Transfusion-related acute lung injury in the critically ill: a translational approach

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Link to publication

Citation for published version (APA):
Developing specific therapeutic strategies for transfusion–related acute lung injury. An overview of potentially useful animal models

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Cardiovasc Hematol Agents Med Chem 2007;5:319-26
Abstract

Transfusion–related (TR)- acute lung injury (ALI) is the leading cause of transfusion–related morbidity and mortality. The pathogenesis of TRALI is thought to be a “two hit”–entity: the “first hit” is (any) proinflammatory pulmonary condition (e.g., pneumonia, sepsis or lung contusion) resulting in activation of lung endothelium with sequestration of polymorphonuclear neutrophils – the “second hit” is provided by transfusion of a blood product. Either antibodies against neutrophils are thought to be implicated in the activation of the sequestrated neutrophils, or bioactive lipids (which accumulate during storage of blood products) induce the “second hit”, finally resulting in lung injury. Preventive measures do not prevent all TRALI cases. Also, TRALI is most probably underdiagnosed. In this review, we call for the development of therapeutic approaches for this potentially life–threatening disease. Several interventions which are beneficial in ALI and may also be beneficial in TRALI are discussed. The application of these interventions requires the development of clinically relevant TRALI animal models. We discuss the present TRALI animal models and their shortcomings and propose future animal models, in which clinically relevant “first hits” can be applied, thereby imitating the complex clinical situation.
Introduction

Transfusion–related acute lung injury (TRALI) is a subcategory of acute lung injury/acute respiratory distress syndrome (ALI/ARDS). ALI/ARDS is characterized by respiratory failure as a consequence of an acute inflammatory pulmonary response following a broad spectrum of underlying conditions, such as pneumonia, sepsis or lung contusion. Per definition, the occurrence of ALI within 6 hours of transfusion of a blood product should be considered as TRALI. It has been hypothesized that TRALI is a “two hit” entity, in which a “first hit” (any proinflammatory pulmonary condition) serves as a priming factor for the “second hit” (human neutrophil antibodies or infusion of blood products containing bioactive material). In this review, the incidence and pathogenesis of TRALI will be discussed. In addition, several in vivo animal models of ALI which can and should be used in the development of (specific) therapeutic strategies for TRALI will be presented. Since many pro-inflammatory pulmonary conditions may serve as a “first hit” for TRALI, including mechanical ventilation, we consider it essential to combine the several existing models for ALI with models for TRALI.

Methods – systematic search of the literature

The Medline database was used to identify medical subjects headings (MeSH) to select search terms. In addition to MeSH terms, we also used free text words. Search terms referred to aspects of the condition (“TRALI”, “Transfusion”) as well as models for ALI/ARDS (“Acute Lung Injury”, “Acute Respiratory Distress Syndrome”, “Animal Models”). Relevance of each paper was assessed using the on–line abstracts. In addition, the reference lists of retrieved papers were screened for potentially important papers.

Definition, incidence and symptoms of TRALI

In 1994, the American-European Consensus Conference defined ALI/ARDS. Criteria are (a) acute onset of symptoms, (b) bilateral infiltrates on chest radiography, (c) pulmonary artery wedge pressure ≤ 18 mm Hg or the absence of left ventricular overload, and (d) $\text{PaO}_2/\text{FiO}_2$ ratio ≤ 300 (ALI) or $\text{PaO}_2/\text{FiO}_2$ ratio ≤ 200 (ARDS). Present consensus for TRALI–diagnosis requires the fulfilment of the definition of ALI within 6 hours after transfusion. Furthermore, other risk factors for ALI must be excluded, such as pneumonia, sepsis or lung contusion. The clinical diagnosis of TRALI appears difficult, since distinguishing markers of this disease are lacking. As a
consequence, TRALI is most probably underdiagnosed. This may apply in particular to intubated and mechanically ventilated patients on the Intensive Care unit (ICU), in whom hypoxemia may occur as a result of a number of conditions, such as volume overload or atelectasis. Clear data on the incidence of TRALI are lacking. At present, the incidence is estimated between 1 in 300 to 5,000 plasma containing transfusions. Other reports show an overall incidence of 0.02% to 0.05% per unit of blood transfused or from 0.08% to 0.16% per patient who received any transfusion. These figures may not apply to ICU-patients, as symptoms may be less apparent during mechanical ventilation. Moreover, this population is highly exposed to the risk of acquiring TRALI, as up to 85% of patients admitted to the ICU receive a transfusion during their stay. Indeed, a recent study from the Mayo Clinic showed a significant higher incidence of TRALI in ICU-patients. In this study, 892 patients were prospectively observed, among whom 9% developed ALI within 6 hours after a transfusion (28 TRALI, 51 possible TRALI), resulting in an incidence of 3% per ICU-patient and 0.4% per unit transfused. This relatively high incidence suggests that ICU-patients are possibly more susceptible to TRALI.

