



## REVIEW

## Transfusion-related acute lung injury (TRALI): Current concepts and misconceptions

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## SUMMARY

Transfusion-related acute lung injury (TRALI) is the most common cause of serious morbidity and mortality due to hemotherapy. Although the pathogenesis has been related to the infusion of donor antibodies into the recipient, antibody negative TRALI has been reported. Changes in transfusion practices, especially the use of male-only plasma, have decreased the number of antibody-mediated cases and deaths; however, TRALI still occurs. The neutrophil appears to be the effector cell in TRALI and the pathophysiology is centered on neutrophil-mediated endothelial cell cytotoxicity resulting in capillary leak and ALI. This review will detail the pathophysiology of TRALI including recent pre-clinical data, provide insight into newer areas of research, and critically assess current practices to decrease its prevalence and to make transfusion safer.

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## Introduction

Transfusion-related acute lung injury (TRALI) is the most common cause of transfusion-related death world-wide. First described in the 1950s the clinical term was not coined until 1985.<sup>1</sup> The initial reports of TRALI occurred in relatively healthy patients with the first large series reported on patients who required transfusion after recent surgery. Newer animal models have been developed and *in vitro* assays using primary human cells to mimic the condition have been used. TRALI can certainly be caused by a number of mediators and each requires some specific constraints and must be thought of in context to the blood product from which it originates.

## Diagnosis and treatment

TRALI is a clinical diagnosis, and while laboratory data may support the diagnosis it is not required.<sup>2,3</sup> TRALI occurs within 6 h of transfusion with the majority of cases presenting during the transfusion or within the first 2 h.<sup>1,4,5</sup> TRALI is the insidious onset of acute pulmonary insufficiency presenting as tachypnea, cyanosis, and dyspnea with acute hypoxemia, PaO<sub>2</sub>/FiO<sub>2</sub> <300 mm Hg, and decreased pulmonary compliance, despite normal cardiac function.<sup>1,4–8</sup> Radiographic examination reveals diffuse, fluffy infiltrates

consistent with pulmonary edema.<sup>1,7</sup> TRALI is the new onset or worsening of pulmonary function with hypoxemia that satisfies the international criteria for ALI (PaO<sub>2</sub>/FiO<sub>2</sub> <300 mm Hg) with a chest X-ray consistent with pulmonary edema occurring during or 6 h within transfusion.<sup>2,3</sup> What differs between the National Heart Lung and Blood Institute (NHLBI) and the Canadian Consensus Conference definitions is that the in the NHLBI definition other risk factors for ALI may be present, while the Canadian Consensus Conference definition designates these conditions as “possible TRALI”.<sup>2,3</sup> In any case, transfusion must be envisioned as the inciting event.<sup>2,3</sup> All blood components have been implicated in TRALI; however, plasma containing blood components are most commonly implicated with fresh frozen plasma (FFP) and whole blood-derived platelet concentrates (WB-PLTs) having caused the largest number of reported cases.<sup>5,7,9</sup> In addition, plasma is considered one of the most hazardous transfused components, mainly because of its association with TRALI, and at most centers the plasma is platelet contaminated plasma because most blood collection facilities do not make platelet concentrates (PCs) from whole blood collections.<sup>10</sup> This use of platelet contaminated plasma is significant for it allows for the possible infusion of platelet fragments and all endogenous growth factors and other mediators which are platelet-derived. Many of these compounds are effective activators of PMNs and innate immunity including soluble CD40 ligand, from platelet membranes, ADP, ATP, and regulated on activation, normal T-cell expressed and secreted (RANTES).<sup>11–16</sup>

The treatment for TRALI is supportive and consists of aggressive respiratory support with supplemental oxygen and mechanical ventilation if required at low enough pressure and tidal volume

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to not induce barotrauma.<sup>4,5,17,18</sup> Two separate consensus conferences have occurred to define TRALI, and in short, diuretics may cause decreases in intravascular volume and are not indicated.<sup>2,3</sup>

### Prevalence and mortality

TRALI has been reported as commonly as 1/1333–1/5000 per unit transfused in North America with lesser rates in Europe.<sup>1,2,5,19,20</sup> The reported mortality from TRALI is 5–35%, with lower mortality rates (5–10%) being more common.<sup>4,7,9,21,22</sup> However, recent prospective data from critically ill patients in the intensive care units have documented TRALI rates as high as 8%; therefore these patients appear to have the highest risk for developing TRALI.<sup>23</sup> Most patients recover within 72 h; however, the data regarding TRALI are limited, and the attendant morbidity and mortality may be under appreciated due to both lack of recognition and under reporting.<sup>4,7,9,21</sup> Autopsy specimens have demonstrated widespread PMN infiltration with interstitial and intra-alveolar pulmonary edema, hyaline membrane formation, and destruction of the normal lung parenchyma consistent with the acute respiratory distress syndrome (ARDS).<sup>4,24–29</sup> In addition, in epidemiological studies of ARDS, transfusion was implicated as the most common predisposing factor for ARDS, and a number of these cases may be TRALI.<sup>24,30–32</sup> A recent analyses of all reported cases of TRALI concluded that antibody-mediated TRALI may represent a more clinically severe form as compared to those reported reactions secondary to lipids and other biologic response modifiers (BRMs).<sup>33</sup> However, because most centers require the presence of antibodies against HLA or granulocyte antigens to make the diagnosis, such an analysis of BRM-mediated TRALI may be invalid due to selection bias.<sup>33</sup> Importantly, this bias probably reflects the availability of antibody (anti-HLA or anti-HNA) testing services and in contrast the scarcity of laboratories that investigate BRMs, such that investigation of the role of BRMs in TRALI should be promoted.

