Transfusion-related acute lung injury: from bedside to bench and back

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ABSTRACT
Over the last 60 years, the transfusion medicine community has attained significant knowledge regarding transfusion-related acute lung injury (TRALI) through the bedside to bench and back to the bedside model. First, at the bedside, TRALI results in hypoxia and non-cardiogenic pulmonary edema typically within 6 hours of transfusion. Second, bedside studies, determined the higher incidence in plasma and platelet products than red blood cell products (fatal TRALI incidence plasma 1:2-300,00; platelet 1:3-400,00; red blood cell 1:2,500,000), as well as an association with donor leukocyte antibodies (~80% of cases). Third, at the bench, antibody and antibody-independent mechanisms have been described, requiring neutrophil and pulmonary endothelial cell activation. Antibodies, as well as alternate substances in blood products, result in neutrophil activation, which in a susceptible patient, result in TRALI (two-hit hypothesis). Fourth, back to the bedside, policy changes based on results of these studies such as minimizing use of plasma and platelet products from donors with leukocyte antibodies have decreased the incidence of TRALI. Thus, steps to mitigate TRALI are in place, yet, a complete mechanistic understanding of the pathogenesis of TRALI and which patients are at highest risk remain to be elucidated.
INTRODUCTION

Popovsky et al in the 1980s coined the term transfusion-related acute lung injury (TRALI), previously called severe pulmonary hypersensitivity reaction\(^1\), clarified the clinical presentation of hypoxia and bilateral non-cardiogenic pulmonary edema usually within 6 hours of a transfusion, and made the association with leukoagglutinins in the donor\(^2\). In 2005-9, 48% of the confirmed transfusion-related deaths in the U.S. reported to the Food and Drug Administration (FDA) were secondary to TRALI\(^3\). At that time, and as TRALI was being increasingly recognized as a potentially fatal transfusion adverse event, multiple definitions were in place. Thus, in order to better understand TRALI, a universally accepted definition was required and was finally created through working groups of the National Heart, Lung, and Blood Institute (NHLBI) and, more universally accepted, via Consensus Panel\(^4\). The Consensus Panel definition is currently used in hemovigilance systems, which tracks transfusion complications, and therefore improves determination of TRALI incidence as well as its blood product associations. The data obtained enables comparing the incidence of TRALI between countries with different practices as well as changes in incidence secondary to policy modifications\(^5\).

This gain in clinical knowledge of TRALI is paralleled with increased knowledge of its pathophysiology. Pathophysiologic studies have included those at the bedside (hemovigilance data and clinical trials) and the bench (laboratory investigations using \textit{in vitro}, \textit{ex vivo} and \textit{in vivo} models). Through these studies, current knowledge surmises that TRALI results from neutrophil and/or endothelial activation by multiple mechanisms in the lung resulting in pulmonary edema and alveolar damage\(^6\). Blood product substances that have been associated with TRALI include HNA (Human Neutrophil Antigen), and HLA class I and II antibodies, CD40-ligand (CD40L), and biologically active lipids. Furthermore, transfusion of these substances alone likely, and usually, does not result in TRALI but require a susceptible patient. Data from TRALI cases and laboratory studies hypothesize a susceptible patient is an individual with activated granulocytes and/or pulmonary endothelium\(^7\).

The benchside knowledge has resulted in developing methods to mitigate this untoward reaction. First steps have focused on blood product modifications. Indeed, U.S. and some non-U.S. countries have removed donors to be considered at high-risk (i.e. those with or having a high likelihood of white blood cell [WBC] antibodies) from donating high volume plasma containing products. Thus, through the last 50 years the transfusion community has gained knowledge regarding TRALI through the bedside to bench, and back to the bedside approach. Each gain in knowledge has resulted in steps to decrease the incidence of TRALI, but further knowledge is needed to mitigate and treat this potentially fatal transfusion reaction.

BEDSIDE

Recognizing the clinical entity
In 1926, WBC incompatibility between the donor and recipient resulting in severe, near fatal reactions was recognized\(^8\). In 1957, recipient WBC antibodies were associated with febrile transfusion reactions, some resulting in cyanosis, weakness, apathy, and prostration. The reactions resulted from the buffy-rich fraction but not the buffy-poor fraction of the blood component\(^9\). In 1966, transient non-cardiogenic pulmonary edema accompanying fever, cough and cyanosis without hypervolemia secondary to blood transfusion was described in three patients\(^10\). Until the mid-1980s this reaction was termed ‘pulmonary hypersensitivity reaction’\(^1,11\) and was associated with leukocyte antibodies in the donor against the recipient or in the recipient against the donor; however the specifics were not known or understood. In 1985, Popovsky and Moore described 36 cases of TRALI with acute respiratory distress characterized by hypoxemia and pulmonary edema with an onset usually within 4 hours of transfusion and accompanied by hypotension with rapid and complete recovery in the majority of patients (81%)\(^2\). In 89% of the patients, granulocyte or lymphocytotoxic antibodies were found in the donor of the implicated product. In 2000, TRALI resulted in 13% of all U.S. transfusion-related fatalities and was the third leading cause of transfusion-related mortality reported and, in 2001, the FDA published a letter alerting clinicians to TRALI\(^12\). By 2006, over 50% of transfusion-related fatalities reported to the FDA were due to TRALI (figure 1)\(^13,14\).