In most cases, patients develop the symptoms of TRALI within 1-2 hours after transfusion, but an incubation period of 6, or even 48 hours has also been described. Symptoms include rapid onset of tachypnea/dyspnea due to hypoxia and fever. Hypotension is not a consistent finding. Radiographic examination shows bilateral infiltrates, indistinguishable from pulmonary edema. The clinical symptoms are identical to ALI/ARDS resulting from other causes. Supportive therapy with intubation and mechanical ventilation is necessary in 70% of reported TRALI-cases. Respiratory symptoms disappear within 96 hours. In survivors, lung injury usually is reversible. Mortality from TRALI varies from 5–15%.

**Pathogenesis of TRALI**

Presently, there are two hypotheses in the pathogenesis of TRALI. The first hypothesis suggests that TRALI is caused by donor antibodies against human neutrophil antigens (HNA) or human leukocyte antigens (HLA). The second hypothesis implicates two independent events – the first event is the clinical condition of the patient, in which neutrophils are attracted to the pulmonary compartment. The second event is the transfusion, in which either donor antibodies directed against host neutrophils or bioactive lipids which have accumulated during blood storage, cause activation of the primed and adherent pulmonary neutrophils, leading to endothelial damage, and ultimately TRALI.
The first hypothesis postulates an antibody-mediated reaction. Popovsky and Moore were the first to report on TRALI in which neutrophil–reactive and lymphocyte–reactive antibodies were involved. Thereafter numerous antibodies and antigens implicated in the development of TRALI have been reported. Most of the reported TRALI–cases are associated with donor antibodies reacting with recipient leukocytes. A minority (<10%) of TRALI case reports describe a reaction of antibodies of the recipient reacting with the donor leukocytes, in which case a sufficient amount of leukocytes have to be transfused to develop a full TRALI. Both HLA class I and II are suggested to be involved in the development of TRALI. Activation of polymorphonuclear neutrophils occurs via HLA class I antigens – mononuclear cells expressing HLA class II are involved by activation through HLA class II antibodies. Human neutrophil alloantigens are likely to serve as a target for neutrophil reactive antibodies. An argument for the implication of antibodies against leukocytes lies in the fact that a higher incidence of TRALI has been reported when blood from multiparous women and donors with a transfusion history was used. This donor population has a higher incidence of HLA antibodies, caused by sensitization during pregnancy and/or previous transfusions. However, a number of events are not solely explained by the antibody theory. First, one study found that female parous donors with high HLA–sensitization rates provided 9,000 transfusions of which none resulted in a TRALI–reaction. Secondly, antigen specificity can be demonstrated in only a small part of the analyzed TRALI–cases, even though 25% of the investigated donors had anti-leukocyte antibodies. Thirdly, many TRALI–cases are reported in which no specific anti–leukocyte HLA or HNA antibodies are detected, nor leukocytes expressing antibody-specific antigens. Therefore, taking into account that all included patients were really TRALI–cases, this suggests that the antibody-mediated reaction is not the only mechanism in the pathogenesis of TRALI.