### Neutrophils and TRALI

Popovsky and Moore first postulated that the PMN is the effector cell in TRALI and the murine and rat models of *in vivo* TRALI have reaffirmed that TRALI is PMN-mediated; thus, a review of PMN physiology is required.<sup>1,9,34,35</sup> In response to an infection in the tissues signals are sent out that cause pro-inflammatory activation of the vascular endothelium in a concentration dependent fashion (Fig. 1A).<sup>36–39</sup> These signals induce the increased surface expression of the selectins on the endothelial cell (EC) surface and shedding of L-selectin on the PMNs, resulting in loose attachment of PMNs to the EC surface, known as capture in the pulmonary circulation.<sup>37,39–41</sup> Closer to the nidus of the infection the ECs, in response to pro-inflammatory stimuli, synthesize and release chemokines, e.g. IL-8, which increase the surface expression of cellular adhesion molecules, especially intracellular adhesion molecule-1 (ICAM-1).<sup>37,39–41</sup> The chemokines cause a rapid conformational change in the  $\beta_2$ -integrins on the PMNs resulting in the firm adherence of PMNs to the vascular EC via PMN  $\beta_2$ -integrins and ICAM-1 on the ECs.<sup>37,39–43</sup> The change from a non-adherent to an adherent PMN is known as priming and not only involves PMN adhesion but also augments the release of microbicidal products from PMNs when activated to kill pathogens.<sup>37,39–44</sup> In response to chemotaxins, the PMNs then diapedese through the EC layer and chemotax along a gradient of mediators to the nidus of infection where they phagocytize and destroy the invading microbes.<sup>36,39</sup> In PMN-mediated ALI the first few steps are identical except that the stimulus is form the intravascular space and not the tissues (Fig. 1B).<sup>36,38,39</sup> PMNs become firmly adherent (primed) but do not marginate due to the lack of a chemotactic gradi-

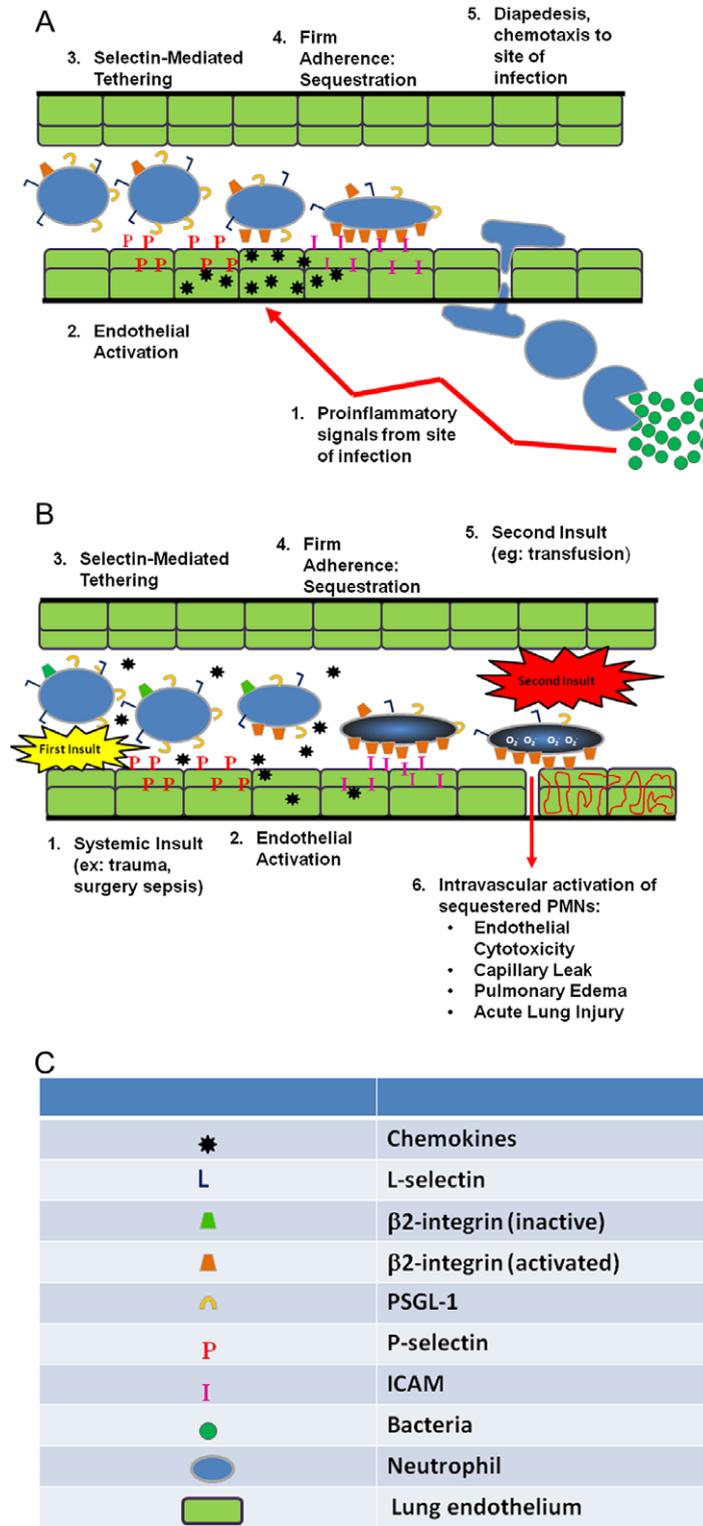
ent.<sup>36,38,39</sup> Therefore, these adherent, functionally hyperactive PMNs are sequestered in the lung and may predispose the host to ALI if a second stimulus, such as BRMs or antibodies in a blood transfusion, activates these primed granulocytes causing release of the microbicidal arsenal, EC damage, capillary leak, and ALI.<sup>36,38,39</sup> Such “activating” agents may not have the capacity to cause activation of the microbicidal arsenal in “quiescent” PMNs but only have the ability to activate adherent, primed PMNs; otherwise, ALI would be a much more common event.<sup>36,38,39,44</sup> Importantly, many clinical insults linked to TRALI may cause pro-inflammatory activation of vascular EC including: viral infections (adenovirus or cytomegalovirus), traumatic injury or major surgery, a “scheduled injury”, which release significant amounts of tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), and massive transfusion, which exposes the host to large amounts of neutral lipids which activate endothelial cells.<sup>42,45–48</sup>