**Defining the clinical entity**

TRALI was increasingly recognized as a serious transfusion adverse event, but was poorly understood. NHLBI recognized the need for a common definition to further the understanding, and allow focused study of TRALI, because creating a common and useable definition permits comparison between reports and clarifies diagnosis\(^15\). NHLBI group defined TRALI as new acute lung injury (ALI) occurring within 6 hours of the end of transfusion of one or more plasma containing blood products in patients without other risk factors for ALI, or in patients with other risk factors for ALI if there was no ALI present pretransfusion and if the new ALI was temporally associated with blood transfusion\(^15\). NHLBI used the definition of ALI put forth by the North American-European Consensus Conference in 1994 (Table 1).\(^16\) However, in 2004, Consensus Panel entitled “Towards an Understanding of TRALI” extended the criteria for diagnosing TRALI by expanding the presence of hypoxia to also include other clinical evidence supporting the conclusion of hypoxia and by creating criteria for “possible TRALI” which include patients with preexisting ALI and ALI occurring more than 6 hours after transfusion (Table 2)\(^4,17\). Currently, Consensus Panel definition is commonly used to define TRALI\(^14,18\).

Although commonly used Consensus Panel definition lacks validation. A study investigating the applicability of this definition to a cardiac surgery registry demonstrated that approximately 65% of patients upon presentation to the ICU had a PaO\(_2\)/FiO\(_2\) ratio of <300\(^19\). Thus, the authors conclude using this criterion may not be appropriate for cardiac surgery patients.
Incidence, outcome and blood component association

Reports use different definitions of TRALI, different blood products and product modifications, and passive reporting systems; therefore, incidence, outcome, and blood product association data vary from country to country. German hemovigilance data includes 44 cases of confirmed TRALI: 80% antibody and 20% presumed non-immune medicated. The fatal 18% of cases were antibody mediated from female donors. TRALI risk was 1:260,000 for all blood components. The rate for antibody mediated TRALI was 1:66,000 for fresh frozen plasma (FFP; 1:285,000 for fatal antibody mediated), 1:420,000 for platelet concentrates, and 1:2,860,000 for red blood cell (RBC) units. Hemovigilance data from other countries reported TRALI rates of 1:250,000 (UK, Denmark and Norway), 1:66,667 (Finland), and 1:55,556 (Sweden) for all transfusions, and 1: 66,667 (Denmark), 1:62,500 (UK), 1:24,390 (Sweden), and 1:11,363 (Finland) for FFP transfusions. The blood products implicated in TRALI were: 49% FFP, 29% RBCs, 13% platelet concentrates, 2% whole blood, 0% solvent/detergent (SD) plasma and 7% mixed. The American Red Cross estimated risk of fatal TRALI per distributed component was 1:202,673 for plasma, 1:320,572 for apheresis platelets, and 1:2,527,437 for RBC units. TRALI mortality rates range from 5-35% in case series.

Antibody mediated

Leukocyte antibodies in products implicated in TRALI cases

In 65-90% of TRALI cases leukocyte antibodies were identified in the implicated donor. A systematic review of case reports with leukocyte antibody testing determined these antibodies contributed to 80% of the cases reported. The distributions of the implicated donor antibody targets are similar in two large case series. In a series of 36 cases, 31 were antibodies mediated; donors had HLA class I (11%), HLA class II (47%), HLA class I and II (8%) and HNA (33%) antibodies. In a series of 44 cases, 35 were antibody mediated; donors had HLA class I (11%), HLA class II (44%), HLA class I and II (37%), and HNA (23%) antibodies. Since transfusion recipients may receive more than one product, identifying a product with antibodies may overestimate its association with TRALI.

Prevalence of antibodies in blood donors

Multiple studies determined donor prevalence of leukocyte antibodies. First, in a study of 1043 donors using five methods to detect HLA class I, HLA class II, and NHA antibodies, 9.8% of females yet no males had antibodies. The incidence of antibodies increased with the number of pregnancies: one 3.8%, two 13.5%, and three or more pregnancies 24.1%. The antibodies were directed against HLA class I antigens 45.2%, class II 33.9%, and both class I and II 20.9%; no NHA antibodies were detected. In a second study of 5332 female parous donors, 8.9% had HLA class I, HLA class II, and/or HNA antibodies. The incidence of antibodies increased with the number of pregnancies: one 4.5%, two 8.6 %, three 12.1%, four 16.7%, and five or more pregnancies 15.4%. 92% of
leukocyte antibodies were against HLA (class I 61%, class II 19%, both class I and II 12%), 5% against HNA and 2% had positive granulocyte agglutination test of unknown specificity. In the Leukocyte Antibody Prevalence Study (LAPS) performed by the Retrovirus Epidemiology in Donor Study II (REDS II) 7920 donors volunteer donors were screened for HLA class I and II antibodies using a multiantigen bead kit\textsuperscript{26}. HLA antibodies were present in 1.7% of transfused males, 1.0% nontransfused males and 17.2% females (1.7% non-parous females and 24.4% parous females). Antibody positivity was correlated with the number of pregnancies: one 11.2%, two 22.3%, three 27.5%, and four or more pregnancies 32.2%. 10.4% of the antibodies were against class I antigens and 11.7% against class II antigens.

