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Experimental Models of Transfusion-Related Acute Lung Injury (TRALI)

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Abstract

Transfusion-related acute lung injury (TRALI) is defined clinically as acute lung injury occurring within six hours of the transfusion of any blood product. It is the leading cause of transfusion-related death in the United States, but under-recognition and diagnostic uncertainty have limited clinical research to smaller case control studies. In this review we will discuss the contribution of experimental models to the understanding of TRALI pathophysiology and potential therapeutic approaches. Experimental models suggest that TRALI occurs when a host, with a primed immune system, is exposed to an activating agent such as anti-leukocyte antibody or a biologic response modifier such as lysophosphatidylcholines. Recent work has suggested a critical role for platelets in antibody-based experimental models and identified potential therapeutic strategies for TRALI.

Cases of non-cardiogenic pulmonary edema due to blood transfusion were described in the literature as early as the 1950s (1–3), but the term transfusion-related acute lung injury (TRALI) was not used until 1983 when Popovsky and Moore described five patients who developed acute lung injury (ALI) following transfusion of blood containing donor-derived leukoagglutinating antibodies (4). In this seminal article, the authors reported a TRALI incidence of 1:3130 per patient transfused and suggested that donors who are anti-leukocyte antibody positive increase the risk of TRALI.

The release of additional reports and the implementation of hemovigilance programs have significantly raised the awareness of TRALI, and TRALI was reported by the FDA as the most common cause of transfusion-related death in the United States during 2005–2009 (5). TRALI has now been formally defined by the Canadian Consensus Conference (6) and a NHBLI expert panel (7) as acute lung injury that develops during or within six hours of the transfusion of any blood product.

Despite relative consensus on the definition of TRALI, the diagnostic ambiguity, rapid progression, and the relatively rare nature of TRALI have made it difficult to study from a clinical and epidemiologic perspective. As the most accessible means of studying TRALI, animal models have significantly advanced our understanding of TRALI pathogenesis and defined those characteristics that separate TRALI from other types of acute lung injury.

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Several theories on the pathophysiology of TRALI have been proposed based on the experimental models and these contributions will be reviewed. In general, we will discuss the rationale for and contributions of animal modeling to our understanding of TRALI and will highlight opportunities for future work and the translation of experimental findings to preventative or therapeutic applications.

EARLY MODELING OF TRALI

The foundations of modern theories about TRALI were established by experimental work done by Geelhoed and Bennett in the 1970's. These investigators used baboons and dogs to investigate the relationship between blood storage and ALI among victims of serious traumatic injury, referred to as "shock lung" (8,9). Autologous blood was harvested from animals and stored for either 24 hours or 21 days, and then perfused into the left lower lobe of the lung *in situ* by cannulating the left pulmonary artery and vein. Perfusion with blood stored for 21 days resulted in increased pulmonary vascular resistance, increased lung wet-to-dry weight ratio, increased end inspiratory bronchial pressure, and decreased arteriovenous pO₂ difference when compared to blood stored for 24 hours.

These studies also demonstrated that the filtration pressure required to force stored baboon blood through a 20-micrometer pore screen was elevated compared to fresh blood. Pulmonary vein sampling of stored blood after one passage through the lungs demonstrated normalized filtration pressure, thus they concluded that the lung filtered out an occluding agent from the stored blood (8). The authors hypothesized that leukocyte-platelet aggregates, which had been measured at up to 200 micrometers in size and shown to form in stored blood after 2–10 days (10,11), might be the responsible occluding agent. When they filtered stored blood through Dacron-wool, they discovered that filtration reduced the effects of storage on vascular resistance, wet-to-dry ratio, compliance, and arteriovenous gradient, providing evidence that microaggregates play a role in ALI. However, the combination of plasma stored 21 days with cells stored 24 hours resulted in mild pulmonary injury, and it was concluded that there is a mutual contribution of both microaggregates and an unknown humoral factor. This work built the foundation for current work on the role of storage-related biologic response modifiers, leukocytes, and platelets in TRALI. It also provided evidence that TRALI may occur in the absence of donor derived anti-leukocyte antibody.