The second hypothesis considers TRALI to be a “two hit” syndrome. In this theory, a pro-inflammatory state of the patient is required as a “first hit”. First, activation of the pulmonary endothelium leads to sequestration of neutrophils. The “second hit” is provided by bioactive lipids (lysophosphatidylcholines, lysoPCs) which accumulate during storage of cellular blood products, such as red blood cells and platelets. This theory was postulated after a study of 10 TRALI patients in which TRALI was linked to the transfusion of lipids with significant neutrophil priming activity. Silliman et al. showed that the concentration of 4 lysoPCs (Lyso-PAF, steatoyl-, palitoyl, oleoyl phosphocholine) increases during storage of red blood cells and platelets. These LysoPCs are likely associated with TRALI as their infusion causes a release of components of the microbicidal arsenal resulting in pulmonary leakage through the alveolar–capillary membrane. Besides lysoPCs, a role may
exist for pro-inflammatory cytokines, (e.g., tumor necrosis factor (TNF), interleukin (IL)-6), chemokines (e.g., IL-8) and/or endotoxins, which may also accumulate during storage of blood products.\textsuperscript{6,38,39}

The above-mentioned 2 etiologies need not to be mutually exclusive. We, and others, propose a continuum Fig. (2), in which one side of the spectrum is the highly specific antibody-antigen matched reaction and the other side of the spectrum is a predisposing “first hit”, after which infusion of antibodies or lysoPCs are required for the development of TRALI.\textsuperscript{16} There may be a gradual course from one side to the other, in which a weak positive antibody–antigen matched transfusion requires a “first hit” to develop into a full clinical TRALI. In support of this view, resting neutrophils express HLA class II antigen at very low levels, whereas activated neutrophils have increased levels of HLA class II antigen expression.\textsuperscript{40,41} Moreover, experimental models of TRALI have shown the requirement of a first hit to induce TRALI.\textsuperscript{8,36,42-44} In addition, it was found that only a fraction of patients who received blood from a donor with an anti-5b antibody (implicated in a former TRALI case) developed TRALI,\textsuperscript{22} suggesting an additive factor. Alternatively, blood which has been stored for up to 6 weeks may contain a high load of bioactive lipids. It can be hypothesized that the predisposing condition needed to develop TRALI, may be

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Pathophysiology of Transfusion Related Acute Lung Injury (TRALI). Polymorphonuclear neutrophils (PMNs) are attracted to the lung by release of cytokines and chemokines released from the upregulated lung endothelium. Lose binding by L-selectin takes place. Firm adhesion is realized by E- and P-selectin and intra cellular adhesion molecules (ICAM-1). These hyperactive PMNs are finally activated by the “second hit” of bio active lipids (Lipids) or HLA/HNA antibodies influx. This results in alveolar capillary damage and leakage of fluid intravascular thorugh the interstitium into the alveolar space, resulting in long edema and finally TRALI.}
\end{figure}
a subtle trigger when older blood products are transfused. In agreement, stored blood products cause lung injury in an animal model of TRALI.\textsuperscript{36,44} Considering the higher incidence of TRALI in mechanically ventilated patients, mechanical ventilation may be a predisposing factor.\textsuperscript{45}

Present insights in the inflammatory processes involved with TRALI

TRALI is clinically and histopathologically identical to ALI.\textsuperscript{2,13,22,36,44,46,47} TRALI is, at least in part, a neutrophil–mediated condition,\textsuperscript{48–51} in which neutrophils are attracted to the pulmonary compartment by the local release of chemokines and cytokines from lung endothelium and the alveolar macrophages Fig. (1). Sequestrated neutrophils bind to the endothelium and migrate to the alveolar space through the interstitium. Finally, activated neutrophils degranulate with the release of oxidants, proteases, leukotrienes and other pro-inflammatory molecules, causing damage to the alveolar epithelium with subsequent leakage of the alveolar capillary membrane and surfactant breakdown. The result is a protein–rich edema containing high concentrations of inflammatory cells.\textsuperscript{52} After the acute inflammatory phase, ALI may pass on to a chronic inflammatory phase represented by extensive lung remodeling, with hyalinization of the alveoli, and fibrin deposition resulting in lung fibrosis.