Presence of TRALI in patients who receive blood product with antibodies
Clinical studies investigated donors with leukocyte antibodies with occurrence of TRALI in recipients. In one report, 26 recipients of blood donated by immunized women resulted in no reported TRALI, although 11 of these patients had the cognate antigen\textsuperscript{25}. In a TRALI lookback study of 103 transfusion recipients from an implicated donor with multiple HLA class I and II antibodies revealed one case of TRALI although 54 of the 55 patients tested had cognate antigens\textsuperscript{15}. Interestingly, 4 of 62 patients with chest radiographs developed new or worse bilateral infiltrates posttransfusion. Look back from a fatal case of TRALI from a donor with an antibody to HNA-3a in 36 previous plasma recipients, revealed 36% had a transfusion reaction (of which 43% were mild/moderate reactions with symptoms of chills, fever, dyspnea, tachycardia, chest pain and hypotension and 58% were severe reactions with symptoms of respiratory failure, dyspnea, tachycardia, pulmonary edema, fever, and hypotension)\textsuperscript{27}. Another lookback study of a TRALI case associated with RBC transfusion from a donor with anti-NB2 and NB2-positive recipient with thrombotic thrombocytopenic purpura revealed none of the previous 21 blood donations had resulted in a transfusion reaction\textsuperscript{28}. Thus, other factors in addition to the antigen-antibody binding likely contribute to the development of TRALI, such as antibody specificity, antibody titer, antigen density, and underlying condition of the patient\textsuperscript{25}.

Male versus female plasma
A retrospective study of the incidence of acute pulmonary edema after transfusion in 8902 intensive care patients demonstrated 25 cases of transfusion associated circulatory overload (TACO; incidence 1:356 units transfused), 7 cases of suspected TRALI (1:1271 units transfused) and 14 cases of possible TRALI (1:534 units transfused)\textsuperscript{29}. Patients who developed suspected or possible TRALI received larger amounts of plasma, especially female donor plasma. In addition the mortality rate was 67% for suspected or possible TRALI compared to 20% for TACO and 11% in matched controls. A recent study from Japan demonstrated less post-transfusion pulmonary dysfunction with transfusion of male only plasma versus mixed gender plasma ($p=0.022$)\textsuperscript{30}. Lastly, a randomized control trial of intensive care unit patients who received one unit of control plasma and one unit of plasma donated from a multiparous female
showed multiparous plasma resulted in a significant decrease in PaO2/FiO2 ratio compared to control plasma (p<0.01). In contrast, a recent retrospective study comparing cardiac surgery patients who received female versus male plasma donor plasma reported less pulmonary dysfunction and improved outcome yet no difference in long-term outcome. The number of plasma units transfused was strongly correlated with increased mortality. The analysis of female versus male plasma did not consider number of plasma or RBC units, or other factors. Thus the balance of data supports female plasma is associated with a higher risk for TRALI or pulmonary dysfunction than male or nonparous female plasma.

Autopsy studies
Autopsy studies aid in the histopathology of pulmonary injury during TRALI. Two autopsy reports of patients who expired secondary to TRALI demonstrated pulmonary edema, neutrophil congestion in the alveolar capillaries without the presence of diffuse alveolar damage; case 1 had HLA class I (B50) and class II (DR16) antigens corresponding to donor’s antibodies and case 2 had HLA class I (A68) corresponding to donor’s antibody (donor also had antibody to HNA-3a, however recipient was not typed although >90% of whites are positive for HNA-3a). In another series of three fatal cases of immune mediated TRALI, autopsy demonstrated pulmonary edema with cholesterol crystals in endothelial membrane of venules (patient 1 had HLA class I A2 antigen corresponding to donor’s antibody, case 2 no WBC antibodies were identified in donor, and case 3 donor had antibody to HNA-3a). The proposed pathogenesis based on these findings is WBCs are coated with antibody then attached to the pulmonary venule endothelium where WBCs are activated. Activation results in lipid membrane oxidation leading to cholesterol and fatty acid release from membranes so that cholesterol crystals form. These crystals pierce the membrane resulting in pulmonary edema. Autopsy studies question the role of HLA class II antibodies in TRALI. In a fatal case of TRALI, pulmonary tissue immunohistochemistry did not demonstrate presence of HLA class II antigens on the vascular endothelium or intravascular WBCs, only on intraaveolar macrophages.