TWO-HIT HYPOTHESIS

The earliest descriptions of "shock lung" implied that severe traumatic injury was the greatest risk factor for lung injury in these patients. By contrast, the term TRALI implies that the primary determinant of post-transfusion ALI is the transfusion itself. Here we will review epidemiologic data and the animal models that have consistently reinforced the requirement for two events to produce post-transfusion ALI. This multi-event model of TRALI involves both immune priming and the introduction of a TRALI-inducing agent, such as anti-leukocyte antibody or a storage-derived biologic response modifier. Indeed, most research done during the past 20 years has focused on understanding the second event. Lysophosphatidylcholine (lyso-PC) levels, MHC Class I/II antibodies, and granulocyte antibodies in donor units have each been identified as risk factors for TRALI (12–14). However, elucidating the contribution of priming in TRALI by identifying at-risk populations and developing preventative strategies may have the greatest clinical impact.

ROLE OF PRIMING OR THE "FIRST HIT"

Priming can be defined as the induction of a state of hyper-reactivity to subsequent activating agents, and may be viewed on a continuum with activation and injury. Indeed many biologic agents that prime immune cells can also lead to activation at higher doses.

Epidemiologic data first highlighted the requirement for immune priming in TRALI. A retrospective case-control study including ten TRALI patients demonstrated an underlying clinical morbidity such as sepsis, cancer, recent surgery, cytokine administration, or massive transfusion in 10/10 TRALI patients. By contrast, only 2/10 patients in the control group had identifiable morbidities (14). A small case-control study by Sanchez *et al* identified spine surgery as another potential recipient risk factor for TRALI (15). Larger case-control series by Silliman *et al* (16) and Gajic *et al* (12), including 46 and 74 patients respectively, identified hematologic malignancy during the induction phase of chemotherapy, cardiac disease requiring surgery, sepsis, and chronic alcohol abuse as risk factors for TRALI. The results of a TRALI case-control study conducted by investigators at the University of California, San Francisco and the Mayo Clinic are expected soon and will have the potential to contribute a greater understanding of recipient and donor risk factors.

Animal models have validated hypotheses about priming that were generated based on the epidemiologic data. Silliman and colleagues have demonstrated induction of ALI *ex vivo* in perfused and ventilated rat lungs using a variety of experimental agents, including purified lyso-PCs, the plasma fraction of older, stored platelets or packed red blood cells (PRBCs), and MHC Class I antibody (17–19). Importantly, in each case, the observed effects required the pretreatment of the rats with intraperitoneal lipopolysaccharide (LPS). Further evidence for the role of priming comes from Sachs *et al* (20). These investigators added fMLP (formyl-Met-Leu-Phe), a neutrophil priming and activating agent, during *ex vivo* perfusion of rat lungs along with anti-HNA-2a antibody and neutrophils with high- or low-expressing cognate antigen. fMLP accelerated lung injury with the high-expressing cognate antigen neutrophils and also unmasked injury not previously observed with low-expressing cognate antigen cells.

In 2006, we reported the first mouse model of TRALI based on MHC Class I antibody challenge in BALB/c mice (21). This model produced robust lung injury in the absence of an overt first event or priming step. However, we were subsequently surprised when we changed the housing conditions of our mice from non-barrier to barrier, specific-pathogen free rooms and the lung injury in our model significantly decreased (Figure 1) (22). Lung injury was restored when the barrier mice were pre-treated with low-dose intraperitoneal (i.p.) or intratracheal (i.t.) LPS prior to antibody challenge. Interestingly, the barrier mice had significantly lower neutrophil counts in the peripheral blood compared with the non-barrier animals (Figure 1) (22). Priming with LPS increased circulating neutrophil counts and increased sequestration of neutrophils in lung microcirculation. From these experiments, we concluded that environmental conditions could significantly influence TRALI susceptibility through modulation of the neutrophil response in the peripheral blood and the lung microcirculation.

