Review Article

Non-antibody mediated transfusion-related acute lung injury an enigma

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Abstract: In 20% to 28% of transfusion-related acute lung injury (TRALI) cases, no leucocyte antibodies have been detected and such cases are described as non-antibody mediated TRALI. In the other 72% to 80% of TRALI cases, leucocyte antibodies are hypothesized to be the "second hit" that precipitates acute lung injury (ALI) in a sick patient. There is a substantial body of evidence demonstrating an association between TRALI and antibodies to human neutrophil antigens (HNA), human leukocyte antigens (HLA) class I and II. Therefore, one of the most widely used TRALI mitigation strategies has been to reduce/avoid use of blood components that contain or are more likely to contain these antibodies. Despite the success of these risk reduction strategies, hemovigilance data show that TRALI remains a serious and sometimes fatal complication of transfusion. This is possibly because the current TRALI risk-reduction strategies all focus on antibody mediated TRALI but do not address non-antibody mediated TRALI. This review discusses the available evidence on non-antibody mediated TRALI.

Keywords: Transfusion-related acute lung injury (TRALI); non-antibody; biological response modifiers (BRMs); storage lesion; microparticles (MPs); animal models

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Introduction

TRALI is hypothesized to follow a two-hit mechanism; the first hit being the patient's underlying illness which activates their pulmonary endothelium and primes neutrophils (1). The second hit is thought to be leucocyte antibodies or biological response modifiers (BRMs) contained in the transfused blood component. The second hit may directly or indirectly activate the primed neutrophils to produce an augmented microbicidal response. This release of reactive oxygen species and granular enzymes damages the pulmonary endothelial leading to the symptoms of ALI.

There is a substantial body of evidence demonstrating the role of antibodies to human neutrophil antigens (HNA), human leukocyte antigens (HLA) class I and II in the pathogenesis of TRALI. HNA-3a and HLA class II antibodies have been associated with more severe TRALI (2-4). Therefore, one of the most widely used TRALI mitigation strategies has been to reduce/avoid use of blood components that contain or are more likely to contain these antibodies. These strategies can include screening donors for the presence of leucocyte antibodies, or limiting the use of plasma or platelets from female donors who are more likely to have leucocyte antibodies due to pregnancies (3,5-7). Another strategy is the use of pooled solvent/detergent plasma where the large volume from pooling dilutes the leucocyte antibody and/or neutralizes the antibodies because of the presence of residual leucocyte

Table 1 Potential BRMs identified in TRALI research

Biological response modifiers (BRMs)	References
Interleukin 8 (IL-8)	(25-27)
Tumor necrosis factor-α (TNF-α)	(25-27)
Soluble CD40 ligand (sCD40L)	(28)
Lysophosphatidylcholines (Lyso-PCs)	(25-27,29,30)
Non-polar lipids (arachidonic acid, 5-, 12- and 15-HETE)	(31)
Microparticles	(32,33)

TRALI, transfusion-related acute lung injury.

antigens (8,9). Reports from the United Kingdom (10) and the United States (11) indicate that introduction of male-predominant plasma strategy reduced the number of TRALI cases. For an overview of the effectiveness of TRALI mitigation strategies, readers are referred to the recent review by Otrock *et al.* (7).

Despite the implementation of various risk reduction strategies, TRALI cases still occur according to published hemovigilance reports (12-14). This is possibly because the current TRALI risk-reduction strategies all focus on antibody mediated TRALI but do not address non-antibody mediated TRALI which is the subject of this review.

Incidence of non-antibody mediated TRALI

Non-antibody mediated TRALI is implicated in cases of TRALI where leucocyte antibodies are not detected. The incidence of non-antibody mediated TRALI ranges from 20% to 28% (5,14-16). An early systemic literature search for TRALI reports prior to December 2007 found that leucocytes contributed to 80% of TRALI cases, hence 20% were likely to be non-antibody related (15). In Australia, a study of 25 TRALI cases which included investigation for antibodies to HNA, HLA class I, and HLA class II detected none of these antibodies in seven cases (28%) (16). A study evaluating the TRALI risk-minimization strategies of excluding female donors with a history of pregnancy or leucocyte antibodies in Germany, demonstrated a reduced risk of antibody mediated TRALI (5). More recently, data from France Hemovigilance Network database identified 100 of the 378 cases of TRALI (26.5%) were non-antibody mediated (14).

