

The pathogenesis of transfusion-related acute lung injury (TRALI)

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Summary

In recent years, transfusion-related acute lung injury (TRALI) has developed from an almost unknown transfusion reaction to the most common cause of transfusion-related major morbidities and fatalities. A clinical definition of TRALI was established in 2004, based on acute respiratory distress, non-cardiogenic lung oedema temporal association with transfusion and hypoxaemia. Histological findings reveal lung oedema, capillary leucostasis and neutrophil extravasation. However, the pathogenesis of TRALI remains controversial. Leucocyte antibodies, present in fresh frozen plasma and platelet concentrates from multiparous donors, and neutrophil priming agents released in stored cellular blood components have been considered to be causative. As neutrophils and endothelial cells are pivotal in the pathogenesis of TRALI, a threshold model was established to try to unify the various reported findings on pathogenesis. This model comprises the priming of neutrophils and/or endothelium by the patient's co-morbidity, neutrophil and/or endothelial cell activation by the transfused blood component, and the severity of the TRALI reaction.

Keywords: transfusion-related acute lung injury, transfusion, granulocytes, neutrophils.

Non-cardiogenic lung oedema as a result of blood transfusion was first described by Barnard (1951). Popovsky *et al* (1983) recognised this transfusion reaction as a distinct clinical entity and coined the term transfusion-related acute lung injury (TRALI). Two years later Popovsky and Moore (1985) analysed a series of 36 patients with TRALI. Minimal criteria for the diagnosis of TRALI were: acute respiratory distress and new bilateral lung infiltrations in the chest X-ray within 6 h of blood transfusion and the absence of evidence for the presence of volume overload or cardiac malfunction. They reported a ventilation rate of 70%, a fatality rate of 6% and the presence

of leucocyte, i.e. neutrophil and human leucocyte antigen (HLA), antibodies in the blood of 89% of implicated donors.

Silliman *et al* (1997) reported the association of biologically active lipids with the development of TRALI. Meanwhile, several neutrophil-priming agents have been identified in stored cellular blood components. In a prospective study of 90 TRALI reactions in 81 patients, patients with haematological malignancies and cardiac disease were identified as patients at risk for TRALI (Silliman *et al*, 2003a). All TRALI reactions were secondary to the transfusion of stored platelets and red cells, with one exception. Mechanical ventilation was required in only 3% of the cases; TRALI lead to death in one patient. However, in this study lung oedema was mainly diagnosed by auscultation and not by chest X-ray. Despite these reports, TRALI remained little known. In 2004 the European Haemovigilance Network (EHN) and the Canadian Consensus Conference (Kleinman *et al*, 2004) proposed criteria for the diagnosis of TRALI, which are summarised in Table I. It has been thanks to the British Serious Hazards of Transfusion (SHOT) haemovigilance system that the recognition of TRALI has gained increased recognition. In recent years, TRALI has been reliably shown to be the most common cause of transfusion-related fatalities in the United States and in the United Kingdom (Holness *et al*, 2004; Stainsby *et al*, 2004). This review summarises the current knowledge on the pathogenesis of TRALI.

Pathological findings in TRALI patients

Histological findings in patients who died from TRALI are consistent with early acute respiratory distress syndrome (ARDS), showing interstitial and intra-alveolar oedema (Felbo & Jensen, 1962; Flury & Reutter, 1966; Kernoff *et al*, 1972; Wolf & Canale, 1976; Popovsky & Moore, 1985; Silliman *et al*, 1997; Dry *et al*, 1999) and an extravasation of neutrophils into the interstitial and air spaces (Kernoff *et al*, 1972; Wolf & Canale, 1976; Silliman *et al*, 1997; Dry *et al*, 1999). In addition, hyaline membranes and destruction of the pulmonary architecture have been reported (Wolf & Canale, 1976; Silliman *et al*, 1997). More important, an increased number of neutrophils within the pulmonary capillary vasculature and small pulmonary vessels

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Table I. Criteria for the clinical diagnosis of transfusion-related acute lung injury (TRALI).

TRALI Consensus Conference Committee 2004 and European Haemovigilance Network

- Acute respiratory distress
- Bilateral lung infiltrations in the chest radiograph
- Occurrence during or within 6-h after completion of transfusion
- No evidence of transfusion-associated circulatory overload/cardiogenic lung oedema
- Hypoxaemia ($\text{PaO}_2/\text{FiO}_2 < 300$ mmHg or O_2 saturation $< 90\%$ or other clinical evidence)
- New acute lung injury (ALI) and no other ALI risk factors present (aspiration, multiple trauma, pneumonia, cardiopulmonary bypass, burn injury, toxic inhalation, lung contusion, pancreatitis, drug overdose, near drowning, shock, severe sepsis)