There are also a number of important issues that are inherent to lung physiology: (1) the lung is the only organ in which PMN margination occurs in the capillaries.<sup>49–55</sup> The capillaries are narrow and leukocytes, which have a larger diameter than the capillary, and need to “squeeze” through, such that mechanical sequestration may occur via the exposure of agents that stiffen PMNs.<sup>49–55</sup> (2) There is controversy as to the mechanism of PMN sequestration whether firm adhesion, selectin-mediated tethering, or mechanical sequestration is required for PMN-induced ALI.<sup>49–55</sup>

### Pathogenesis

Three basic mechanisms have been proposed for the pathogenesis, and all require PMNs. Antibody-mediated TRALI: the infusion of donor antibodies specific for HLA class I and human neutrophil antigens (HNA) expressed by the recipient. The first clinical series of TRALI found donor antibodies against HLA class I antigens and granulocyte antigens in 89% and 72% of cases, respectively.<sup>1</sup> Most of the granulocyte antibodies did not exhibit specificity but 59% of the HLA class I antibodies did, and the infusion of specific HLA antibodies has been confirmed in 50–65% of TRALI cases.<sup>1,7,56–58</sup> However, in most investigation of antibody-linked TRALI, the investigators do not determine if the recipient expresses the cognate antigen, although it is a prerequisite for the proposed pathogenesis.<sup>1</sup> Antibody-mediated TRALI requires that donor antibodies bind the cognate antigen in the host leading to the activation of complement, which causes pulmonary sequestration and activation of PMNs resulting in the release of cytotoxic agents, endothelial damage, capillary leak, and ALI.<sup>1,7,58</sup> These antibodies are likely to be cytotoxic or pro-inflammatory, because HNA-3a is not cytotoxic, to confer such biologic activity upon binding the leukocyte antigen.<sup>1,5,59–62</sup> Antibodies have caused TRALI in healthy humans and these clinical details supported this pathogenesis.<sup>63,64</sup>

The *in vivo* relevance of antibodies to HLA class I antigens was confirmed in a murine model of TRALI in which a monoclonal antibody to mouse MHC class I antigens caused reproducible ALI at a dosage of 4.5 mg/kg.<sup>35</sup> PMNs were required to recognize antigen:antibody complexes deposited onto the surface of the pulmonary EC and PMNs cytotoxicity was accomplished via activation of the murine Fc receptors because Fc knockout animals did not manifest TRALI when administered this antibody.<sup>35</sup> This study mimicked the clinical time course for TRALI for the mice developed ALI within 2 h of infusion; however the mortality was 50% (vs. 10–20% in clinical TRALI), and despite such a high mortality no blood pressure (BP) recordings or other physiologic parameters were included from the time of infusion to time of death/euthanasia. Furthermore, there was no dose–response to the infused antibody, i.e. only the one concentration caused ALI. It would be improbable for a human to be transfused with a specific antibody



**Fig. 1.** (A) PMN Physiology: The normal response to infection. From an infection (green circles) in the tissues:<sup>1</sup> pro-inflammatory signals (LPS) are sent out (arrows) that activate ECs<sup>2</sup> causing chemokine synthesis and release (black stars), increases in P-selectin (red P's) and increased surface expression of intercellular adhesion molecule-1 (ICAM-1, pink I's). This activation elicits<sup>3</sup> PMN attraction and selectin-mediated tethering (capture) of PMNs between the PMN P = selectin glycoprotein ligand-1 (PSGL-1, tan C's) and endothelial P-selectin. Capture is then followed by<sup>4</sup> firm PMN CD11b/CD18 (orange trapezoids) : ICAM-1 endothelial cell adherence resulting in PMN pulmonary sequestration.<sup>5</sup> PMNs diapedese through the endothelial cell layer and chemotax to the site of infection and kill the pathogens in the tissues. (B) PMN Physiology: PMN-mediated ALL. In response to intravascular stimuli due to a systemic (first) insult, ECs become<sup>1</sup> activated and synthesize and release chemokines (black stars) and increase the surface expression of P-selectin (red P's) and ICAM-1 (pink I's).<sup>2</sup> This endothelial activation causes tethering.<sup>3</sup> of PMNs via the PMN PSGL-1 (tan C's) and P-selectin followed by firm adherence<sup>4</sup> via the PMN CD11/CD18 : ICAM-1 from the endothelial surface resulting in pulmonary PMN sequestration. A second intravascular stimulus (insult), transfusion of specific antibodies against host PMN antigens or BRMs activate these primed, adherent PMNs<sup>6</sup> causing release of the microbicidal arsenal from the PMNs, including O<sub>2</sub><sup>-</sup>, resulting in EC damage, capillary leak, and TRALI. Panel C is the key for this figure and denotes the abbreviations used in panels A and B.

at a concentration of 4.5 mg/kg, for this specific antibody would have to comprise >10% of all IgG in 200 ml of FFP, the average dosage to a 70 kg adult, assuming the normal IgG concentrations (1060–1274 mg/dl) in the plasma and in the transfused host.<sup>35</sup> Interestingly, complement activation was not required for TRALI in this murine model, and the authors did state that the antibodies did not cause activation of the NADPH oxidase using a flow-based assay system. This last result is to be expected if the IgGs prime the PMN oxidase because priming agents, especially those implicated in TRALI, do not activate the respiratory burst.<sup>35,36</sup> Lastly, a recent *in vivo* model employing the identical antibody from Looney et al. demonstrated a requirement for the capture of platelets and a requirement of E-selectin to produce TRALI.<sup>65</sup> Although this model represents an elegant mechanistic representation of antibody-induced inflammation, the pathogenesis represented differs widely from TRALI because of the aforementioned reasons and does not take into account the requirement for Fc receptor-mediated PMN activation through recognition of immune complexes on the endothelial surface.<sup>65</sup>