Identifying causes of non-antibody mediated TRALI

The clinical similarity between TRALI and acute respiratory distress syndrome (ARDS), led Silliman *et al.* to investigate whether these pulmonary events shared similar mechanisms (17). They detected neutrophil (PMN) priming activity in the post-transfusion plasma of TRALI patients, all of whom had clinical conditions such as active infection and inflammation, recent surgery, cytokine administration and massive transfusion. HPLC separation of plasma lipids identified three active species. That study concluded that TRALI was the result of two events: (I) predisposing clinical condition and (II) transfusion of biologically active lipids in stored blood. Date-of-expiry packed RBC (PRBC), whole blood and platelet concentrates were found to contain PMN priming agents with platelet activating factor (PAF) like activity (18).

Storage lesion

PRBC units are routinely stored refrigerated for up to either 35 or 42 days depending on the country. During this time a number of changes occur at both the cellular and biochemical levels, collectively referred to as the storage lesion. This PRBC storage lesion has been linked to adverse outcomes associated with the transfusion of older PRBC units, but the underlying pathogenic mechanism remains unclear and is the subject of considerable research (19-21). Platelet units are stored routinely at room temperature for up to either 5 or 7 days depending on the country, and they too develop a storage lesion (22,23). Although not as well researched as the PRBC storage lesion, it is an area of growing interest. Several of the factors associated with the storage lesion of PRBC and/or platelet units have been shown to prime or activate neutrophils, and hence are considered as potential BRMs that might contribute to non-antibody mediated TRALI (24). Data from laboratory and animal models supports the identity of several of these potential BRMs (*Table 1*). Interestingly, there is some thought that donor-based differences in BRM levels and accumulation during storage may be a more important factor than the absolute storage duration of blood components (34); however, specific donor-related factors that increase the risk of TRALI have not been identified (35).

Leukoreduction has been shown to reduce the

concentration of some potential BRMs, but not others. For example, lyso-PC accumulation does not occur in leukoreduced PRBC, whereas the accumulation of arachidonic acid and its metabolites occurs in both leukoreduced PRBC and non-leukoreduced PRBC (25). Data from an *in vivo* rat model showed no difference in TRALI development with leukoreduction (25); however, preliminary data from an *in vivo* sheep model showed a reduction in TRALI development with leukoreduction (26). UK haemovigilance data appears to show a decreased TRALI incidence with the introduction of leukoreduction; however, the introduction of TRALI mitigation strategies in the same time period makes it difficult to determine which of these activities generated the benefit (10). Intuitively, based upon the threshold hypothesis of TRALI development (27), the reduction of BRM concentration with leukoreduction should reduce the inflammatory load of the transfused blood component making the development of TRALI less likely.

Animal models of non-antibody mediated TRALI

The low incidence of TRALI, and the inability to predict its occurrence has led to the use of animal models to try and better understand non-antibody mediated TRALI. One of the earliest models of non-antibody mediated ALI was a 'two hit' rat model (28). Rats pre-treated with a "first hit" of endotoxin [lipopolysaccharides (LPS)] followed by either plasma or extracted lipids from day 42 human PRBC (HuPRBC) developed ALI. Subsequent experiments with human whole blood and aphaeresis platelet supernatants (29) produced similar results as HuPRBC supernatant demonstrating the need for two hits and an aged blood product. As these experiments involved used of human blood products in a rat model, it was unclear if cross-species factors were a confounding factor.

This question was addressed by two *in vivo* rat models which were transfused with syngeneic rat blood products (30,31). Vlaar *et al.* first demonstrated that LPS treated rat transfused with aged rat PRBC (rPRBC) developed ALI and pulmonary coagulopathy (30). To determine the source of the injuring second insult, they also transfused LPS treated rats with either washed aged rPRBC or the supernatant of aged rPRBC. The latter produced worse pulmonary inflammation and coagulopathy. Experiments with the supernatant of aged rat platelets produced ALI, but not with washed aged rat platelets (31). This set of experiments showed firstly that animal models that use human products are valid and that importantly showed that the injuring second insult is contained in the supernatant of aged blood products.