If one or more ALI risk factors are present, possible TRALI should be diagnosed.

was observed in lung sections from TRALI patients (Felbo & Jensen, 1962; Dry *et al*, 1999), and a positive correlation between the degree of capillary leucostasis and the amount of proteinaceous fluid within the alveolar air spaces has been demonstrated. On electron microscopic pictures, neutrophils were degranulated and focally in direct contact with denuded stretches of the capillary wall; in addition, a correlation between capillary leucostasis and desquamated epithelial cells were reported (Dry *et al*, 1999). From these observations, it seems reasonable to speculate that after sequestration in the early stages of TRALI, neutrophils and endothelial cells of the pulmonary microvasculature establish close contacts, which lead to firm neutrophil adhesion and retention. Once the neutrophils are immobilised in the alveolar capillaries, they activate their microbicidal arsenal and induce endothelial damage and capillary leak. This allows transit of proteinaceous fluid from the vessels into the air spaces, resulting in acute pulmonary oedema. In the later stages, especially of severe TRALI, neutrophils can extravasate from the capillary into the alveoli and induce pulmonary injury. Accordingly, neutrophils and endothelial cells of the lung capillaries are the key players in the pathogenesis of TRALI.

Physiological aspects of neutrophil passage through the pulmonary microvasculature

Neutrophil transit

An adult human with a cardiac output of 5 l/min pumps approximately 7200 l of blood through the pulmonary circulation in 24 h. As each litre of circulating blood contains about 10^9 leucocytes, their traffic through pulmonary microvessels is enormous. The alveolar capillary bed is a complex interconnecting network of short capillary segments. From quantitative histological studies it was calculated that each of the estimated 100 million alveoli in the adult human lung contains about 1000 capillary segments. The path from arteriole to venule

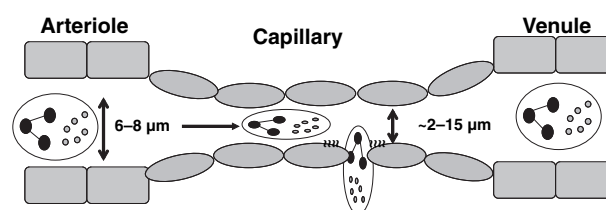


Fig 1. Neutrophil passage through pulmonary microvasculature. During passage of the pulmonary microvasculature, neutrophils have to pass more than 50 segments of the alveolar capillary bed. As the diameter of spherical neutrophil is larger than about 50% of the capillary segments, neutrophils, on their path from arteriole to venule, encounter many segments that require them to change shape in order to squeeze through. In contrast to the vasculature of many other organs, the principal site of neutrophil emigration in response to inflammation is the alveolar capillary bed.

crosses several alveolar walls (often > 8) so that a blood cell encounters > 50 capillary segments, each with an average length of $14.5 \mu\text{m}$. Approximately 50% of the pulmonary capillaries ($2\text{--}15 \mu\text{m}$) are narrower than the diameter of a spherically shaped neutrophil ($6\text{--}8 \mu\text{m}$). Thus, neutrophils often encounter a capillary segment that forces them to pause and to deform and assume a 'sausage' shape before squeezing through the narrow capillary segment (Fig. 1). Median transit times of neutrophils were 26 s compared with $1.4\text{--}4.2$ s of erythrocytes, because, although the erythrocyte has a discoid shape with a similar diameter to the spherical neutrophil, the erythrocyte can rapidly reduce its diameter by folding when it encounters a narrow capillary (Gebb *et al*, 1995). Computational models of the capillary bed support the concept that the structure of the capillary bed and the deformation of neutrophils are critical under normal conditions (Huang *et al*, 2001). Consequently, the transit time of neutrophils through the pulmonary capillary bed is mainly affected by their deformation time. The increased transit time also accounts for the significant neutrophil accumulation ('marginated pool') in the lungs (Doerschuk, 1999). The pulmonary circulation contains about 28% of the blood neutrophil pool that is available on demand for host defence against bacterial infections (Peters, 1998).

Neutrophil sequestration and retention

In the microvasculature of many organs, neutrophil recruitment from blood into tissue at sites of inflammation occurs in postcapillary venules. In contrast, the principal site of leucocyte emigration in the lung is the capillary bed (Loosli & Baker, 1962; Downey *et al*, 1993; Lee & Downey, 2001). As neutrophils do not roll in lung capillaries, the conventional model of neutrophil tethering, rolling and arrest on inflamed endothelium is not applicable to lung capillaries (Burns *et al*, 2003). The stimulus-induced decrease in deformability is thought to be important for neutrophil sequestration in the lung and probably replaces the role of selectin-mediated rolling in stopping neutrophils within capillaries (Doerschuk, 1999; Burns *et al*, 2003; Reutershan & Ley, 2004). The role of