BENCH
Although studies provided significant insight into the clinical characteristics of TRALI, the pathogenesis of TRALI remained enigmatic. While anti-HLA and anti-HNA appear to facilitate TRALI development, mechanisms whereby antibodies induce ALI were unknown. Furthermore, antibody-independent mechanisms also likely exist as a significant number of antibody-independent cases have been reported. Clinically, TRALI resembles other forms of ALI, which suggests that mechanisms involved in TRALI development might bare some similarities to other forms of ALI. In other ALI models, neutrophils appear to play a critical role. Indeed, ALI appears to reflect the rapid accumulation and activation of pulmonary neutrophils following significant perturbation of common regulatory pathways. However, whether similar events surround TRALI pathogenesis remain unknown.
Innate inflammatory responses

Neutrophils provide a critical component of the innate immune response to invading microbial pathogens following tissue injury\(^3\). However, exuberant neutrophil-mediated inflammatory events often induce significant tissue damage and are commonly associated with a wide variety of deleterious sequelae\(^3\). Indeed, neutrophil mediated inflammation can be associated with liquefactive necrosis, which commonly reflects significant neutrophil necrosis and release of a broad repertoire of factors which cannot only rapidly destroy pathogen, but also viable tissue\(^3\). Due to their destructive potential, neutrophils appear to have evolved distinct mechanisms of turnover, not found in other leukocyte populations\(^3\), which likely reduce unregulated release of neutrophil contents. In addition to neutrophil necrosis-mediated tissue injury, activation of viable neutrophils in the absence of infection can cause significant tissue injury. Indeed, reperfusion injury, which commonly occurs following iatrogenic or naturally occurring recannalization of an occluded vessel, often occurs in the absence of microbial pathogens\(^4\).

In addition to factors regulating neutrophil turnover, proper immunological homeostasis requires significant regulation of neutrophil activation and recruitment. Endothelial cells play a central role in the proper activation, localization and extravasation of neutrophils to an area of tissue injury or pathogen invasion\(^2\) (Figure 2). Several key mediators appear to be responsible for these events. For example, once activated following hypoxic challenge, inflammatory cytokines or pathogen metabolites, endothelial cells rapidly mobilize vascular adhesion molecules, such as P-selectin, to the apical surface\(^2,4\). Alterations in the vascular lumen diameter induces margination of leukocytes which facilitates engagement of neutrophil ligands, such as PSGL-1, to endothelial and platelet derived selectins, inducing loose neutrophil adhesion to vascular wall\(^5\). Cytokine mediated activation of these neutrophils induces conformational changes in integrin molecules which allow for firm adhesion to the vascular wall followed by rapid extravasation\(^2\). Chemotactic factors released by pathogens and injured tissue then direct neutrophils to areas of primary tissue damage where they neutralize pathogens and remove necrotic tissue\(^3\) (Figure 2).

Proposed pathogenesis of antibody-mediated TRALI

While several studies suggested that transfusion-induced dysregulation of neutrophils may in part be responsible for the development of TRALI\(^1,4,5,6\), only recently have several of the mechanisms responsible for these alterations been elucidated. Given the clinical correlations implicating antibodies in the development of TRALI\(^17,23\), early studies examined the potential involvement of antibody-mediated perturbation of normal neutrophil function. Several studies demonstrated that anti-HNA-3a and anti-HLA-A24 possessed the ability to not only recognize and activate fMLP primed neutrophils, but also cause significant release of granule contents in vitro\(^47,48\), which suggested that significant infusion of neutrophil reactive antibodies may prematurely activate neutrophils.
intravascularly resulting in significant neutrophil-mediated damage to the vascular endothelium (Figure 3). In addition to directly activating neutrophils, other studies demonstrated that donor serum negative for neutrophil reactive antibodies, yet positive for HLA class II antibodies, may induce monocytes and possibly platelets to secrete a variety of inflammatory mediators, including LTB₄, TNF-α, and IL-8, which subsequently activate neutrophils. In contrast, antibody complexes might activate a variety of leukocyte populations through engagement of cell surface Fc receptors as opposed to direct engagement of antibody target antigens. Consistent with this, several studies demonstrated that antibody-soluble HLA antigen complexes may induce neutrophil activation in vitro through direct engagement of cell surface Fc receptors (Figure 3).

**Antibody-independent mechanisms of TRALI**

While early studies primarily focused on potential mechanisms whereby antibodies might induce TRALI, several studies uniquely examined antibody-independent pathways that might also induce neutrophil activation and subsequent TRALI. For example, supernatants harvested from stored platelet units induced significant activation of neutrophils. Although several studies failed to identify consistent accumulation of lipid mediators in stored blood products, several studies utilizing HPLC analysis demonstrated several fractions capable of mediating neutrophil activation displaying significant lipid content, similar to those observed in other forms of neutrophil-mediated tissue injury. In addition, synthetic lysophosphatidylcholines, analogous to lipids identified in stored units, also induce neutrophil activation, further suggesting that transfusion-associated lipids may in part induce TRALI-like pathways (Figure 4).