Rodents are dissimilar to humans in many ways, and it can therefore be difficult to translate findings from mouse/rat models to the clinical setting of human patients (32). To address this limitation, Tung *et al.* established a large animal model using sheep (33,36). Of the LPS-treated sheep which were transfused with date-of-expiry (day 5) human platelet supernatant, 80% developed ALI (33). Similarly, 80% of LPS-treated sheep also developed ALI when transfused with aged (day 42) HuPRBC supernatant (36), indicating that the age of the product was a determinant. The larger size of sheep enabled frequent blood sampling and the use of clinical monitoring techniques. This allowed for a detailed investigation of the hematological, hemodynamic and respiratory changes associated with the development of non-antibody mediated TRALI. By comparing the haemodynamic changes of aged platelet supernatants and aged HuRBC, this study revealed that the latter induced a more severe injury with larger increases in pulmonary arterial pressure as well as larger decreases in pulmonary compliance and partial pressure of oxygen (36).

To directly compare the pathological events of immune versus non-immune TRALI, Tamarozzi *et al.* used mouse models (37). They observed similar TRALI histology, increased keratinocyte-derived chemokine and macrophage inhibitory protein-2 (MIP-2) in both forms of murine TRALI. Unique to the non-immune form was a dramatic increase in interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-α). This indicates that the pathways identified for antibody-mediated TRALI development in mice (38-43) may be different to those by which non-antibody mediated TRALI develops.

Microparticles (MPs)

MPs are small (0.05–1 μm diameter) membrane-bound vesicles shed from cells upon activation and/or apoptosis (23,44,45). Depending on the cell of origin and the method of formation, MP may contain phosphatidylserine and various protein or lipid BRMs (46). MP play a role in inflammation, immune responses and thrombosis (45). MP are released from red blood cells and platelets during the routine storage of PRBC and platelet units respectively (23,47).

The quantity of platelet MPs (PMP) in human aphaeresis platelets increases after 3 days of storage when the levels of

soluble CD40 ligand (sCD40L) are also seen to increase (48). PMP have also been shown to prime the fMLP-activated PMN respiration burst (48). Xie *et al.* then went on to demonstrate that PMP promoted PMN mediated damage in a two hit (first hit of LPS) *in vitro* human pulmonary microvascular endothelial cell (HMVEC) model (49), thus, presenting a potential mechanism for how PMP contribute to the development of TRALI.

A study of associated blood components reported higher concentrations of red blood cell derived MPs (RMP) in TRALI cases relative to post-transfusion reactions with dyspnoea (50). RMP accumulate in HuPRBC over the 42-day storage period, with significantly higher counts from 28 days (46). The isolated RMP and supernatant from HuPRBC stored for 28 or 35 days primed PMN respiratory burst. Using a two-hit mouse model (first hit of LPS), they demonstrated that these stored products also were able to induce ALI.

Human trials

The *in vitro* and animal model data on non-antibody mediated TRALI as a two-hit model and its association with aged blood products led Peters *et al.* to conduct a rare human trial (51,52). In a two-hit model, 18 healthy male volunteers received 2 ng/kg of LPS as a first hit, all of whom were confirmed to fulfil the criteria of sepsis. The second-hit varied across three groups of six people each: a control group received saline, one test group received one unit of 2-day-old autologous PRBC, and the other test group received one unit of 35 day old autologous PRBC. As they had also demonstrated that non-polar lipids increased after PRBC were stored for 21 days, the second test group could also reveal the effect of higher levels of these lipids. Careful hourly monitoring surprisingly detected no change in either hemodynamic or respiratory variables compared with the control groups. The authors pointed out that many animals models relied on human blood products thus introducing an inter-species confounding factor. They acknowledged the limitation of only transfusing one unit of PRBC, when most TRALI events involve transfusions of multiple blood components. Furthermore, in the clinical setting the vast majority of transfusions are allogeneic rather than autologous.

Conclusions

Non-antibody mediated TRALI remains an enigma of transfusion. The persistent incidence of TRALI cases despite the introduction of mitigation strategies confirms the need to better understand the underlying mechanisms of non-antibody mediated TRALI. Until we understand the underlying mechanism we will not be able to test for it nor avoid it.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/aob.2019.03.02). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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