The fact that TRALI is neutrophil mediated was confirmed by adoptive transfer experiments indicating that the Fc\g receptor on neutrophils is critical in the development of a TRALI reaction.\textsuperscript{42} However, activation of neutrophils expressing HLA class I antigens does not explain all the supposed antigen-antibody mediated

![Threshold model of TRALI](image-url)

**Figure 2.** Threshold model of TRALI edited from Bux et al.\textsuperscript{79} To overcome a threshold to induce a TRALI reaction, several factors play a role: the strength of the neutrophil-priming activity of transfused mediators (light box) and the clinical status of the patient (grey box).
TRALI reactions. Also, considering the cytokine profile found in TRALI, it is conceivable that cellular mediators other than neutrophils are involved. Monocytes which express HLA class II may be involved by activation through HLA class II antibodies. Indeed, monocytes of TRALI patients show enhanced activation, as demonstrated by increased expression of TNF-\(\alpha\) and tissue factor, when incubated with serum from an implicated donor compared to serum that did not cause TRALI. The acute inflammatory lung injury initiating and maintaining ALI of any origin involves the same pro-inflammatory cytokines (IL-6, TNF-\(\alpha\), IL-8) which accumulate during storage of RBCs and platelets. Furthermore, increased levels of IL-8, IL-6 and TNF-\(\alpha\) are found in both syndromes. Although cytokine profiles in TRALI and ALI/ARDS appear to be similar, inflammation in TRALI needs further exploration.

Disturbances in coagulation and fibrinolysis have clearly been demonstrated in patients with ALI/ARDS or pneumonia. Indeed, alveolar fibrin deposition is a hallmark of ALI/ARDS and pneumonia, and is the result of activation of coagulation and inhibition of fibrinolysis. Disturbed fibrin turnover plays a central role in the pathogenesis of pulmonary inflammation, and probably is (at least in part) comparable to intravascular deposition of fibrin in patients with sepsis. In TRALI, knowledge on coagulation and fibrinolysis is limited. Of special interest, however, are protease activated receptors (PARs, transmembrane proteins that are expressed on the surface of leukocytes and endothelial cells). Activation of PARs by thrombin enhances inflammation by increased production of pro-inflammatory cytokines. Another example of activated coagulation in TRALI is shown by an animal model in which TRALI was reduced by infusion of platelet activating factor (PAF)-receptor antagonist, suggesting that this receptor is an important mediator in the development of TRALI. Of interest, PAF is similar in composition to LysoPCs, which accumulate during blood storage and are implicated in TRALI. These LysoPCs are thought to be able to activate neutrophils through the PAF receptor.

In summary, ALI and TRALI both are characterized by an enhanced inflammation which is neutrophil mediated. Knowledge about the precise mechanism of neutrophil activation and of other inflammatory pathways involved in the pathogenesis of TRALI is limited. In ALI, coagulation is activated and fibrinolysis impaired. Considering the similarities between ALI and TRALI, we speculate that activated coagulation and impaired fibrinolysis is also present in TRALI. Furthermore, TRALI and ventilator induced lung injury may be interrelated. It is conceivable that strategies aimed at limiting ventilator induced lung injury in ALI may also apply in TRALI. To further address TRALI pathogenesis and possible therapeutic approaches, animal models provide a valuable tool. The innate immune response can be investigated by depletion of specific cellular mediators using antibodies. Also, transgenic and knock-out
mouse strains (e.g. TNF-α) can be used in \textit{in vivo} TRALI models, which allow to study specific pathways in TRALI pathogenesis.

\textbf{Presently used animal models of TRALI}

Despite limitations, TRALI animal models are helpful in elucidating the pathogenesis of this uncommon transfusion reaction. An overview of TRALI models is given in table 1. Animal models have been developed for TRALI even before the name TRALI was coined in 1985.\textsuperscript{13} Bennet et al.\textsuperscript{56} performed research on “shock lungs”, an ALI-model that was seen after multiple transfusions in the surgical setting. They hypothesized that micro–aggregates were causing ALI. In an \textit{in vivo} lung model in dogs and baboons they compared fresh autologous blood versus whole blood of 21 day old.\textsuperscript{57} After filtering, the 21–days old blood products induced ALI after 2 hours of perfusion in comparison with the fresh blood which did not show any effect. As filtering did not prevent ALI, the theory of micro–aggregates was turned down and ALI was explained by “toxic serum borne factor”.