Antibodies to granulocyte specific antigens have also been implicated in TRALI, and the *in vivo* relevance to these antibodies was confirmed in isolated, perfused rabbit lungs.<sup>62</sup> ALI was characterized by pulmonary edema, and TRALI was precipitated by the infusion of a mixture of human PMNs (HNA-3a (5b) positive), HNA-3a antibodies, and rabbit plasma as a complement source.<sup>62</sup> Pulmonary edema occurred 3–6 h following the infusion of the admixture; however, if any one of the three components were deleted or antibodies without defined specificity were used, TRALI did not occur.<sup>62</sup> The need for complement has been obviated in a rat model with the infusion of monoclonal antibodies (Mab) to HNA-2a (CD177) eliciting TRALI if they are infused with PMNs that express a high density of the HNA-2a antigen.<sup>66</sup> The observed mild pulmonary edema with the Mab to HNA-2a and PMNs which express a high density of HNA-2a was significantly augmented with the addition of N-formyl-Met-Leu-Phe.<sup>66</sup> In addition, using a flow-based assay the Mabs to HNA-2a demonstrated the ability to activate the PMN oxidase in PMNs that express a high density of HNA-2a without affecting those PMNs with low density or lack of HNA-2a expression.<sup>66</sup> Although eloquently done, one must consider the effects of the tubing on the infused PMNs in this *ex vivo* model because the tubing used to introduce PMNs is known to prime them, and such stiff leukocytes may become non-specifically sequestered due to their inability to “navigate” the pulmonary circulation.<sup>55,67,68</sup> In addition one must be careful with antibodies to granulocyte specific antigens because animal models of human disease and inflammation routinely employ granulocyte specific antibodies to PMN deplete these rodents, and infusion of this antibody did not cause ALI.<sup>35,69</sup> Transfusions which contain PMN-reactive-antibodies to specific host antigens may result in alloimmune neutropenia depending upon the characteristic of the antibody:antigen interaction.<sup>70</sup>

#### *Antibody-mediated TRALI: the infusion of donor antibodies specific for HLA class II antigens expressed by the recipient*

Antibodies specific for HLA class II antigens have been implicated in a large number of patients with TRALI who express the cognate antigens.<sup>17,23,71–73</sup> Binding of specific HLA class II antibodies to their cognate antigens on monocytes *in vitro* resulted in intracellular synthesis of TNF $\alpha$ , IL-1 $\beta$  and tissue factor over a 4-h time period as compared to identical monocytes incubated with control sera.<sup>74</sup> Moreover, incubation (20 h) of monocytes that express the cognate antigens with antibodies to these HLA class II antigens resulted in the production of both cytokines and chemokines (IL-8, GRO $\alpha$ ); the latter peptides could certainly activate PMNs sequestered in the lung.<sup>75</sup> Recent *in vitro* studies demon-

strated that co-cultures of human pulmonary microvascular endothelial cells (HMVECs) and monocytes stimulated with antibodies to HLA class II antigens expressed on the monocytes lead to the release of leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and TNF $\alpha$  into the supernatant with concomitant apoptosis of HMVECs.<sup>76,77</sup> These experiments also identified the importance of monocytes and the HMVECs together because if alone minimal LTB<sub>4</sub> was produced, and if an interfering anti-Dr. antibody was introduced, HMVEC apoptosis and mediator release was inhibited.<sup>76,77</sup> Lastly, because HLA class II antigens may be expressed on ECs the infusion of class II antibodies into a recipient with the cognate antigen present on the pulmonary EC may manifest TRALI due to antibody-mediated EC activation, fenestration, and mild leak.<sup>74</sup>

Although attractive, this model of TRALI raises a number of issues: (1) while the synthesis of cytokines by circulating monocytes has the potential to cause TRALI, there is a significant time delay (4–20 h) for the synthesis of these cytokines and chemokines, and at 4 h these cytokines were never released extracellularly.<sup>74,75</sup> By definition, TRALI occurs within 6 h of transfusion with most of the reactions occurring during or 1–2 h following transfusion; thus, the synthesis of chemokines and cytokines over a 20 h time period may have little to do with TRALI.<sup>2,3,74,75</sup> However, the production of LTB<sub>4</sub> and TNF $\alpha$  in the model of Nishimura et al. is temporally consistent with TRALI. This model requires further elucidation because the number of circulating monocytes in contact with the pulmonary EC must be relatively high as monocytes contain the LTA<sub>4</sub> hydrolase to synthesize LTB<sub>4</sub> from LTA<sub>4</sub>; whereas, ECs alone can only synthesize the cysteinyl leukotrienes via activation of the LTA<sub>4</sub> synthetase and glutathione (LTC<sub>4</sub>, LTD<sub>4</sub> & LTE<sub>4</sub>).<sup>78–82</sup> (2) Pathologic examination of lungs from a fatal TRALI reaction attributed to HLA class II antibodies documented that there were no HLA class II antigens on the pulmonary EC.<sup>25</sup> In addition, although prolonged (72 h) *in vitro* cytokine exposure has led to the surface expression of HLA class II molecules on PMNs, HLA class II antigen expression on the PMN surface has only been demonstrated in patients treated with G-CSF or GM-CSF, and although such cytokine exposure may predispose these individuals to TRALI, this group of patients is largely neutropenic from chemotherapy and may not manifest PMN-mediated ALI.<sup>4,83–88</sup> These data are important because some interaction must occur between the pulmonary EC and the monocytes to produce clinically relevant amounts of LTB<sub>4</sub> and the expression of HLA class II molecules is one potential factor.