In vitro models have also shed light on the mechanism of priming and its relationship to lung injury. In response to inflammatory cytokines, neutrophils and the pulmonary endothelium undergo adaptive changes that result in neutrophil sequestration in the lung microvasculature. Under normal physiologic conditions, neutrophils deform and elongate to fit through the many, narrow lung capillary segments (23,24). However, after inflammatory challenge with fMLP (23), IL-8 (24), activated plasma (25) or LPS (21), neutrophils sequester in the lungs through both mechanical and adhesive mechanisms. Re-organization of cellular actin results in stiffening of the cell membrane and the neutrophils become lodged in the pulmonary microvasculature (23). However, cytoskeletal reorganization does not result in permanent sequestration without subsequent changes in endothelial and neutrophil adhesion molecules (24–27).

Augmented neutrophil and endothelial expression of adhesion molecules may be required for prolonged neutrophil recruitment to the lungs. Blocking neutrophil L-selectin or CD11/CD18 (Mac-1) does not alter the rate of sequestration, but does alter retention time (23,26–27). Endothelial cells increase expression of Inter-Cellular Adhesion Molecule 1 (ICAM-1) in response to LPS treatment or thromboxane (27,28), and adding monoclonal antibody against CD18 or ICAM-1 to human microvascular endothelial cell (HMVEC) and neutrophil co-cultures blocks neutrophil adhesion and lyso-PC stimulated damage of HMVECs (27). E-selectin is not constitutively expressed on the pulmonary endothelium, but it can be induced on the pulmonary endothelium, along with E-selectin ligand-1 (ESL-1) on neutrophils, by a number of inflammatory stimuli including TNF- α , IL-1, G-CSF, and IL-17 (29–31), and may mediate neutrophil secondary capture of additional cellular elements to sites of inflammation (32).

In addition to sequestering neutrophils in the lung microvasculature, priming agents may also augment subsequent neutrophil respiratory burst (27,33). Wyman *et al* showed that pretreatment with LPS potentiated subsequent lyso-PC- or fMLP-induced superoxide and elastase production and augmented neutrophil mediated HMVEC injury *in vitro* (27). This same group also demonstrated that pretreatment of HMVECs with LPS and subsequent co-culture with anti-HNA-3a antibody and cognate antigen positive neutrophils potentiates HMVEC cellular injury (27). Thus, pretreatment with LPS may augment neutrophil-mediated killing through both neutrophil-dependent and endothelium-dependent mechanisms.

Immune priming may affect cellular recruitment through stiffening of neutrophils and increased expression of surface adhesion molecules. It may also affect the magnitude of injury through modulation of neutrophil effector functions, increased expression of cognate antigens, or recruitment of additional cellular elements necessary for injury amplification. Of note, in our MHC Class I antibody model of TRALI, increased expression of cognate antigen with LPS priming was not observed in lung endothelial cells or neutrophils (22). However, increased cognate antigen expression is still a plausible mechanism of priming in TRALI cases with MHC Class II or HNA antibody. Additional experiments should define the full range of factors that are capable of priming animals for TRALI, including modeling of trauma or surgical intervention and further exploration of the role of bacterial infection on leukocyte-endothelial interactions.

ACTIVATING AGENTS OR THE “SECOND HIT”

Biologic Response Modifier Hypothesis

The term biologic response modifier is a general term used to describe any TRALI-inducing agent, but it has been applied most frequently when discussing lipids derived from stored, cellular blood components. In the early 1990s, Silliman and colleagues demonstrated in a series of experiments that the lipid fraction of stored, cellular blood components can prime neutrophils for increased respiratory burst upon subsequent stimulation with fMLP (34,35). Isolation and characterization of the lipids by mass spectrometry determined that they were lyso-PCs, and that certain subspecies of lyso-PCs accumulate rapidly in stored components (36). Even commercially purchased lyso-PCs can prime neutrophils similar to lipids isolated from stored, cellular blood products (36). The ability of lyso-PCs to prime neutrophil respiratory burst could be consistently blocked with an antagonist to the platelet activating factor receptor.