\textbf{Ex vivo TRALI models}

The first animal models of TRALI were based on the infusion of HLA and HNA antibodies in \textit{ex vivo} lung models of rats and rabbits.\textsuperscript{58,59} Seeger et al used a leuko–agglutinating anti–5b neutrophil antibody extracted from multiparous donor plasma, which was reported to be involved in non–cardiogenic lung edema during transfusion. \textit{Ex vivo} isolated rabbit lungs were perfused with albumin buffer, and human granulocytes were admixed to the circulating perfusate. In the presence of 5b-positive neutrophils and rabbit plasma as a source of complement, severe lung edema occurred after 3–6 hours when anti–5b antibody was introduced. In the absence of one of these three substrates lung edema did not occur. Therefore, the presence of both a specific antibody–antigen match and plasma is required for the development of TRALI. This model however has many limitations. The \textit{ex vivo} conditions hamper extrapolation to the human situation. Also, the need for complement does not mimic the clinical situation. Lastly, human antibodies in an animal may induce unwanted reactivity.

The previous \textit{ex vivo} lung model was modified by Sachs and co-workers, by introducing mouse anti-human IgG HNA-2a-antibody with human HNA-2a neutrophils. Resting neutrophils (expressing less then 30% HNA-2a) did not induce lung injury after perfusing the lungs with anti-HNA2a. However, when co-stimulated with formyl-Met-Leu-Phe (fMLP), these neutrophils were able to induce lung injury. In agreement with the previous model, the presence of the cognate antigen was mandatory for the development of TRALI, However, complement was absent, which was a necessary component in the rabbit model of Seeger. This modified
Table 1. Overview of the Transfusion Related Acute Lung Injury (TRALI) animal models with their experimental and clinical relevance.

<table>
<thead>
<tr>
<th>Author</th>
<th>Experimental design</th>
<th>Species</th>
<th>Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bennet et al. 56,57</td>
<td>In vivo model In an ALI-model fresh autologous blood versus whole blood of 21 day old was compared and the effect of filtering the blood.</td>
<td>Dogs and Baboons</td>
<td>They hypothesized that micro-aggregates were causing ALI and filtering the blood would prevent this. Outdated whole blood caused TRALI compared to fresh blood. Filtering the blood did not effect the results. Although the hypothesis was turned down, this was the first TRALI model.</td>
</tr>
<tr>
<td>Seeger et al. 59</td>
<td>Ex Vivo isolated lung perfused with anti 5b neutrophil antibody (human), human neutrophils, and plasma</td>
<td>Rabbit</td>
<td>Evidence for the requirement of both plasma and an antibody-antigen match to induce TRALI. Ex vivo conditions and preheating of plasma hampers extrapolation to clinical situation. Inter species difference results in aspecific activation. This model is a single ‘hit’ model.</td>
</tr>
<tr>
<td>Sachs et al. 58</td>
<td>Ex Vivo isolated lung perfused with HNA 2a antibody (human) after neutrophil stimulation with fMLP.</td>
<td>Rat</td>
<td>Evidence of ‘two hit’ theory. Ex vivo conditions and fMLP stimulation hamper extrapolation to clinical situation</td>
</tr>
<tr>
<td>Silliman et al. 44</td>
<td>Ex Vivo isolated lung perfused with outdated human blood products after priming with LPS</td>
<td>Rat</td>
<td>Evidence of “two hit” theory. Evidence for a role of lysoPCs in the pathogenesis of TRALI. Use of clinical relevant transfusion material. Ex vivo conditions, preheating of plasma and the use of LPS hampers extrapolation to clinical situation</td>
</tr>
<tr>
<td>Silliman et al. 61</td>
<td>In Vivo transfusion with outdated red blood cells (human)</td>
<td>Rat</td>
<td>Evidence of “two hit” theory. Evidence for a role of lysoPCs in the pathogenesis of TRALI. Use of clinical relevant transfusion material. In vivo conditions resemble clinical situation. The use of LPS and pre heating of plasma hampers extrapolation to clinical situation</td>
</tr>
<tr>
<td>Looney et al. 42</td>
<td>In Vivo transfusion with Anti-H2Kd antibody MHC Class I</td>
<td>Mouse</td>
<td>Evidence for a role of antibodies and the Fcg receptor in the pathogenesis of TRALI. In vivo conditions resemble clinical situation. High mortality hampers extrapolation to clinical situation. This model is a single ‘hit’ model.</td>
</tr>
</tbody>
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Abbreviations: HNA (Human Neutrophil Antibody), fMLP (formyl-Met-Leu-Phe), LysoPC (Lysophosphatidylcholine), LPS (Lipo Poli Sacharide).
ex vivo model was the first evidence of the requirement of a ‘first hit’ to induce TRALI, provided by the neutrophil co-stimulation with fMLP. Also this model has limitations. Again, there are ex vivo conditions, which are not representative of the clinical situation. No time-relation effects can be studied, nor the effect of therapeutic interventions. For this purpose, in vivo models of TRALI mimicking the clinical surroundings are warranted.