#### *The two-event model of TRALI*

TRALI has been documented in cases in which antibodies have not been detected in either the donor or the recipient;<sup>1,4,5,7,9</sup> moreover, if the infusion of specific anti-leukocyte antibodies were sufficient to cause TRALI, then blood from donors with specific antibodies against common leukocyte antigens should elicit TRALI reactions in the vast majority of recipients who express the cognate antigen. In “look-back” studies of donors with known high-titer “TRALI-implicated” antibodies to a) HLA class I alone, b) HNA-3a, c) HLA class I and class II, or d) HLA class II alone, few of the transfused patients who expressed the cognate antigens developed TRALI.<sup>18,57,89–92</sup> Therefore, the clinical condition of the patient appears important and may serve as the first event in the two-event model.<sup>4,5,57</sup> The second event is inherent to the blood product and may be either donor-derived anti-leukocyte antibodies which recognize a cognate antigen in the recipient or BRMs that accumulate during blood storage.<sup>4,36,93</sup> During storage of cellular components, effective PMN priming agents accumulate that are lipids as determined by solubility in chloroform.<sup>94,95</sup> Identification of these lipids determined that a mixture of lysophosphatidylcholines (lyso-PCs) accumulates in the plasma fraction of all cellular components and reaches a relative maximal concentration on the

day of component outdate (day 42) in the 100 units of packed red blood cells (PRBCs) tested.<sup>94,95</sup> These compounds effectively prime the PMN oxidase, activate primed, adherent PMNs *in vitro*, and may serve as the second event in a two-event model of PMN cytotoxicity and PMN-mediated ALI.<sup>44,94–97</sup>

The two-event model of TRALI was initially verified in an isolated perfused lung model and then re-confirmed in an *in vivo* rat model.<sup>28,69,98</sup> A two-event rat model was employed with saline (NS) or endotoxin (LPS) as the first event and the infusion of plasma from PRBCs or antibodies (OX18 and OX27) against MHC class I antigens as the second event.<sup>69</sup> The plasma from stored PRBCs, both pre-storage leukoreduced and unmodified [10% D28 and 5–10% D42]<sub>FINAL</sub>, and antibodies directed against MHC class I antigens caused ALI as the second event in this two-event *in vivo* model. ALI was demonstrated in a concentration-dependent manner in multiple ways including Evans Blue Dye (EBD) leak, lung histology, increases in total protein and CINC-1 in the bronchoalveolar lavage (BAL), and for antibody-mediated TRALI increases in EBD in the pulmonary interstitium. Importantly, in NS-injected rats neither the plasma from stored PRBCs nor the antibodies caused ALI, even when the concentration of OX27 was increased to 4.5 mg/kg, a level at which a monoclonal antibody induced ALI alone *in vivo* corroborating a two-event mechanism.<sup>35</sup> In addition, lipopolysaccharide (LPS) was not lethal and did not cause (1) EBD leak into the BAL or the pulmonary interstitium, (2) increases in total protein or CINC-1 in the BAL or histological evidence of ALI, but did induce pulmonary sequestration of PMNs.<sup>28</sup> LPS was employed as the first event because acute, active infection (bacterial or viral) was implicated as a predisposing clinical event in TRALI.<sup>4</sup> Bacterial and viral infections induce pro-inflammatory activation of the vascular endothelium which leads to adherence/sequestration of PMNs.<sup>42,44,99–101</sup> Other first events have been implicated including: cytokine administration, massive transfusion, recent surgery (especially cardiovascular surgery), and induction chemotherapy, but only infections have been studied to date *in vivo*.<sup>4–6,38,102–105</sup> PMNs were also required because granulocyte depletion inhibited ALI. Furthermore, the OX18 and OX27 antibodies demonstrated both antigen recognition and the ability to prime the fMLP-activated respiratory burst of rat PMNs, which were not affected by Fc receptor blockade, implying that these interactions were specific to antibody:antigen binding.<sup>69</sup> Lastly, a third antibody, which was granulocyte-(PMN) specific, caused immunodepletion of PMNs from rats, did not elicit ALI, when used in the two-event model, nor did it prime rat PMNs.<sup>69</sup> Both clinical TRALI and this model are similar with the onset of ALI within 6 h, mortality of 5–10%, identical histological evidence of ALI, and a dose–response relationship of antibodies or plasma from stored PRBCs to elicit TRALI as the second event such that lower concentrations did not cause TRALI or elicited milder ALI.<sup>7,34,39</sup> PMN reactive antibodies have varying effects on PMN physiology *in vivo* for they may prime or immunodeplete PMNs. A number of donor “look-back” studies which demonstrated that transfusion of a donor antibody to a recipient that expressed the cognate antigen caused TRALI in the minority of cases; thus, these antibodies appear to induce TRALI as a “second event”; hence, the clinical condition of the patient may be determinant for its genesis.<sup>18,57,89</sup> In addition, these antibodies were found on the surface of PMNs that were sequestered in the lung, but not on the surface of pulmonary vascular endothelium as demonstrated in the murine model.<sup>35,69</sup> Both OX18 and OX27 primed the rat PMN oxidase *in vitro*, identical to previous data which demonstrated that antibodies to HNA-3a could prime PMNs that expressed HNA-3a on their surface; moreover, Fc receptor blockade did not affect priming activity or cognate antigen immunoreactivity, implying specificity for these antigen:antibody interactions.<sup>69</sup> Therefore, a number of specific prerequisites appear to be required for antibody-induced TRALI which may explain its

relatively low prevalence: (1) the recipient has an underlying clinical condition that predisposes to TRALI, (2) the donor antibody must recognize the cognate antigen on the host's leukocytes which are sequestered in the lung, and (3) the antigen:antibody interaction must cause a pro-inflammatory change in the PMN.<sup>69</sup> The presented model is superior to other *in vivo* modeling because it better approximates clinical TRALI: (1) the first event, LPS, approximates acute active infection, a proposed risk factor for TRALI and causes pro-inflammatory activation of the pulmonary capillary bed without causing death or other organ injury.<sup>28,37,98,106–109</sup> LPS has been used in many animal models and even injected into human volunteers and mimics infection.<sup>28,37,98,106–111</sup> (2) ALI was induced by two separate second events, like clinical TRALI, and the second events demonstrated a concentration:response relationship in ALI using multiple parameters, unlike other *in vivo* models.<sup>35</sup> (3) The MHC class I antibodies localized to the antigen on the PMN membrane, identical to Popovsky's proposed antibody pathophysiology, and did not implicate Fc-mediated indirect activation, reminiscent of immune complex disease, not currently implicated in clinical TRALI.<sup>1,7,35</sup> (4) The novel pathophysiology of antibody- and BRM-mediated TRALI in this model are due to direct pro-inflammatory changes (priming) of PMNs induced by the second events, synonymous with *in vitro* studies using human pulmonary endothelium, PMNs, granulocyte antibodies, sCD40L or lipids.<sup>28,44,94,95,112</sup> This pro-inflammatory requirement may explain why not all antibodies may cause TRALI and induce TRALI. (5) Disparate from the murine model, this model used antibody concentrations 15- to 30-fold less and did not require supra-physiologic concentrations of a specific antibody. If one calculates the amount of this specific antibody needed to cause clinical TRALI, then this antibody must comprise 10% of all IgG in 200 ml of transfused FFP.<sup>35,69</sup>