In addition to lipid mediators, several studies identified additional antibody-independent pathways. For example, platelet storage appears to increase levels of CD40L. Although commonly thought to primarily regulate adaptive immune responses, CD40 also resides on neutrophils and engagement by storage-derived CD40L induces significant activation. Importantly, leukoreduction eliminated accumulation of CD40L, strongly suggesting that CD40L is a leukocyte derived factor within platelet products (Figure 5). In contrast, RBCs themselves appear to possess the ability to scavenge inflammatory mediators, a property that may be lost during storage. Consistent with this, aged RBCs display reduced capacity to scavenge CCL2, a chemokine capable of recruiting neutrophils in vivo, likely due to altered ligand recognition and reduced expression of Duffy antigen (Figure 4). Taken together, these results importantly illustrate that several transfusion-associated factors possess ability to significantly modulate neutrophil function with obvious implications in the development of TRALI (Figures 3,4).

**Animal models of TRALI**

Although several in vitro studies suggested that a variety of mechanisms might be responsible for neutrophil activation, whether similar activation-induced
events occur in vivo, in the presence of key variables unique to the in vivo setting, remained unknown. For example, although neutrophils may induce injury and apoptosis of endothelial cells in vitro following significant activation\(^\text{47,49,50,52}\), neutrophils commonly extravasate following activation, allowing movement of the potentially deleterious effects of neutrophil activation away from the intravascular compartment and into the extravascular tissue, a process that is significantly limited in vitro\(^\text{63}\). Furthermore, neutrophils encounter endothelial cells under the presence of significant shear forces that can significantly impact the signaling and activation of neutrophils in vivo\(^\text{64}\). In addition, blood flow itself can modulate the effect of secreted factors by several mechanisms including directly engaging blood components and immediate dilution of secreted factors\(^\text{65}\). Although analysis under flow chambers in vitro may more adequately control for several of these factors\(^\text{64}\), in vivo models provide a practical alternative when evaluating the potential influence of these variables.

Early studies employed ex vivo lung preparations to evaluate the potential involvement of antibodies and neutrophils in TRALI pathogenesis. Explanted rabbit lungs transfused with human neutrophils and human anti-HNA-5b induced significant alterations in synthetic arachodonic acid metabolites, endothelial permeability and lung edema following co-transfusion of 5b positive, but not 5b negative neutrophils\(^\text{66,67}\). Similar results occurred using neutrophil reactive antibodies targeting the HNA-2a epitope\(^\text{68}\). Development of TRALI utilizing ex vivo lung was not limited to antibodies as lipid mediators generated during prolonged platelet storage appeared to likewise mediate significant vascular compromise and increased pulmonary pressure\(^\text{69}\). Taken together, these results strongly suggested that both antibody and lipid mediators possess the capacity to induce TRALI-like changes in ex vivo lung models.

While several ex vivo models suggested that transfusion factors possessed the ability to directly induce TRALI\(^\text{66,67}\), subsequent studies suggested that priming events prior to transfusion might be required\(^\text{70}\). This two-hit model suggests that a first hit, secondary to preexisting pulmonary pathology, must occur first followed by a second transfusion-associated injury. For example, in one model, lipids generated during prolonged storage of RBCs only induced TRALI-like changes in animals if previously challenged with LPS\(^\text{58}\). Similarly, subsequent studies demonstrated that MHC class I antibody and CD40L only induced TRALI following a similar priming event\(^\text{59,71}\). Although several of these studies employed ex vivo lungs\(^\text{69}\), most of these studies utilized intact lungs in vivo\(^\text{61,71-73}\). Differences in the activation state of the pulmonary vascular endothelium and possible priming of neutrophil during contact with synthetic conduits in ex vivo models may alter priming requirements in vivo\(^\text{74}\). Alternatively, subtle, yet significant differences in husbandry practices may also influence outcomes in in vivo models. Indeed, animal housing conditions, whether in a barrier protection or barrier free environment significantly influenced priming requirements for the development of TRALI\(^\text{72}\). Furthermore, differences in antibody concentrations used in various animal models may also partially account for variability in priming
requirements for the development of TRALI\textsuperscript{74}. Importantly, although LPS and other priming regimens may alter the expression of target antigens in anti-MHC class I models of TRALI, low dose LPS, while capable of priming models, does not appear to alter MHC class I expression, suggesting that other factors, such as toll like receptor-dependent endothelial cell activation may be required for these priming events to occur\textsuperscript{73}. As neutrophils and endothelial cells respond to a broad range of activating factors, differences in animal models, priming regimens and distinct activation signals initiated following transfusion likely result in unique thresholds for the development of TRALI in distinct settings.