In vivo antibody TRALI model

Recently, the first in vivo model of TRALI was published, based on infusion of MHC Class I monoclonal anti-H2Kd antibodies in wild type BALB/c mice expressing the cognate antigen. The model is straightforward: after sampling an equal volume of blood, antibodies were infused in the vena jugularis. Iso-type matched anti-IgG2a,κ antibodies and phosphate buffered saline were used as controls. In this model, peripheral blood neutropenia developed, reflecting neutrophil sequestration. Pulmonary histology and immunochemistry showed edema, inflammation and neutrophil sequestration. Depletion of the neutrophils by isotypic control antibody before starting the experiment, as well as introducing knock out mice for the Fcγ receptors resulted in an absence of lung injury, pointing to Fcγ receptors and neutrophils as essential in antibody mediated TRALI. Unfortunately, 50% mortality occurred 2 hours after administrating the antibodies, making the model difficult to handle. Also, mortality in this model is much higher then the reported mortality in the human situation of 5 - 15%, making comparison difficult. Furthermore, this model provides a solely antibody-mediated TRALI. As previously discussed, antibodies are not involved in a number of TRALI cases, suggesting the need of a priming event. Therefore, the development of TRALI models consisting of ‘two-hits’ is warranted.

“Two hit” TRALI models

Silliman and his group, developed an isolated perfused rat lung model using human peripheral red blood cells, human whole blood and platelets. The “first hit” in their models was achieved by an intraperitoneal injection of lipopolysaccharide (LPS) after which lungs were isolated and mechanically ventilated in an ex vivo experimental setting. After 2 hours, the “second hit” was caused by perfusion of the isolated lungs with plasma from outdated blood products (42 days old red blood cells or 5 days old platelets). The contained plasma was preheated to 56 °C to eliminate complement and fibrogen. Plasma from outdated products resulted in a strong increase in pulmonary artery pressure. Histology showed influx of neutrophils in the lung and pulmonary damage, even in the absence of any neutrophils in the lung perfusate. Also, when instead of outdated blood products,
lungs were perfused with lysoPCs dissolved in 1.25% human albumin solution, this resulted in TRALI, indicating that lysoPCs may be the responsible component in the development of TRALI. Recently, the first \textit{in vivo} TRALI model in rats based on the “two hit” theory was presented. In this model, the “first hit” was similar as in previous models, by intraperitoneal injection of LPS 2 mg/kg. After two hours the “second hit” was induced by infusion in the vena femoralis of 42–day old human red blood cell plasma or 42–day old human leukocyte depleted red blood cells. Also in this model, plasma was preheated to eliminate complement. An equal volume of blood was taken before transfusion of the circulating volume (10%) to prevent an overload edema. Outdated red blood cells, whether leuko-depleted or not, resulted in increased pulmonary leakage in comparison with fresh plasma and controls. This \textit{in vivo} model of TRALI demonstrates the necessary step of priming in the development of lung injury, underscoring the “two hit” model. However, it is not a clinically relevant situation, considering the use of preheated human transfusion material and a first hit by LPS injection.