Two clinical studies support the two-event model. The first identified 4 separate first events that may predispose patients to TRALI that were not present in a control group of patients with febrile or urticarial transfusion reactions including: (1) active infection, (2) recent surgery, (3) cytokine therapy, and (4) massive transfusion.<sup>4</sup> These first events have been documented by other investigators in both antibody-mediated TRALI and TRALI caused by BRMs, including the seminal article of Popovsky and Moore in which all TRALI patients had a surgical operation 24 h prior to the transfusion.<sup>1,104,105,113–117</sup> In addition, a nested case control study documented that two patient groups were at particular risk for TRALI: patients who had cardiac surgery and patients with hematological malignancies in the induction phase of therapy (all with absolute PMN counts >500/ $\mu$ l).<sup>5</sup> The second event was the infusion of bioactive lipids from the stored products as supported by the following data: (1) the implicated units were stored longer than control units that did not cause transfusion reactions, (2) the implicated blood products demonstrated significant plasma PMN priming activity as compared to the identically stored control units, and (3) there was PMN priming activity in all TRALI patients at the time of recognition, which consisted of neutral lipids and lyso-PCs, compared to pre-transfusion plasma.<sup>5</sup> These cases of TRALI were not antibody-mediated because of the donors tested, only 1/28 exhibited a leukocyte antibody with specificity (HLA-A26).<sup>5</sup> Furthermore, patients with hematological malignancies and patients requiring cardiac surgery comprised the majority of patients with fatal TRALI in a recent FDA report on transfusion-related mortality.<sup>102</sup> “Healthy” patients who experience TRALI would seem to disprove the two-event model; however, by definition, patients who require transfusion are not healthy. Lastly, a case of autologous TRALI has been reported in a patient who required a radical prostatectomy and transfusion. No leukocyte antibodies were detected but significant amounts of lipid priming activity were present in his PRBC units and in the post-transfusion plasma at the time TRALI was recognized.<sup>6</sup>

## Merging the pathogenesis

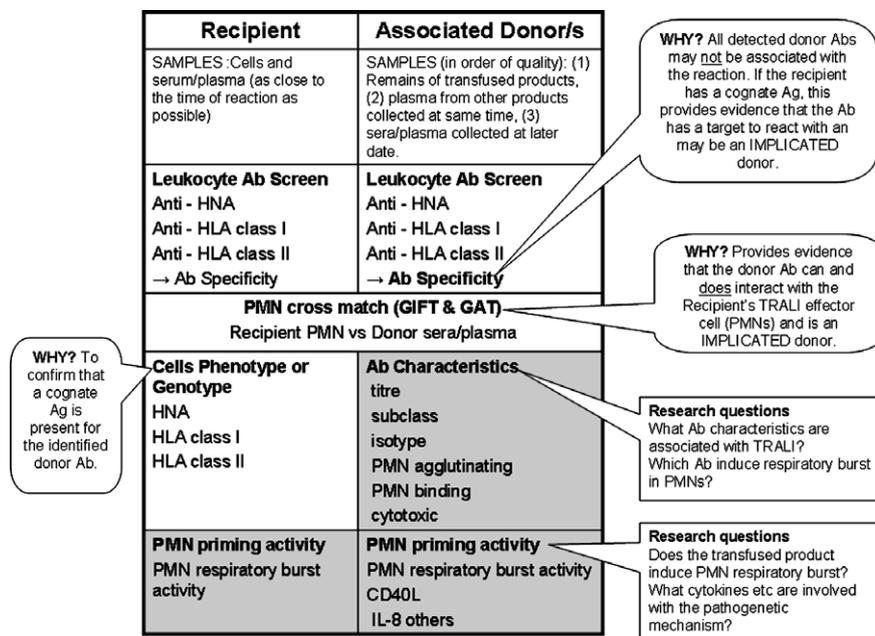
Lipids, sCD40L, antibodies to HNA-3a, and antibodies to MHC class I antigens that cause TRALI *in vivo* have been linked to clinical TRALI and prime isolated PMNs that exhibit the cognate ligand whether it be a G-protein coupled receptor, lipids and sCD40L, or the cognate antigen, HNA-3a or OX18 or OX27.<sup>28,44,69,96,112,118</sup> Importantly, immunoglobulins used to immunodeplete rats recognize the rat PMNs but do not induce TRALI in the two-event model *in vivo*, and do not prime PMNs. Moreover these BRMs and antibodies that primed PMNs caused PMN cytotoxicity of pulmonary endothelial cells as the second event in an *in vitro* model of two-event PMN-mediated cytotoxicity or TRALI *in vivo*.<sup>28,44,69,96,112,118</sup> Thus, antibodies that cause pro-inflammatory changes in PMNs may induce TRALI and those that do not may cause immunodepletion and lead to immune neutropenia.

## Laboratory work-up of TRALI

The combination of the granulocyte immunofluorescence test (GIFT) and the granulocyte agglutination test (GAT) is an effective approach for detecting PMN reactive antibodies in serum or plasma (Fig. 2).<sup>119,120</sup> These methods detect antibodies to both HNA and HLA class I, and possibly HLA class II, although further work is needed;<sup>119,121–123</sup> moreover if antibodies to HNA-3a are suspected GAT is required.<sup>120</sup> GAT provides the best indication as their physiologic relevance because it demonstrates direct antibody agglutination which is the results of PMN chemotaxis and homotypic PMN:PMN interactions, distinct from passive IgM cross-linking.<sup>124,125</sup> The combination of GAT and GIFT applied to panels of phenotyped PMNs enable identification of HNA antibody specificity. These techniques are limited to reference laboratories in the Granulocyte Working Party (GWP) of the ISBT ([www.isbt-web.org](http://www.isbt-web.org))

is a necessary reference for finding the appropriate laboratories in different geographic locales.