\textit{Mechanisms of TRALI in vivo}

Although the exact molecular mechanisms of TRALI may be difficult to elucidate \textit{in vivo}, numerous studies suggest that several key players may be involved in distinct models of TRALI. For example, while certain antibodies appear to directly activate neutrophils or endothelial cells \textit{in vitro}\textsuperscript{47,48}, whether antibodies possessed the ability to directly activate inflammatory cascades responsible for TRALI remained unknown \textit{in vivo}. To examine direct antibody as opposed indirect pathways, several animal models employing target deletions of putative endogenous factors were employed. Injection of MHC class I antibodies induced significant TRALI in a wild-type (wt) animal model, while the same priming and injection regiment failed to induce TRALI in Fc knockout mice\textsuperscript{72}. Importantly, while MHC class I antibodies induced TRALI in Fc null mice transfused with wt neutrophils, antibody infusion into wt mice following transfusion of Fc null neutrophils failed to result in TRALI\textsuperscript{72}, which strongly suggested a role for neutrophil Fc receptors in antibody-dependent TRALI. Furthermore, in addition to engaging neutrophil Fc receptors, antibodies appeared to recognize endothelial MHC receptors \textit{in vivo}\textsuperscript{72}, strongly suggesting antibody mediated tethering of neutrophils \textit{in vivo}. Consistent with this, antibody mediated TRALI does not appear to require P-selectin-PSGL-1 or integrin interactions for neutrophil sequestration and TRALI development\textsuperscript{72}, which suggested that Fc mediated neutrophil interactions may bypass normal adhesion pathways and in part may be responsible for the premature intravascular release of neutrophil derived factors (Figure 3). Although conflicting data exist concerning the requirement for complement in antibody-mediated TRALI\textsuperscript{66}, C5a null animals remain sensitive to antibody-induced TRALI\textsuperscript{72}, strongly suggesting that complement-mediated pathways may not be required. In addition, while several studies demonstrate a central role for neutrophils in TRALI pathogenesis\textsuperscript{47,53,56,58,59,69,71,73}, some recent studies also suggest that platelets, which can also mediate neutrophil adhesion to vascular walls\textsuperscript{75}, might also facilitate neutrophil-mediated injury\textsuperscript{73}, since depletion of platelets and anti-platelet treatment with aspirin prevented TRALI development following MHC class I antibody injection\textsuperscript{73}.

Although \textit{in vitro} and \textit{in vivo} studies clearly implicate neutrophils in TRALI pathogenesis\textsuperscript{47,53,56,58,59,69,71,73}, few studies have examined actual signaling pathways initiated by putative TRALI-inducing factors. Previous studies
demonstrated that neutrophils possess multiple signaling pathways capable of inducing robust activation38 (Figure 5). Indeed, distinct receptors with unique signaling pathways likely evolved to enable rapid neutrophil response to the wide range of potential pathogens38. The ability of the broad range of putative factors implicated in TRALI suggests that distinct and possibly disparate pathways of activation may be engaged during events surrounding TRALI47,48,57,59,76. As several of these pathways are distinct, different factors may work synergistically, independently or require different levels or distinct mechanisms of neutrophil priming in order to sufficiently activate neutrophils to cause injury (Figure 5). As a result, seemingly disparate findings regarding TRALI etiology may simply reflect the multitude of pathways capable of resulting in one common outcome, neutrophil activation. Regardless of the mechanism of neutrophil activation, once activated, neutrophils appear to be capable of inducing endothelial injury and apoptosis47,49,50,52, likely through the release of soluble factors50,52, which ultimately results in loss of vascular integrity and pulmonary edema, hallmarks of ALI. In summary, as neutrophils appear to play a central role in the development of TRALI, additional studies will be needed to develop mechanisms of identifying and mitigating those factors which may increase TRALI risk, both in the patient and product.

BACK TO BEDSIDE: DECREASING THE INCIDENCE
Through bench studies, both antibody as well as non-immune mediated pathogenesis appears clinically relevant. In addition, most studies support transfusion of the antibody as a second hit in a susceptible patient. Because antibodies are present in most TRALI cases, especially severe and fatal cases, most policy changes made to mitigate TRALI have targeted antibody mediated TRALI.

Decreasing incidence of TRALI through changes in policies in blood products is supported through biovigilance data77. Multiple approaches have been proposed and are in place, including male only plasma, resuspending pooled buffy coat platelets in male only plasma, and screening female donors for leukocyte antibodies (some screen all, and some only if donor has history of pregnancy and/or transfusion)78, 79. Potential risk of these policies on the available blood supply should be weighed against the benefit of implementation.

ISBT (International Society for Blood Transfusion) published recommendations for screening donors for leukocyte antibodies80. Antibody detection should include antibodies against HNA (HNA-1a, -1b, -2, and -3a), HLA class I (HLA-A2) and class II.

In addition, ISBT recommended screening donors at risk for leukocyte antibody formation, i.e. parous female, transplanted and transfused individuals79. Blood components with high plasma fraction (plasma, apheresis platelets and whole blood) should not be prepared from these donors. Lastly, donors with anti-HNA-3a should not donate because of the high rate of fatality associated with this
antibody, even in the receipt of RBCs. Donor testing only needs to be repeated if exposure to leukocyte antigens occurs.