In conclusion, existing TRALI models are limited by the use of different species, the absence of “two hits” or the use of non-clinical first hits such as LPS.

\textbf{Potentially useful TRALI models combine multiple ‘hits’}

It is conceivable that many other “first hits” for the development of TRALI exist in the clinical situation.

\textit{“First hits” in TRALI}

Risk factors for the development of ALI may also apply to TRALI, such as sepsis, aspiration, pneumonia or major surgery. To test this hypothesis, animal models using clinical risk factors for ALI should be used in TRALI models. Clinically relevant TRALI models may include pneumonia, aspiration or sepsis as a “first hit” and require combining existing animal models with the induction of TRALI. In such models, lung injury induced by aspiration, pneumonia or sepsis can be compared to lung injury caused by the same hits with the addition of the induction of TRALI using either antibodies or bioactive lipids. Such an approach may provide an explanation for the high incidence of possible TRALI found in these patients. Another important priming condition may be mechanical ventilation. As discussed before, ICU patients are at risk for the development of TRALI. In a recent prospective study, a much higher incidence of TRALI was found in ICU patients, suggesting that mechanical ventilation may predispose for the development of TRALI. Furthermore, as mechanical ventilation adds to lung injury, it should be considered as potential
harmful and therefore as a factor for developing ALI and perhaps TRALI. Also, mechanical ventilation itself has been found to induce ALI.\textsuperscript{63,64} Therefore, the role of mechanical ventilation should be addressed in transfusion mediated lung damage by including ventilated induced lung injury (VILI)\textsuperscript{65,66} in combination with present in vivo TRALI models. In such a model, mechanical ventilation provides the “first hit”. The “second hit” may consist of the infusion of antibodies or lysoPCs.

“Second hits” in TRALI

Conceivably, as antibodies and lysoPCs need not be exclusive in TRALI pathogenesis, combinations could be tested. Alternatively, \textit{in vivo} transfusions of red blood cells, fresh frozen plasma or platelets products of the animals own species could be used. In our laboratory, the latter concept is currently developed. A TRALI model using \textit{in vivo} transfusion material would be the first model without cross species difference simulating TRALI in clinical conditions.

Another issue to be assessed in TRALI models is the concept of a continuum in TRALI pathogenesis (Fig. 2), ranging from a strong specific antibody-antigen mediated TRALI to TRALI caused by significant pulmonary priming, in conjunction with a second hit, which may be either a (weak) antibody mediated reaction or lipids from (outdated) blood products. The titer of antibody or the amount of bio active lipids to induce the second hit is not known and should be addressed. Conceivably, threshold dose of either antibody or lipids may differ, depending on the priming status of the host. Infusion of a weak second hit may be sufficient to cause TRALI in the presence of a strong first hit of any origin. The concept of a continuum may clarify the fact that antibodies from the same donor cause TRALI in some patients but not in others.

\textbf{Therapy for TRALI}

Specific treatment for TRALI does not exist. Treatment consists of supportive care, which may require mechanical ventilation, or oxygen supplementation. At present, prevention of TRALI seems the best approach to reduce the incidence, such as the application of restrictive transfusion guidelines and the exclusion of women and persons who have previously received a blood transfusion from donorship,\textsuperscript{67-71} as well as donors who have been implicated in a TRALI case. However, these measures are insufficient in preventing TRALI, emphasizing the importance of the development of TRALI research.
Potential therapies to be tested in the clinically relevant ‘two hit’ TRALI–models

As discussed above, patients exposed to multiple “hits” resulting in priming of pulmonary neutrophils may be at risk for developing TRALI. Whether specific clinical conditions causing ALI also predispose to TRALI remains to be established and can be addressed in clinically relevant animal models. In these models, the attributable risk related to transfusion can be assessed, as well as interventions aiming at limiting lung injury.