mechanical factors in the initial sequestration of neutrophils in the alveolar capillaries is supported by direct evidence that neither L-selectin nor β_2 -integrins are required (Kubo *et al*, 1999). Activated neutrophils lose their ability to deform mainly because of the intracellular polymerisation of actin filaments. In contrast, events following the initial sequestration, i.e. retention and transendothelial migration, are apparently influenced by adhesion molecules. As mediator-induced decreases in neutrophil deformability are temporally correlated with a conformational change of β_2 -integrins from non-adhesive to adhesive, physical trapping goes along with neutrophil attraction to the endothelial surface. Intercellular adhesion molecule 2 (ICAM-2; CD102) which is constitutively expressed on vascular cells, is a ligand of the β_2 -integrin CD11b/CD18 and appears to play a role in neutrophil transendothelial migration (Issekutz *et al*, 1999). Transendothelial migration occurs mainly by penetrating interendothelial junctions at bicellular or tricellular corners of endothelial cells, although there is an alternative transcellular route (Burns *et al*, 2003). Platelet/endothelial cell adhesion molecule-1 (PECAM-1; CD31), junctional adhesion molecules, vascular/endothelial-cadherin, and CD99 are thought to regulate neutrophil transendothelial migration (Chavakis *et al*, 2003).

Fluid leakage in the pulmonary microvasculature

Fluid and protein leakage occur in the normal lung primarily through small gaps between capillary endothelial cells. The filtered fluid enters the alveolar interstitial space but not the alveoli because the alveolar epithelium is composed of very tight junctions. From the alveolar interstitium the fluid moves into the peribronchovascular space where it is removed by the lymphatics and returned to the systemic circulation (Ware & Matthay, 2005). Increased transvascular fluid filtration is the hallmark of acute cardiogenic or volume overload oedema (transudate). By contrast, non-cardiogenic pulmonary oedema is caused by an increase in the vascular permeability resulting in an increased flux of fluid and protein into the lung interstitium and air spaces. Non-cardiogenic pulmonary oedema has therefore high protein content (exudate).

Pathogenic aspects of TRALI: priming and activation of neutrophils

In general, contacts with injurious agents are a prerequisite of neutrophil activation. Activated neutrophils will respond by phagocytosis, the release of preformed granular enzymes and proteins, and by the *de novo* synthesis of a range of ephemeral, but highly toxic reactive oxygen species (ROS). In order to be become fully activated, neutrophils need to be primed.

Priming

Circulating neutrophils do not express anywhere near their full microbicidal capacity when challenged with biological activating

agents unless they have first been primed. Priming refers to a process whereby the response of neutrophils to an activating stimulus is potentiated; it facilitates the clustering of relevant surface receptors, such as Fc γ RIIa and β_2 -integrins, and the formation of the NADPH oxidase complex, which is responsible for the synthesis of ROS. The release of harmful ROS that occurs in response to an agonist is enhanced up to 20-fold by prior exposure of the cell to a priming agent (Guthrie *et al*, 1984).

Priming agents do not elicit effector functions on their own, except when they are applied at very high concentrations. As neutrophil activation is of central importance in the pathogenesis of TRALI, we have to assume that neutrophils experience both priming and activation, usually triggered by different stimuli, and that one of these stimuli must come from the transfused blood component.

Priming of neutrophils owing to underlying co-morbidity

A number of studies and epidemiological data from haemovigilance systems indicate that a large percentage of patients who develop TRALI had recent surgery. In the initial study by Popovsky and Moore (1985), 31 out of 36 TRALI cases occurred in patients with surgical procedures, requiring blood transfusion either during surgery ($n = 24$) or caused by postoperative blood loss ($n = 7$), and only five out of 36 TRALI cases occurred in patients with anaemia for chronic or stable conditions. These data were corroborated by others, and active infection, cardiovascular disease and leukaemia were identified as additional risk factors (Silliman *et al*, 1997; Silliman *et al*, 2003a; Holness *et al*, 2004). It should be kept in mind that linking TRALI to any specific clinical event needs valid denominator data. However, some of these observations are in accordance with *in vivo* evidence from studies demonstrating that surgical procedures and active infections induce neutrophil priming in patients (Bass *et al*, 1986; Krause *et al*, 1988; Kawahito *et al*, 2000). A variety of neutrophil priming agents that are released either by dying/necrotic cells or by stimulated endothelial cells, monocytes and lymphocytes have been described, including platelet activating factor (PAF) (Vercellotti *et al*, 1988), tumour necrosis factor- α (TNF- α) (Berkow *et al*, 1987), interleukin 8 (IL-8) (Daniels *et al*, 1992), granulocyte/macrophage-colony stimulating factor (Fleischmann *et al*, 1986), and interferon- γ (Tennenberg *et al*, 1993). Neutrophils can also be primed by a number of infectious agents, such as influenza A virus (Busse *et al*, 1991) and bacteria-derived lipopolysaccharides (LPS) (Guthrie *et al*, 1984).

