappreciated sequelae of alloimmunization.: Transfusion medicine, in general, and blood centers in particular, have focused almost exclusively on the development of alloantibodies. However, cellular responses to alloantigens may also have significant sequelae in some settings. It is very unlikely that cellular immunity plays a role in the posttransfusion destruction of transfused RBCs or PLTs. However, recent basic animal studies suggest that T-cell responses to blood group antigens may play a role in rejection of subsequent transplants that express the same alloantigens.^{21,22} In addition, T-cell responses to PLT antigens can be the main effectors in an animal model of ITP.²³ As neither traditional nor current clinical tests in the blood bank can detect such T-cell responses, it is entirely unclear from the existing medical data if these responses are clinically relevant. Evaluation of the potential sequelae and pathophysiology of such cellular responses is a promising area of future research with potential medical significance.

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ABBREVIATIONS:

NHLBI National Heart, Lung, and Blood Institute

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