Silliman and colleagues next published a retrospective study demonstrating increased potentiation of neutrophil respiratory burst by lipids extracted from post-transfusion plasma of TRALI patients (14). This activity was not present in the pre-transfusion plasma of

TRALI patients or the pre- or post-transfusion plasma of control patients who experienced febrile or urticarial reactions. Presumably, the activating agent came from either the patient's response to the transfusion or the transfused blood, and they concluded that it was likely conferred by transfusion of lipids generated during blood storage.

Using an *ex vivo* rat model of lyso-PC-induced TRALI, it was demonstrated that perfusion of rat lungs with plasma or lipid extracts from human packed RBCs (PRBC) or platelet units on the day of expiration resulted in increased pulmonary artery pressures, lung edema, and histologic evidence of lung injury and the findings were replicated by perfusion of purified lyso-PCs (17,18). Of note, these *ex vivo* experiments required pretreatment of the animal with LPS prior to explantation of the lungs. The LPS administration resulted in neutrophil sequestration in the lung microvasculature, which was demonstrated on histologic sections.

In 2006, Khan and colleagues validated another potential biologic response modifier, CD40 ligand (CD40L), which is a platelet derived pro-inflammatory mediator that may be cell-associated or exist as a soluble protein (37). CD40, the receptor for CD40L, is a transmembrane glycoprotein member of the TNF- α family that is expressed on endothelium, monocytes, and macrophages. Observational studies have suggested that patients experiencing a transfusion reaction after platelet transfusion are more likely to have received units with higher soluble CD40L levels (38). Additionally, blocking the CD40L-CD40 interaction has been shown to decrease injury in LPS- and hyperoxia-induced models of ALI in mice (39). Khan and colleagues reported that soluble CD40L levels were increased in older platelet units and non-leukoreduced PRBCs (which contained 100-fold more platelets than leukoreduced PRBCs in their study), and others have confirmed that the rate of CD40L accumulation is directly related to the platelet content (40). Units implicated in TRALI had two- to four-fold more CD40L than control units, but increased peripheral blood CD40L could not be demonstrated in the post-transfusion blood of TRALI patients. These investigators also demonstrated that CD40 (the receptor) was located on the neutrophil membrane, that ligation of CD40 could prime neutrophil oxidative burst *in vitro*, and that addition of CD40L to co-culture of LPS-primed HMVECs and neutrophils augments injury to the endothelial cells.

Using experimental models Silliman and colleagues have constructed a convincing case that lyso-PCs and CD40L can stimulate primed neutrophils to damage the pulmonary endothelium. The clinical impact of this phenomenon is not yet clear, but the relatively high TRALI risk associated with FFP administration (5,12,41) indicates that at a portion of TRALI may not be mediated by these modifiers that accumulate during storage of cellular blood components.

Antibody Hypothesis

In their original case series Popovsky and Moore reported 5/5 donors positive by a leukoagglutination assay and they characterized MHC antibodies in 4/5 donors (1). Since this initial report, the theory that a significant percentage of TRALI cases results from the transfusion of donor-derived cognate antibodies has been supported by several observations. Units implicated in TRALI reactions have a higher incidence of anti-leukocyte antibodies than control units. The prevalence of MHC antibodies has been reported to be as low as 1.0% in males (42), 1.7% in nulliparous females (43), greater than 25% in multiparous donors (42,43), and as high as 65% in donor units implicated in TRALI (41). It is clear that alloimmunized donors are a risk factor for TRALI (44,45) and FFP and platelets, plasma-rich components that may contain donor-derived antibodies, are associated with an increased risk of TRALI (12). Based on the hypothesis that anti-leukocyte antibodies are responsible for TRALI reactions, several groups have based animal models of TRALI on transfusion of antibodies against neutrophil and MHC antigens.