AABB Bulletin #07-03 recommended implementing measures to minimize the preparation of high plasma-volume components from donors known to be leukocyte-immunized or at increased risk of immunization. These measures were to be implemented by November 2007 for plasma components and November 2008 for platelet components.81

**Male only plasma**
The UK hemovigilance system from 1996 to 2004 diagnosed TRALI by respiratory distress and hypoxemia associated with transfusion of plasma-containing blood components and in the absence of fluid overload or cardiac failure82.

In 2006, the UK modified their definition based on Consensus Panel to limit time of onset from 24 to 6 hours after transfusion77. In addition in 2003 they created four groups: highly likely, probable, possible, and unlikely based on other causes of symptoms and serologic investigations. Reports of TRALI averaged 14 per year through 2001, then rose to 26 per year in 2002 and 36 in 2003 (36 deaths and 93 cases of major morbidity). TRALI was 5-7 more times as likely to be associated with a plasma-rich component (FFP, platelets, and whole blood) then a component containing small amounts of plasma (RBCs). Because TRALI is usually secondary to donor HLA or HNA antibodies, which are more common in females than males, the UK moved to male donor plasma and resuspension of buffy coat-derived platelets in male plasma. Since 2003, 80-90% of the UK FFP has been male plasma. In 2004 the UK started using SD plasma for plasma exchange procedures in thrombotic thrombocytopenic purpura patients. These changes resulted in a decrease in number of TRALI reports and deaths in the UK (figure 6); risk decreased from 1:65,000 to 1:317,000 (p<0.001) for FFP and 1:71,000 to 1:173,000 (p=0.068) for platelets; risk for RBCs (1:949,000) and cryoprecipitate (1:104,000) remained similar.

Likewise, American Red Cross reported decrease in fatal and nonfatal TRALI cases after implementing male-only plasma in 2007 (from 26 cases in 2006 to 7 in 2008).83

**Solvent/detergent (SD) plasma**
SD plasma is a pooled human plasma product from 500-1600 donations which undergoes virally inactivation. Leukocyte antibodies are not detected in SD plasma because the process dilutes WBC antibodies and soluble HLA antigens are present in the product neutralizing the antibodies83,84. Importantly, SD plasma has not been associated with TRALI, although over 13 million units have been transfused5.
FUTURE DIRECTIONS
Through the last 50 years, knowledge of TRALI has significantly increased. Changes are occurring which decrease its incidence; yet much remains unknown. First, patients at risk for TRALI are not well characterized and nonimmune mechanisms are poorly understood. Therefore, blood product modifications to prevent TRALI secondary to these factors have yet to be developed. Next, Consensus Panel definition of TRALI may need validating. Last, TRALI incidence and outcome are largely based on passive reporting systems. Thus, TRALI is likely significantly under-reported and therefore the incidence may be significantly higher than published. Coordinated efforts between bench researchers and translational researchers, clinicians, epidemiologists, and donor centers will be necessary in future years to further minimize the risk of this potentially fatal transfusion complication.

AUTHORSHIP
Beth H. Shaz, Sean R. Stowell, and Christopher D. Hillyer have all contributed to the writing of this manuscript. The authors have no conflict of interest.
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Table 1: The North American-European Consensus Conference definition of ALI\textsuperscript{15,16}

1. Timing: Acute onset
2. a) Pulmonary artery occlusion pressure: $\leq 18$ mm Hg when measured, or
   b) A lack of clinical evidence of left atrial hypertension
3. Chest radiograph: Bilateral infiltrates seen on frontal chest radiograph
4. Hypoxemia: Ratio of $\text{PaO}_2/\text{FiO}_2 \leq 300$ mm Hg regardless of positive end-expiratory pressure level, or
   Oxygen saturation of $\leq 90\%$ on room air (added by working group)
Table 2: Recommended criteria for TRALI and possible TRALI⁴

1. TRALI criteria
   a. ALI
      i. Acute onset
      ii. Hypoxemia
         Research setting:
         \[\text{PaO}_2/\text{FiO}_2 \leq 300\],
         or \[\text{SpO}_2 < 90\%\] on room air
         Nonresearch setting:
         \[\text{PaO}_2/\text{FiO}_2 \leq 300\]
         or \[\text{SpO}_2 < 90\%\] on room air
         or other clinical evidence of hypoxemia
      iii. Bilateral infiltrates on frontal chest radiograph
      iv. No evidence of left atrial hypertension (i.e., circulatory overload)
   b. No preexisting ALI before transfusion
   c. During or within 6 hr of transfusion
   d. No temporal relationship to an alternative risk factor for ALI

2. Possible TRALI
   a. ALI
   b. No preexisting ALI before transfusion
   c. During or within 6 hr of transfusion
   d. A clear temporal relationship to an alternative risk factor for ALI
FIGURE LEGENDS