Consequences of circulating primed neutrophils with regard to TRALI

In response to priming agents, neutrophils undergo polarisation or shape change, a process that has originally been suggested to represent frustrated chemotaxis (Haslett *et al*,

1985). This 'stiffening' of the cells augments the physiological mechanism of mechanical retention of neutrophils within the pulmonary capillary bed (sequestration), and prolongs the process of neutrophil squeezing through the narrow capillaries (Worthen *et al*, 1989). Prolonged, close contact of the stiff, i.e. primed, neutrophil with the endothelial cells allows the neutrophil to effectively sample the endothelial surface and provides a micro-environment in which transmembrane receptors and released mediators of each cell type can easily influence the other. Exogenous stimuli present in the blood bag can have impact on both, neutrophils and endothelial cells (Fig. 2). In addition, after having been primed in the circulation, sequestered neutrophils can now be activated to express their full microbicidal activity by exogenous activating substances present in the blood bag. These include neutrophil-binding antibodies, cytokines and bioactive lipids (see below). If a stimulus is no longer present, the primed neutrophil will be deprimed. Studies on human peripheral blood neutrophils *in vitro* suggest that the primed status is maintained for at least 24 h (Ichinose *et al*, 1990), a finding that fits well with the epidemiological observation that surgery (as a priming event) is a risk factor for TRALI if it was performed recently, i.e. <48-h previous to the transfusion event (Silliman *et al*, 1997).

As mentioned above, some priming substances may also induce full activation of the neutrophil when applied at high concentrations. Besides chemokines/cytokines, cross-linking antibodies to neutrophil surface receptors, namely to L-selectin and CD18, have been demonstrated to induce neutrophil priming efficiently (Waddell *et al*, 1994; Liles *et al*, 1995).

More important, ligation of neutrophil receptors may not only result in neutrophil priming, but can also afford neutrophil activation either in concert with or independent of soluble activators (Berton *et al*, 1992; Crockett-Torabi *et al*, 1995). We and others have demonstrated that antibodies to neutrophil-specific antigens involved in TRALI cases are able to prime and even activate neutrophils as well (Kopko *et al*, 2004; Sachs *et al*, 2004; Sachs *et al*, 2006; Silliman *et al*, 2006). This, as discussed below, explains why even completely healthy individuals can develop TRALI upon neutrophil antibody infusion (Dooren *et al*, 1998).

Pathogenic aspects of TRALI: activation of pulmonary endothelial cells

There is both clinical and experimental evidence that TRALI induction does not always start from the primed neutrophil, but may also be triggered by an activated pulmonary endothelium (Fig. 2, right panel). In addition to the constitutively expressed ICAM-2, activated endothelial cells upregulate surface membrane receptors that allow neutrophil adhesion, including ligands for L-selectin (Spertini *et al*, 1991), P-selectin, and ICAM-1 (Gerritsen & Bloor, 1993; Klein *et al*, 1995; Scholz *et al*, 1996). Neutrophils that pass through the narrow lung capillaries may well get stuck if the endothelium is activated and ligands for L-selectin have been upregulated, as the process of retaining neutrophils within the pulmonary capillaries has been described to be selectin-dependent (Yamaguchi *et al*, 1997; Kubo *et al*, 1999). Once