The laboratory investigation of HLA class I and II antibodies will not be discussed in detail as the techniques are widely and there are excellent, recent reviews available.<sup>126,127</sup> It is important to note that many of the assays employed are highly sensitive techniques to detect the presence of any antibodies in donor or recipient sera that could be clinically relevant leading to graft rejection and or graft versus host disease. Because HLA antibody screening is widely available it is often the first step in many TRALI investigations when screening implicated donors; however, two reports revealed that a disproportionately small number of HLA class I antibodies actually induce TRALI.<sup>89,128</sup> With this in mind and the high sensitivity of current HLA techniques, one questions the clinical relevance of all of the HLA antibodies detected in TRALI because most of the current flow-based assays are so sensitive that a relatively high percentage of non-transfused males demonstrated antibodies.<sup>129,130</sup> Similarly, flow-based assays used to detect antibodies to HLA class II antigens in donors are designed to detect very small amounts of antibodies using a PRA luminex bead assay (One Lambda, Canoga Park, CA). These tests may overestimate the role of antibodies to HLA class II antigens in TRALI because: a) no titers of antibody concentration are employed, b) without sufficient modeling of HLA class II antibody-mediated TRALI it is not known what concentration is applicable and c) the role of the antibody has been thought to be PMN-dependent although PMNs do not “naturally” express HLA class II antigens.<sup>1,35,69</sup> Recently, the current Leukocyte Antibody Prevalence Study (LAPS) determined the prevalence of HLA class I and II antibodies in large cohorts of non-transfused males, transfused males, nulliparous females, and parous females.<sup>130</sup> Separate analyses were generated for both antibodies to HLA class I and class II antigens and stratified by donor group, and the data was log transformed such that positives were



**Fig. 2.** Laboratory investigations into the antibody (Ab) mediated and priming mechanism of TRALI. Patient samples (cellular, serum or plasma) should be as close to the time of the TRALI event as possible to reflect the physiological circumstances of the event. The remains of the transfused blood product provide the most ideal sample for the investigation (primary donor sample), followed by samples from other products manufactured at same collection date from that donor (secondary donor sample) or finally a new sample from the donor (tertiary donor sample). This is because antibody titers and priming/biological response modifiers (BRM) effect can be significantly different between various blood products from the same donor e.g. FFP versus PRBCs, and antibody titers of a donor can vary between blood collected on different dates. Laboratory investigation for Ab mediated TRALI begins with screening for leukocyte antibodies in associated donations and the recipient. Specificities of donor antibodies need to be determined as typing of recipient cells will confirm if there is a cognate Ag on the recipient the donor Ab to interact with. A PMN cross-match (GIFT and GAT) provides *in vitro* evidence that the donor Ab does react with the recipient's cells and reveals they type of interaction – agglutination or binding. Primary donor samples should be investigated for their PMN priming activity, especially if leukocyte Abs are not detected.

defined as three standard deviations above the mean. Samples were considered antibody positive with normalized background ratios of >10.8 for HLA class I antibodies and >6.9 for HLA class II antibodies. Antibody positivity for transfused males (1.7%) was similar to that of non-transfused males (1.0%) and nulliparous, non-transfused females (1.7%).<sup>130</sup> Such criteria of a less sensitive cut-off designed to detect passively transfused antibodies rather than an amamnestic response has never been implemented with respect to the prior data which implicated antibodies to HLA class II antigens in TRALI that employed a 8–10% shifts by flow as positive although these studies did not employ the Luminex Bead technology.<sup>17,25,74</sup> These very small flow shifts may be of import for successful organ transplantation but their relevance is not defined in TRALI. Effective HLA antibody screening methods for blood donors from various testing sites reveal challenges in differentiating background noise and defining suitable cut offs such that if these new normal range of cutoffs are employed, some antibodies implicated in TRALI never reach a positive titer.<sup>130–133</sup> Furthermore, the clinical effects of transfused HLA antibodies may be attenuated due to absorption by lymphocytes or platelets, and neutralization by soluble HLA class I molecules which comprise the majority of HLA class I antigens in the blood.<sup>134</sup> One notable exception is HLA-A2 which has been implicated in many TRALI cases and thus should be included in any first line TRALI investigation screen.<sup>1,135–137</sup>

It is important to determine if the cognate antigen is present in the transfused host, and currently there is great variability in how TRALI cross-matches are performed. Techniques employed include the lymphocytotoxicity test (LCT),<sup>1</sup> GIFT alone or GIFT and GAT combination.<sup>123</sup> The LCT is based on lymphocytes which are stable and storable making them investigator friendly, however lymphocytes are not the primary target cell in TRALI and do not express PMN-specific antigens (HNA-1 and 2) and thus will not detect any incompatibilities with these antigens. Therefore, reports using the LCT cross-match alone need to be interpreted with these potential limitations in mind. Importantly, while confirmation of the presence of a cognate antigen implies that the donor antibody has a target or substrate to react with, it does not confirm that the antibody contributed etiologically to the TRALI reaction, for in three look-back reports the minority of confirmed antigen:antibody pairs developed TRALI.<sup>74,138,139</sup>

In the majority of cases, antibodies implicated in TRALI are donor-derived. Consequently, an appropriate cross-match strategy involves only the testing of the associated donor serum/plasma with the recipient's effector cells (PMNs). In the rare case where the antibody is present in the TRALI-affected transfusion recipient, the cross-match should, in addition, test recipient serum/plasma with the associated donor's PMNs, especially for TRALI linked to granulocyte transfusions.<sup>137</sup> In a PMN cross-match, serum or plasma from associated donors should be incubated with the recipient's PMNs using both the GIFT and GAT techniques.<sup>123,137,140</sup> The PMN cross-match is very valuable because positive cross-matches or incompatibilities provide *in vitro* evidence of a reaction between recipient PMNs and associated donor serum/plasma antibodies. This is especially useful in cases where antibody specificities cannot be defined, a common phenomenon in PMN serology, because of the very small number of well defined and characterized HNAs. In determining if a donor antibody did cause TRALI a number of important points need to be considered and should be the subject of ongoing research including: (1) the method of detection, is the antibody an agglutinating (GAT) or a binding antibody (GIFT), (2) the antibody titer, (3) epitope specificity, and (4) antibody isotype, (5) its ability to prime PMNs that express the cognate antigen. Such detailed antibody information may shed more light on the role of allo-antibodies in the TRALI mechanism.