The first clinical case involving acute lung injury after transfusion of an anti-neutrophil antibody was reported by Yomtovian *et al* in 1984 (46). Additional reports followed and in 1990 Seeger *et al* published the first TRALI animal model when they ventilated and then perfused rabbit lungs *ex vivo* with anti-HNA-3a antibody (previously known as “5b”) and human neutrophils expressing cognate antigen in complement active rabbit plasma (47). They noted a lasting increase in vascular permeability and lung weight after six hours of perfusion. Heat-inactivation of complement, omission of antibody or neutrophils, or substitution of HNA-3a negative neutrophils abrogated these effects. These experiments were conducted prior to the broad appreciation that a first-hit may be required for TRALI reactions to occur, and the effects of lung and neutrophil isolation and *ex vivo* perfusion may have been significant, but were not quantified. However, many consider that the HNA antibodies, and perhaps HNA-3a in particular, may be capable of severe TRALI reactions that do not require priming (45,48). The molecular target for HNA-3a antibody has recently been discovered by two groups as a single nucleotide polymorphism in the choline transporter-like protein-2 (CTL-2) (48,49). HNA-3a antibody was able to agglutinate and activate HNA-3a positive neutrophils, but it is still not known how antibody binding to CTL-2 can mediate neutrophil activation (48).

More recently, Sachs *et al* (20) ventilated and perfused rat lungs *ex vivo* with monoclonal anti-HNA-2a and HNA-2a high-expressing human neutrophils. There was a dramatic increase in the filtration coefficient and lung weight, which were accelerated by addition of fMLP. The HNA-2a antibody used in this study was also capable of direct neutrophil activation using a flow cytometric assay of reactive oxygen species (ROS) generation. Complement was not used and the authors concluded that their model involves complement-independent direct antibody-induced neutrophil activation. Interpretation of these experiments is complicated by the potential for cross-species reactivity or immune priming of lungs or neutrophils. These data indicate that direct antibody-mediated activation of cognate antigen expressing neutrophils may be largely responsible for lung injury due to anti-HNA antibodies but provide contradictory evidence regarding the role of complement.

Popovsky's original report demonstrated a match of donor MHC Class I/II antibody to recipient cognate antigen in 3/5 donor units implicated in TRALI (1), and MHC antibodies have since been repeatedly implicated in TRALI clinical cases. We reported an *in vivo*, single species model of TRALI (21), wherein transfusion of a monoclonal antibody against MHC Class I into mice carrying cognate antigen results in increased extravascular lung water, increased lung vascular permeability to albumin, and a 50% mortality within two hours. We observed simultaneous peripheral blood neutropenia and accumulation of neutrophils in the lung microvasculature and demonstrated that injury was blocked if neutrophils were depleted with anti-neutrophil antibody. Fc γ receptor deficient mice were protected from TRALI and adoptive transfer of wild-type neutrophils into the Fc γ receptor deficient animals restored lung injury. We concluded that neutrophil engagement of MHC Class I antibody via the Fc γ receptor was critical to the development of lung injury. Transfer of MHC Class I deficient neutrophils into the Fc γ receptor deficient mice also restored injury, indicating that intact neutrophil MHC Class I antigen was not required. We did not observe any direct neutrophil activation by MHC Class I antibody binding, nor did we find a role for activated complement.

Our data indicates that the pathophysiology of anti-MHC Class I-induced ALI may not be identical to that of anti-HNA. The effect of anti-MHC Class I in our model is likely via binding to the lung endothelium and exposing the Fc portion of the antibody to the circulating neutrophils and their Fc γ receptors. This conclusion is supported by an elegant case-report of TRALI in which a lung transplant patient developed unilateral acute lung injury after transfusion of an MHC Class I antibody that could only recognize antigen in the

transplanted lung (50). Several valuable critiques have been made of this work. The dose of antibody administered may be higher than that found in blood components and the rate of antibody administration is greater than in most transfusions in clinical practice. Future studies may investigate the mechanisms through which neutrophils injure the lung endothelium and we will discuss later the interactions between neutrophils and platelets.