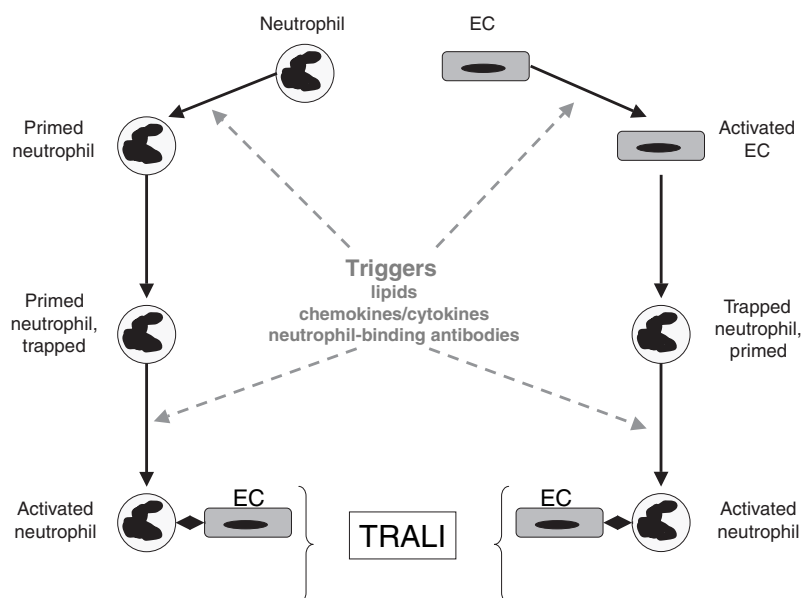


Fig 2. Possible pathomechanism of transfusion-related acute lung injury (TRALI). Neutrophils and pulmonary cells are key players in TRALI. Activation of each cell type may lead to TRALI. On the one hand, neutrophils may become primed, most probably as a result of endogenous triggers, such as those present during infections. Primed cells are trapped in the lungs' microvasculature, where they experience activation via substances present in the blood component, e.g. antibodies or bioactive lipids. On the other hand, an activated endothelial cell can induce neutrophil trapping within the lungs, where they are primed and finally activated because of triggers present in the blood component. In either case, neutrophil/endothelial cell interaction is necessary to finally induce TRALI.

trapped, the neutrophil will incorporate selectin-dependent signals with other extracellular inflammatory stimuli in order to achieve a preactivated (primed) status (Williams & Solomkin, 1999). Additional stimuli come from endothelial cells as well, because, once activated, they produce a broad range of chemokines/cytokines, including PAF, leukotriene B₄, and IL-8. These substances are not only secreted, but remain also bound to the endothelial cell surface, where they have access to their corresponding receptors on the surface of sequestered and/or adherent neutrophils (Kuijpers *et al*, 1992). Various substances have been identified as endothelial cell activators, including TNF- α , IL-1 β , and other mediators released during inflammatory processes, as well as exogenous LPS and antibodies.

One interesting example of TRALI because of antibody-mediated endothelial cell activation was reported by Dykes *et al* (2000). In a patient who underwent lung transplantation and became dyspneic after transfusion of two units red blood cells, a chest X-ray revealed a unilateral white-out of the transplanted lung. An antibody to HLA-B44 was present in the donor of one of the transfused units, and the cognate antigen was expressed on the transplanted lung but not on the patients' tissues. Obviously, the TRALI reaction was triggered by antibody binding to the endothelium of the transplanted lung. Recently, Looney *et al* (2006) presented *in vivo* data on the mechanism of endothelial cell-dependent TRALI in a mouse model. Transfusion of a major histocompatibility complex (MHC) class I monoclonal antibody to mice expressing the cognate antigen induced TRALI and acute peripheral blood neutropenia. Mice lacking neutrophils and mice lacking the Fc γ -receptor (Fc γ ^{-/-} mice) were resistant to MHC class I antibody-induced TRALI, but transfer of wild-type neutrophils into Fc γ ^{-/-} mice restored TRALI following antibody infusion. Accordingly, disease pathogenesis in this model was a result of immune recognition of MHC class I antibodies bound to lung endothelium, as the protection observed in Fc γ ^{-/-} mice argues against direct neutrophil activation by the antibody. The antibody seems to bind to the endothelial cell of the lungs (the first vascular bed encountered after injection); subsequently, neutrophils become sequestered to the lung via Fc γ -receptor interaction, which then leads to neutrophil activation and lung injury. Elevation of a number of cytokines in this murine model (including TNF- α and the murine IL-8 homologues) indicates that endothelial cell activation may participate in additional neutrophil recruitment and activation.

Primary activation of endothelial cells has also been suggested as the mechanism responsible for TRALI induction after infusion of bioactive lipids (Silliman *et al*, 1998; Silliman *et al*, 2003b). In this model, rats were treated with LPS to simulate active infection. Lungs were ventilated, isolated and perfused with buffer or plasma containing bioactive lipids that can develop during storage of blood components (Silliman *et al*, 1994). Lungs from LPS-treated animals perfused with plasma from stored packed red blood cells or stored platelet

concentrates, but not plasma from identical fresh packed red blood cells or platelet concentrates, caused TRALI. Lungs pretreated with vehicle instead of LPS did not develop TRALI with any of the perfusates. Although LPS is a well-known stimulator of the endothelium, it also efficiently primes neutrophils (Guthrie *et al*, 1984). Thus, although these experiments demonstrate clearly that bioactive lipids are capable of inducing TRALI, it cannot be dissected whether activation of the endothelium, priming of the rat neutrophils, or both is necessary to allow these lipids to commence the TRALI reaction.