## Investigation of BRMs implicated in TRALI

These PMN priming assays are only performed in a few laboratories world-wide including the Research Department at Bonfils Blood Center, Denver, CO and by the Australian Red Cross Blood Service, Brisbane, Australia. Such assays have their inherent quirks and require trained personnel, but the assays themselves are not difficult. These and comparable laboratories usually will test samples as part of their research endeavors. When priming activity is found then further identification is required including lipid identification or measurement of chemokines or sCD40L by commercial ELISA.

## TRALI avoidance

### *Manipulation of blood products*

Washing of cellular blood products removes all of the implicated mediators in TRALI, which are present in the plasma fraction; however, washing is expensive, time consuming, and the time constraints in critically ill patients may not allow for the time to remove the plasma from stored cellular components. Decreases in the plasma in cellular blood products has been employed with some success; however, in a number of TRALI cases only small amounts of plasma are required to elicit TRALI.<sup>141,142</sup> Although pre-storage leukoreduction does decrease a number of leukocyte and platelet-derived mediators, it was not effective in inhibiting BRM-mediated TRALI *in vivo*.<sup>69</sup> In Norway no TRALI cases have been reported since the use of solvent-detergent plasma (SDP), a product manufactured from enormous pools of plasma in which leukocyte antibodies are thought to be diluted and neutralized during processing, and cellular fragments are removed during filtration.<sup>143</sup>

### *Donor selection*

The use of male-predominant plasma in Great Britain has significantly decreased the number of the total cases of TRALI particularly the cases of fatal TRALI.<sup>19</sup> Although there may be selection bias in these studies for antibody-mediated TRALI as nicely demonstrated by Dr. Hume, this intervention appears to be effective.<sup>19,144</sup> Not all antibody-mediated TRALI is caused by female donors as emphasized by the look-back study of reported TRALI cases by Middleburg et al. that demonstrated that 48% of the implicated donors who gave antibody positive units were male.<sup>145</sup> Rapid methods of screening for donor HLA and HNA antibodies appears pertinent, especially for apheresis donors; however, the cost and time required is not insignificant and many of the assays employed have a level of sensitivity related to organ transplantation and have not been validated for Transfusion Medicine. In addition, HNA antibody testing presents a major hurdle as there is currently no mass screening technology available.

## Management of implicated donors

Look-back studies have confirmed that donors of implicated products do cause TRALI in other recipients.<sup>18,131</sup> As discussed previously, screening implicated donors for HLA class I, class II and HNA antibodies, followed by confirmation of the cognate antigen in the recipient or incompatible PMN crossmatches between donor and recipient identifies TRALI-associated donors. Blood centers need to have effective systems to identify implicated donors so that future donations do not cause TRALI. If the antibodies recognize common leukocyte antigens the donors may be excluded or their products used for plasma poor products.

## Conclusions

TRALI is the leading cause of transfusion-related morbidity and mortality.<sup>102</sup> Although much of this review has focused on immunocompetent hosts, TRALI has been reported in neutropenic patients and the etiology is thought to involve the transfusion of permeability agents, such as vascular endothelial growth factor, VEGF, or antibody-mediated activation and fenestration of the pulmonary endothelium both resulting in mild lung leak.<sup>146,147</sup> Proper evaluation of antibodies and BRMs implicated in TRALI using *in vivo* and *in vitro* models may lead to novel clinical strategies to preclude patient exposures or methods to identify TRALI risk factors and enable their removal to make transfusions safer and to not needlessly disqualify blood donors. Other than the presence of antibodies, little convincing evidence supporting the danger of female plasma has been presented. Recently, Palfi et al. demonstrated a statistical decrease in blood oxygenation in patients following transfusion of female plasma versus the transfusion of male plasma, but this “decrease” was not out of the normal range for human subjects.<sup>148</sup> Gajic et al. prospectively investigated the transfusion of blood components from male donors versus blood transfusion with one or more female donors to patients in the intensive care unit and found a statistical decrease in oxygenation in the female containing transfusion group. Interestingly, there was more ALI in the male-only transfusion group with none being diagnosed with TRALI.<sup>149</sup> Other investigators have refuted the inherent danger of female plasma; however more work is required to determine if male-predominant plasma transfusion is clinically warranted.<sup>150,151</sup> In addition, if the new transfusion guidelines for military trauma, which included a 1:1 ratio of FFP units to PRBC units for optimal survival, are implemented in civilian trauma, there will not be nearly enough male-only plasma to transfuse injured patients.<sup>152</sup> Although the use of male-only plasma transfusion may decrease a handful of fatalities, this practice may be disastrous to locales in which multiparous women comprise a large proportion of the donor pool, such as in the Western United States,<sup>153,154</sup> and newer methods to remove antibodies and other BRMs from the plasma/plasma fraction of blood components are required.

## Practice points

TRALI is a complex clinical syndrome that appears to require at least two clinical events for its development. TRALI is a clinical diagnosis and should be made on clinical grounds although laboratory test may be supportive. Antibody testing via flow cytometry may be too sensitive and requires modification for its use in Transfusion Medicine. Male-predominant plasma transfusion appears effective to reduce TRALI and further work is needed to include all cases and not just those with donor antibodies.

## Research agenda

TRALI appears to affect the critically ill and further work is needed to determine appropriate transfusion strategies in this patient population. Further work is need with regard to the role of antibodies to HLA class II antigens in TRALI for the pathophysiology is unknown. The at-risk populations for TRALI remain undefined.

## Conflict of Interest Statement

The authors do not have any relevant conflict of interest pertaining to the material presented in this review.

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