Antibodies against MHC Class II antigens have been implicated in a significant percentage of TRALI reactions (12,51). Thus far, no animal model of anti-MHC Class II has been reported, but several hypotheses have been suggested regarding the pathophysiology of MHC Class II-induced TRALI. One theory is that direct antibody binding to monocytes promotes cytokine release and subsequent sequestration and activation of neutrophils in the lung microvasculature. Several *in vitro* studies utilizing samples from clinical TRALI cases have demonstrated that incubation of monocytes with TRALI-implicated serum results in release of TNF- α , IL-1, tissue factor, IL-8 and GRO- α if the serum contains antibody against cognate antigen expressed on the monocyte (52,53). Co-culture experiments of HMVECs with monocytes and anti-MHC Class II demonstrate release of leukotriene B₄ only if the monocytes contain the cognate antigen (54). One critique of this theory is the potential inconsistency with the timing of observed TRALI reactions, which frequently are noted within 1–2 hours after transfusion. In contrast, cytokine release from anti-MHC Class II stimulated monocytes may take 4–20 hours.

An alternate theory states that MHC Class II antigen may be expressed on neutrophils or endothelium. It has been well documented that MHC Class II may be induced on neutrophils *in vitro* and *in vivo* by a variety of agents including GM-CSF or IFN- γ (55). Furthermore, this observation is physiologically feasible, as demonstrated by the increased expression of intracellular MHC Class II in synovial fluid neutrophils of rheumatoid arthritis patients (56), which can be translocated to the cell surface upon activation by LPS, phorbol myristate acetate, fMLP, or CD11b/CD18 (Mac-1) cross-linking (57). Therefore, it is plausible that underlying inflammatory processes may induce sufficient MHC Class II expression to allow direct activation of neutrophils sequestered in the lung microvasculature. However, to date there have been no reports of MHC Class II expression on the neutrophils of a TRALI patient, either from post-mortem lung tissue or in peripheral blood. Human pulmonary endothelial cells do constitutively express MHC Class II protein and therefore do represent a plausible site of antibody binding and direct injury (58).

The preponderance of evidence has implicated antibodies as a TRALI-inducing agent in transfused blood. However, transfusion of antibody into a patient with cognate antigen does not always produce clinical TRALI (13,59). Priming dose, type of antibody, antibody dose, the presence of additional biologic response modifiers, and the presence or absence of protective patient characteristics may all influence the clinical outcome. Animal models have confirmed that anti-MHC and HNA antibodies may each induce ALI through distinct mechanisms.

ROLE OF PLATELETS IN ALI

The hypothesis that platelets may be influential in the pathophysiology of acute lung injury dates back to the 1960's (10,11) when Swank hypothesized that leukocyte-platelet aggregates in stored blood create a structural occlusion of the pulmonary microvasculature. Recently, the function of platelets as dynamic mediators of inflammatory responses has been better appreciated (60–61).

In 1999, Nieswandt *et al* demonstrated a potential role for platelets in TRALI by reporting the effects of a monoclonal antibody against platelet glycoprotein IIb/IIIa (62). Upon intravascular bolus with this antibody, wild type mice develop hypothermia,

thrombocytopenia, and acute lung injury. Prior platelet depletion blocked antibody induced hypothermia, while neutrophil depletion partially blocked it. Introduction of an Fcγ receptor-blocking antibody completely blocked hypothermia, and Fcγ RIII deficient mice did not become hypothermic after antibody bolus. Their findings are consistent with a reaction resulting from Fcγ RIII-mediated interaction of antibody-bound platelets and another cell type, most likely neutrophils. Since anti-MHC Class I-induced lung injury also requires intact Fcγ receptors, it is reasonable to propose that the pathophysiology of the two models may be similar.