Pathogenic aspects of TRALI: neutrophil/endothelial cell interplay

Although in early TRALI it might be possible to differentiate between those mechanisms that primarily lead to neutrophil priming, trapping and activation and those which primarily lead to endothelial cell activation, neutrophil trapping/priming and activation (Fig. 2), it is likely that the spatio-temporal interplay between neutrophils and endothelial cells, once it has been started, contributes largely to lung damage. Neutrophils respond to endothelial cell-derived mediators by activating and expressing integrins and by releasing pro-inflammatory mediators and granule contents. Released mediators activate the endothelium, endothelial cells mobilise selectins, upregulate adhesion proteins, and produce inflammatory mediators; thereby, they enhance neutrophil adhesion and neutrophil priming and activation. It is within this interplay that the lung barrier breaks down and allows transit of proteinaceous fluid and, later, of neutrophils into the alveolar space. ROS may play a relevant role in this process, as it is likely that they accumulate in this situation. Both, neutrophil specific antibodies and bioactive lipids have been demonstrated to prime the formyl-methionyleucylphenylalanine (fMLP)-activated respiratory burst reaction of the neutrophil (Silliman *et al*, 1997; Sachs *et al*, 2006). These partially reduced molecules of oxygen are known to induce several adhesion receptors on endothelial cells *in vitro*, including PECAM-1, an important molecule in neutrophil transmigration (Rattan *et al*, 1997). Upregulation of PECAM-1 has been observed on endothelial cells of pulmonary vasculature from a patient who died from TRALI (Kao *et al*, 2003). More important, in an animal model of lung injury, blockade of ROS protected the lungs from oedema and vascular leak (Mulligan *et al*, 1992), indicating that ROS-dependent activation of the endothelium does not only induce efficient recruitment of additional neutrophils, but is also involved in permitting generous transit of both protein-rich fluid and white blood cells into the alveolar space.

Priming and activating substances present in blood components

As outlined in the previous section, substances that are contained in blood components can induce TRALI by either

priming and/or activating the neutrophil, or by activating endothelial cells of the pulmonary vasculature.

Leucocyte antibodies

In two large series of TRALI, where pulmonary infiltrates were apparent in chest radiographs, leucocyte antibodies were detected in 61–89% of cases, with the causative antibody being identified in the donor of the transfused blood component (Popovsky & Moore, 1985; Popovsky & Haley, 2001). As early as 1957, it was reported that leucocyte antibodies can induce post-transfusion pulmonary reactions in volunteers (Brittingham, 1957): a healthy volunteer received plasma from two patients that contained a weak leucoagglutinin, and a mild respiratory reaction occurred after both transfusions. Subsequently, the same volunteer received 50 ml of whole blood from an alloimmunised patient with strong leucoagglutinins, and he developed neutropenia, fever, and a marked respiratory reaction with bilateral pulmonary infiltrates on a chest X-ray. A similar reaction occurred when a leukaemic patient was injected with 10 ml of sterile serum obtained from a patient with a strong leucoagglutinin (Brittingham, 1957). Severe pulmonary oedema was induced in a healthy volunteer who received an experimental gamma globulin concentrate, prepared from plasma containing leucocyte-reactive antibodies (Dooren *et al*, 1998). Although the antibodies in these experiments performed on humans were not well characterised, it appears that some leucocyte antibodies, especially leucoagglutinins, in transfused blood components are capable of inducing TRALI in a 'one-step' reaction, probably because they are strong enough to induce both neutrophil priming and activation. Although these antibodies are often referred to as leucoagglutinins, most of them cause neutrophils to aggregate in an active process and do not clump (agglutinate) them passively. Passive agglutination is usually restricted to antibodies of the IgM class.

From a pathogenetic point of view, it is necessary to distinguish between antibodies that recognise human neutrophil alloantigens (HNA), expressed on neutrophils (but not on the endothelium) and antibodies that recognise HLA, expressed on both the neutrophil and the endothelium.

Antibodies to human neutrophil antigens

Serological work up of TRALI patients identified antibodies to a HNA in a number of cases (Yomtovian *et al*, 1984; Nordhagen *et al*, 1986; Bux *et al*, 1996; Leach *et al*, 1998); a description of HNA antigens and their implication in TRALI has been published previously (Bux, 2005).

It is known to the authors of this review that one of these antibodies, a neutrophil agglutinin directed against HNA-3a, induced acute dyspnoea even in a healthy human volunteer after injection of a small volume of donor plasma. Further details of the mechanism were studied in an *ex vivo* rabbit lung model (Seeger *et al*, 1990). Severe vascular leakage was