Because of the pathophysiologic similarity of TRALI and ALI, it is conceivable that effective therapies in ALI/ARDS might be effective in TRALI. In ALI, a limited number of strategies exist. In late ALI/ARDS (i.e., after two weeks), high doses of methylprednisolone reduce lung injury and mortality in a small prospective randomized placebo controlled trial. A reduction in mortality of 12% in the methylprednisolone group versus 63% in the placebo group was found.

Protective strategies using low tidal volumes in mechanical ventilation have been proven to reduce mortality with 22% in ALI/ARDS. Injurious mechanical ventilation with high tidal volumes has been postulated to play a role in TRALI pathogenesis. Therefore, lung protective ventilation strategies should be applied in TRALI patients requiring mechanical ventilation. Whether low tidal ventilation reduces morbidity and mortality in TRALI remains to be determined.

The application of a fluid-restrictive management has been shown to reduce ventilation days in patients with ALI. It is conceivable that these results are also applicable in TRALI.

Other anti-inflammatory measures may include interfering with coagulation. Procoagulant strategies have been shown beneficial in sepsis and may be promising in patients with primary lung pathology, be it ALI, VILI or TRALI. However, clinical studies are needed to test this hypothesis. At present, several clinical trials evaluate anticoagulant therapy in lung injury, including a trial comparing recombinant human activated protein C to placebo in ALI patients and a trial comparing tissue factor pathway inhibitor in pneumonia patients. As deposition of fibrin is increased in pulmonary inflammation, stimulating fibrinolysis may prove beneficial in TRALI. TRALI has been found to be reduced by infusion of platelet activating factor (PAF)–receptor antagonist. Whether PAF receptor antagonists reduce mortality or ventilation days remains to be determined.

Several approaches have been shown to reduce neutrophil-mediated lung injury in experimental settings. Ca\textsuperscript{2+} channel blockers, such as verapamil and nifedipine both are able to inhibit the respiratory burst and bactericidal activity of neutrophils \textit{in vitro}. Whether Ca\textsuperscript{2+} channel blockers inhibit neutrophil mediated lung injury in TRALI needs further investigation. Another therapeutic possibility may be interfering with the complement system. Type I antibodies, which are often found
in TRALI, are thought to cause neutrophil influx into the lung through activation of complement. In an animal model of lung injury, administration of a C1 inhibitor resulted in a reduction of pulmonary neutrophil influx.\textsuperscript{78} Whether inhibition of complement reduces lung injury in TRALI is unknown.

In conclusion, therapeutic approaches which are beneficial in ALI also have a rationale in TRALI. Specific measures aimed at reducing neutrophil influx and activation are promising in ALI, but are still highly experimental and should be further investigated in experimental TRALI.

**Conclusion**

TRALI is the leading cause of transfusion–related morbidity and mortality. TRALI is thought to be either antibody-mediated or a “two-hit” event, in which a pulmonary priming condition is required. We propose a continuum in TRALI pathogenesis, ranging from a strong antibody-antigen mediated reaction to TRALI caused by significant priming, in conjunction with a second hit, which may be a (weak) antibody mediated event or caused by lipids from blood products. No specific therapy exists for TRALI. However, several interventions which are beneficial in ALI have a rationale in TRALI, such as lung protective ventilation as well as anti-inflammatory measures including corticosteroids, blocking of coagulation and stimulation of fibrinolysis. Development of such therapeutic strategies require clinically relevant TRALI models. The present TRALI models comprise an *in vivo* antibody model, in which the presence of a “first hit” is not provided. Existing “two hit” models are limited by their *ex vivo* experimental design, or, in the *in vivo* model, the use of cross species difference with manipulated transfusion products and the use of a non-clinical “first hit”. Clinically relevant TRALI models may include pneumonia, sepsis or mechanical ventilation as a “first hit”. The “second hit” may consist of the infusion of antibodies or lysoPCs or blood products of the animals own species.
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