In a recently published follow-up to our initial report of a mouse model of TRALI, we demonstrated that platelets were critical for development of injury following injection of anti-MHC Class I (22). We observed peripheral blood thrombocytopenia and sequestration of platelets in the lungs following antibody administration, and depletion of platelets prior to antibody administration prevented ALI. We also demonstrated that prior depletion of neutrophils prevented platelet sequestration in the lungs, implying that neutrophils are required for platelet sequestration. Platelets and neutrophils are known to interact through several potential receptor-ligand combinations, but we were not able to identify that targeting the P selectin or the CD11b/CD18 (Mac-1) pathways were protective. However, pretreatment with aspirin did reduce lung injury, it reduced platelet sequestration in the lungs without affecting neutrophil sequestration, and it reduced plasma thromboxane B₂ levels (Figure 2). This was the first demonstration of a successful intervention to prevent TRALI using a safe, widely available therapy and identifies platelets and platelet activation as attractive therapeutic targets in TRALI.

Additional evidence of neutrophil-mediated recruitment of platelets during TRALI comes from Hidalgo *et al* (32). Using multifocal intravital microscopy, these investigators examined the behavior of neutrophils rolling in cremasteric venules using L-selectin as a marker of the trailing edge of the cells. They observed neutrophil-platelet interactions at the leading edge of the rolling cells, co-localized with a focus of CD11b/CD18 (Mac-1) expression. These interactions were diminished when either endothelial E-selectin deficient mice, neutrophil ESL-1 deficient mice, or mice treated with a Src kinase inhibitor were substituted. Furthermore, mice deficient in CD11b demonstrated reduced heterotypic interactions, implying that E-selectin signaling through ESL-1 and Src kinase on neutrophils regulates CD11b/CD18 (Mac-1) dependent interaction of neutrophils with platelets. Complement C3 deficient mice also demonstrated a partial reduction in neutrophil-erythrocyte interactions. Vascular permeability and ALI were reduced in the anti-MHC Class I-induced model of TRALI when E-selectin or CD11b/CD18 (Mac-1) were blocked with monoclonal antibodies or by administration of a Src kinase inhibitor.

In 2006, Zarbock *et al* studied the role of neutrophil-platelet interactions during acid-induced lung injury. This work demonstrates commonalities and key differences from the anti-MHC Class I TRALI model (28). These investigators demonstrated that platelets are required for neutrophil recruitment during acid-induced lung injury, which is the opposite relationship that we observed with the anti-MHC Class I model. They reported reduced ALI and neutrophil sequestration after administration of anti-P-selectin antibody, while we were unable to block injury in the anti-MHC Class I model. These effects were dependent on bone marrow derived P-selectin (versus endothelial P-selectin) as demonstrated by chimera experiments. Acid lung injury increased the percentage of neutrophil-platelet aggregates as assessed by flow cytometry. Also, it was demonstrated that activation of platelets or neutrophils before co-culturing with human pulmonary microvascular cells increased neutrophil adhesion, but that adhesion was reduced by treatment with a thromboxane receptor blocker. Furthermore, blocking thromboxane receptors *in vivo* reduced ALI and intra-alveolar neutrophil accumulation following acid lung injury.

Platelets play a crucial role in acid-induced and anti-MHC Class I-induced ALI, likely through an interaction with neutrophils. However, platelets do not seem essential in all models of ALI, especially the neutrophil-independent models such as acute hyperoxia (22). The nature of neutrophil-platelet interactions seems to be specific to the experimental ALI model. Further work is needed to define the nature of this cooperative recruitment in each model, and to define the mechanism of injury and inflammation. Targeting neutrophil-platelet signaling may be a successful therapeutic strategy in several types of ALI.