reproduced in isolated rabbit lungs by application of HNA-3a antibodies. In the presence of HNA-3a positive neutrophils and complement, severe lung oedema occurred after a latent period of 3–6 h. In contrast, no such reaction was noted in the absence of HNA-3a antibodies, HNA-3a positive neutrophils, or a complement source. From these experiments it was concluded that leucoagglutinating antibodies and concomitant complement activation are key players in the initiation of TRALI. Complement activation is not thought to occur as a result of neutrophil-antigen interaction. In addition, it was not found to be a prerequisite for TRALI induction in other *ex vivo* experiments, where the induction of TRALI because of CD177-specific antibodies was dependent on the density of the cognate antigen (see below), but occurred in a complement-free environment (Sachs *et al*, 2006). It seems reasonable to speculate that antibodies to HNA-3a were capable of priming and activating neutrophils in the model published by Seeger *et al* (1990). Indeed, it was demonstrated recently that HNA-3a antibodies are able to prime neutrophils *in vitro* (Kopko *et al*, 2004; Silliman *et al*, 2006), as are antibodies to HNA-4a and HNA-2a (Sachs *et al*, 2004; Sachs *et al*, 2006). Antibodies directed against the neutrophil-specific surface marker CD177 (HNA-2a) were recently used by ourselves to demonstrate that TRALI induction in an *ex vivo* rat lung model is dependent on the density of the cognate antigen and can be enhanced efficiently by the addition of fMLP, a substance that mimics the activity of bacterially derived peptides and is used to approximate active infection (Sachs *et al*, 2006). HNA-2a expression is heterogeneous in man, with HNA-2a being expressed on either a large subpopulation (70%) or a small subpopulation (30%) of neutrophils, varying from individual to individual. We used neutrophils from each group of healthy donors in an *ex vivo* rat lung model, and application of the corresponding antibody induced TRALI only if CD177 was present on the majority of neutrophils. Obviously, under these conditions, neutrophil-antibody interaction was capable of fully activating the cells. In contrast, if CD177 was present on the minority of cells, no TRALI reaction could be induced by antibody addition. However, if fMLP was added to the buffer in the circuit, TRALI induction was promoted in the presence of neutrophils from individuals expressing CD177 on more than 70% as well as those expressing CD177 on <30% of their cells. These findings indicate that additional stimuli (fMLP) can overcome the inability of a stimulus to activate neutrophils. Thus, it might be deduced from these findings that, if antibodies are present in a blood component, they may be able to induce TRALI in an otherwise healthy individual, whenever the antigen/antibody ratio allows appropriate neutrophil activation. This finding is also in accordance with reports from healthy volunteers developing TRALI upon antibody infusion (Brittingham, 1957; Dooren *et al*, 1998). However, if the individuals' neutrophils encounter additional stimuli, as outlined in the previous

section (and mimicked by fMLP in our experiments), it appears that TRALI can develop more readily, a finding that is in accordance with the fact that most TRALI patients are not 'healthy', but suffer from an active infection or had recent surgery.

Antibodies to human leucocyte antigens

In contrast to antibodies to HNA antigens, HLA antibodies are reported frequently. However, only few studies have investigated the mechanisms by which these antibodies can induce a TRALI reaction. In contrast to HNA antibodies, which will exclusively bind to neutrophils or leucocytes, HLA class I antigens are also present on the surface of endothelial cells. An elegant study performed on mice implicates that a major mechanism induced by MHC class I antibodies is, once they have bound to the endothelium, accumulation of neutrophils within the lung capillaries via Fc receptors, and subsequent activation of neutrophils and endothelial cells (Looney *et al*, 2006). In this study, it appeared that a direct (Fab-dependent) interaction between MHC class I antibodies and the neutrophils does not contribute to the TRALI reaction. One constraint of this model is that a monoclonal antibody was used which probably was unable to induce neutrophil priming and/or activation. Unfortunately, there is no systematic evaluation of HLA class I antibodies with regard to their priming activity. However, it is known that some HLA antibodies, such as anti-HLA-A2, induce neutrophil aggregation *in vitro*, whereas others do not. As neutrophil aggregation implies neutrophil activation, these HLA antibodies must be able to prime and activate neutrophils. In addition, it has to be kept in mind that antibodies of the same specificity are not always functionally alike, as we have demonstrated for human antibodies recognising HNA-4a on CD11b. Some of these antibodies were capable of inducing the respiratory burst reaction, whereas others were not, although their serological reactivity was identical (Sachs *et al*, 2004). It should thus not be excluded that antibodies to HLA class I, besides their possible role in trapping neutrophils via Fc receptors and activating the pulmonary endothelium, can also prime and/or activate neutrophils; currently, there are only case reports from neutrophil transfusions indicating that HLA antibodies are capable of interacting with neutrophils directly (see below), and this issue awaits further experimental clarification.