Figure 1: Transfusion-related fatalities reported to the FDA
The 3 leading causes of known and reported allogeneic blood transfusion-related deaths, based on data reported passively to the US FDA over 32 years (1976-2007). For each of the 5 periods for which data have been made available, the figure shows the mean annual number of deaths deemed to be due to TRALI, transfusion associated sepsis (TAS), or ABO hemolytic transfusion reactions (HTRs), along with the mean total number of deaths reported to the FDA plotted on a logarithmic scale. Deaths reported to the FDA include donor fatalities, recipient fatalities in which allogeneic blood transfusion (ABT) was not deemed to be the likely or major cause of death, and recipient fatalities due to TRALI, TAS, ABO HTRs, as well as other transfusion complications. Data on TRALI and TAS are not available for the period 1996 to 2000. (reprinted with permission)

Figure 2: Regulatory pathways which govern neutrophil activation and extravasation. Following tissue injury or pathogen invasion, cytokines produced by resident leukocytes or metabolites generated by pathogens induce endothelial cell activation. Activated endothelial cells mobilize P-selectin and E-selectin to the apical cell surface which facilitates loose neutrophil adhesion through interactions with PSGL-1. Activation of neutrophils during loose adhesion results in conformational changes in integrins that mediate firm adhesion and extravasation. Once extravasated, neutrophils respond to chemotactic stimuli, neutralize pathogens and remove necrotic tissue through the collaboration of a wide variety of factors, including enzymes and free radicals.

Figure 3: Direct antibody-mediated TRALI. Priming events may or may not be required for antibody-induced TRALI, but appear to significantly exacerbate TRALI when present. Transfusion of blood products containing antibodies against human neutrophil antigen (HNA) and MHC class I can result in direct activation of intravascular neutrophils. Anti-MHC class I antibodies recognizing endothelial MHC class I may also directly tether neutrophils to the endothelium independent of selectin or integrin-mediated events. Immune complexes of anti-HNA or anti-MHC class I and soluble HNA or MHC class I may also be recognized by Fc receptors resulting in neutrophil activation. Intravascular activation of neutrophils results in damage to endothelial cells, vascular leakage and pulmonary edema.

Figure 4: Indirect-antibody mediated and antibody independent mechanism of TRALI induction. Priming events secondary to underlying pulmonary pathology, often induced by LPS injection in experimental animal models, activate endothelial cells, resulting in significant sequestration of neutrophils within the pulmonary vasculature. Following pulmonary priming events, transfusion associated factors induce rapid intravascular neutrophil activation with subsequent endothelial damage, vascular compromise and pulmonary edema. Factors responsible for transfusion-induced activation are shown and
include soluble CD40L, antibody-mediated monocyte activation and cytokine release, lipid mediators and impaired chemokine scavenging by aged red blood cells. Duffy antigens are specifically shown on the surface of red blood cells, where Duffy* on aged red blood cells indicates Duffy receptors with impaired capacity to bind intravascular chemokines.

**Figure 5: Transfusion-associated neutrophil activation.** Transfusion associated factors ranging from antibodies to lipid mediators engage distinct neutrophil receptors resulting in significant intravascular neutrophil activation. Although the signaling pathways for several receptors, such as the Fc receptors, have been independently examined, the signaling mechanisms responsible for most of these activation events remain unknown.

**Figure 6: Decreased incidence of TRALI resulting from male only plasma**

All reports of TRALI (n = 195) and deaths (n = 40) from 1996 through 2006 inclusive. Reporting years from 1996 until 2000 each cover 12 months from October 1 until September 30; 2001 covers 15 months from October 1, 2001, to December 31, 2002; 2003 and subsequently cover calendar years. (reprinted with permission)
Figure 1: Transfusion-related fatalities reported to the FDA$^{14}$
Figure 2

- Endothelial activation — Endothelial contact — Activation — Extravasation

Blood Flow

Endothelial cells

Neutrophil

PGSL-1

P-Selectin

E-selectin

IL-8 mediated activation

Extravasation

Neutralization of pathogens and removal of necrotic tissue
Figure 3

Direct antibody-mediated TRALI

Priming event       Transfusion       Activation       Endothelial injury

Neutrophil → Fc receptor → anti-HNA → anti-MHC Class I

MHC Class I → HNA

Immune complex formation

Newtrophil activation → Elastase

Direct tethering to endothelium

+ H2O2

Endothelial injury

Blood Flow

Vascular leakage → Pulmonary edema → TRALI
Figure 4

Indirect antibody-mediated and antibody-independent TRALI

- Priming event
- Transfusion
- Activation
- Endothelial injury

Blood Flow
Figure 5

Transfusion-associated neutrophil activation

- Anti-HNA antibodies
- Cytokines
- Extracellular space
- Cytosol
- Cytokine Receptor
- Neutrophil Antigens
- MHC class I
- Fc Receptor
- Neutrophil activation
- Anti-MHC class I antibodies
- Immune complex and antibody recognition of cell surface antigens
- Chemokines
- Chemokine Receptor
- CD40L
- Lipid Receptor
- CD40
- Lipid
Figure 6: Decreased incidence of TRALI resulting from male only plasma\textsuperscript{77}
Transfusion-related acute lung injury: from bedside to bench and back
Beth H. Shaz, Sean R. Stowell and Christopher D. Hillyer