CONCLUSIONS

TRALI is a unique type of acute lung injury that in most cases requires two inflammatory events before injury. The first event may be initiated by a diverse set of priming stimuli, but the common endpoint is neutrophil recruitment and sequestration in the lungs. The second event typically involves activation of neutrophils resulting in endothelial injury, protein leak, and pulmonary edema, although the mechanisms of neutrophil (and potentially platelet) activation may be unique to the model of TRALI (Figure 3). The relative contribution of biologic response modifiers and anti-leukocyte antibodies to clinical TRALI remains unclear, and may be difficult to answer using model systems. However, animal models hold great promise as tools to understand the nature of immune priming and the dynamics of platelet and neutrophil recruitment to the lungs during ALI. Animal models and *in vitro* models should be used to further define the nature of inflammatory signaling and injury amplification in ALI, including the role of complement, and cell-cell signaling between platelets, neutrophils, monocytes, and the endothelium.

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Figure 1. Effect of Immune Priming in anti-MHC Class I-mediated TRALI

BALB/c mice (H2K^d) housed in a barrier, specific pathogen-free room are protected from TRALI and have lower circulating neutrophils versus non-barrier animals. **(A)** Extravascular lung water and extravascular plasma equivalents (EVPE) measured 2h post-injection of MHC I mAb (4.5 mg/kg, i.v.). ** $p < 0.05$ Barrier (“B”) vs. Non-Barrier (“NB”). **(B)** Baseline peripheral white blood cell (WBC) and neutrophil counts in NB and B mice. ** $p < 0.05$ B vs. NB.

Modified from reference ²².

Figure 2. Treatment with Aspirin prevents TRALI

(A–B) Extravascular lung water (A) and survival curves (B) in mice treated with aspirin (ASA, 100 µg/g, i.p.) or vehicle (DMSO, 1 µl/g, i.p.) 30 min prior to LPS (0.1 mg/kg, i.p.) and again 2h prior to MHC I mAb (0.5 mg/kg) or control (PBS). Data were obtained 2h post-injection of MHC I mAb or PBS. ** $p < 0.05$ vs. other groups. (C) Plasma thromboxane B₂ levels in WT mice challenged with LPS (0.1 mg/kg, i.p.), MHC I mAb (0.5 mg/kg), and ASA (100 µg/g). ** $p < 0.05$ vs. all other groups. † $p < 0.05$ vs. all other groups. Modified from Reference ²².

Figure 3. Proposed Mechanisms of TRALI

LPS, surgery, trauma, or sepsis can prime neutrophils and endothelial cells, inducing neutrophil stiffening, increased expression of endothelial cell adhesion molecules, and sequestration of neutrophils in the pulmonary microvasculature. Secondary activation can proceed through indirect neutrophil activation by MHC antibody (1), biologic response modifiers (2), or direct neutrophil activation by HNA antibody (3). With MHC Class I antibody, neutrophils are activated indirectly through Fc γ receptor engagement of endothelial-bound antibody. Neutrophil inside-out signaling can lead to polarized activation of Mac-1 and subsequent platelet capture. With HNA antibody, primed or unprimed neutrophils may be directly activated. Endothelial injury is ultimately produced through neutrophil and platelet activation leading to protein leakage and pulmonary edema.

Table 1

Summary of Animal Models of TRALI

Lead Investigator	Species	Antibody Hypothesis	Storage Hypothesis
Seeger (47)	Isolated, perfused rabbit lungs Human neutrophils	Yes HNA-3a	No
Silliman (17)	Isolated, perfused rat lungs	No	Yes Human RBCs Lyso-PCs
Silliman (18)	Isolated, perfused rat lungs	No	Yes Human platelets Lyso-PCs
Looney (21,22)	Mice, <i>in vivo</i>	Yes MHC I mAb	No
Sachs (20)	Isolated, perfused rat lungs	Yes Mouse anti-human HNA-2a	No
Kelher (19)	Rats, <i>in vivo</i>	Yes MHC I mAb	Yes Human RBCs Lyso-PCs

HNA (human neutrophil antigen); Lyso-PCs (lysophosphatidylcholines); MHC (major histocompatibility complex); mAb (monoclonal antibody)