In 2001, antibodies to HLA class II were reported to be associated with TRALI in a series of 11 patients (Kopko *et al*, 2001). In this study, HLA class II antibodies were detected in combination with HLA class I antibodies in five cases, and as the only entity in two cases. Although there is growing epidemiological evidence for TRALI induction by HLA class II antibodies, the biological mechanism by which these antibodies induce TRALI remains to be elucidated. Corresponding HLA class II antigens are not expressed on resting human neutrophils, although they may be expressed upon

neutrophil stimulation (Gosselin *et al*, 1993). Expression of HLA class II antigens has also been described for the activated endothelium. However, expression of HLA class II antigens was not present on vascular endothelium of pulmonary capillaries or intravascular neutrophils in a patient who experienced fatal TRALI (Kao *et al*, 2003). Thus, binding of HLA class II antibodies to monocytes with subsequent release of cytokines and neutrophil activation has been suggested to constitute an alternate pathway of TRALI induction (Kopko *et al*, 2003). Further evidence is required especially regarding whether local monocytes are able to produce sufficient amounts of cytokines to really alter the activity of neutrophils and/or the endothelium. Furthermore, the question needs to be addressed whether this multistep-pathway is fast enough to explain the rapid onset of TRALI (within 1–2 h). Finally, HLA class II can be found on intra-alveolar macrophages, and anti-HLA class II binding to these cells may induce release of cytokines and subsequent activation of neutrophils and/or endothelial cells. However, it seems unlikely that antibodies have access to the alveolar space through an intact endothelium. Still, once the endothelium has been destroyed, such a reaction may exacerbate TRALI, but none of these hypotheses has yet been investigated. Finally, antibodies to HNA and HLA could act as surrogates for antibodies to other cell types, e.g. monocytes. Alloantibodies to these or other cells might explain some apparently antibody-negative cases.

Bioactive lipids

Blood components are biological material derived from humans and may thus, as outlined above, contain antibodies to antigens present on neutrophils, endothelial cells, or both. In addition to these unwanted alloantibodies, blood components may accumulate intermediate metabolic products, such as bioactive lipids, during storage. These substances are breakdown products of membrane lipids, including lysophosphatidylcholine species (C16, C18 lyso-PAF), and act on neutrophils through the cells' PAF receptors in order to prime the respiratory burst reaction (Silliman *et al*, 1994). As these neutrophil-priming agents do not develop in stored acellular plasma, their generation is dependent on the presence of blood cells. In a small series of TRALI patients, it has been demonstrated that post-transfusion sera from these patients contained significantly more neutrophil-priming activity than the controls (Silliman *et al*, 1997), and they have also been reported to induce TRALI after the transfusion of stored autologous blood (Covin *et al*, 2004). As outlined above, in an *ex vivo* rat lung model of TRALI, addition of both LPS and plasma containing neutrophil-priming lipids obtained from stored red blood cells was necessary to induce TRALI (Silliman *et al*, 1998), which is well in accordance with the pathophysiological model that priming (in this model, via LPS) advances efficient activation of neutrophils (in this model, via biologically active lipids).

Later, the same group demonstrated that plasma from stored platelet concentrates, which also contains neutrophil-priming lipids, causes TRALI in an identical animal model (Silliman *et al*, 2003b).

Other factors

Another neutrophil-priming breakdown product that does not belong to the family of lipids was identified recently, CD40-ligand (CD40L). CD40L is a primarily platelet-derived pro-inflammatory mediator found in cell-associated and soluble (sCD40L) forms. It has been described to be present in platelet concentrates, where it accumulates during storage (Phipps *et al*, 2001). CD40L binds to CD40, which is present on the surface of monocytes and macrophages; only recently, CD40 was also reported to be expressed by neutrophils (Khan *et al*, 2006). These authors demonstrated that CD40L primes neutrophils through CD40, and identified CD40L as a possible co-factor in TRALI because its concentration in transfused platelet concentrates that were involved in TRALI cases was significantly higher than in the control units. *In vitro*, human microvascular endothelial cells (HMVECs) preincubated with LPS experienced severe damage when sCD40L-primed neutrophils were added, whereas unprimed neutrophils did not induce HMVEC damage.

Activation of neutrophils by immune complexes (ICs) has been demonstrated to activate neutrophils as well, because, after incubation with ICs, they produced TNF- α and induced the apoptosis of HMVECs *in vitro* (Nishimura *et al*, 2004). However, it remains speculative whether antibodies present in blood components form ICs with their corresponding soluble HNA or HLA antigens in the recipient's circulation.

'Inverse TRALI': transfusion of neutrophils

In most cases of TRALI, antibodies or neutrophil-priming agents present in the blood component are causative for the pulmonary reaction. However, it should not go unmentioned that TRALI has also been described in alloimmunised patients receiving blood components which contain neutrophils. This has particular relevance to patients receiving neutrophil transfusions (O'Connor *et al*, 1988; Sachs & Bux, 2003). In one of the case reports, a recipient was immunised against HLA-A2 and the transfused neutrophils were HLA-A2 positive (Sachs & Bux, 2003). It must be claimed that these HLA-antibodies interacted directly with the transfused cells, because, as an alloantibody, these antibodies will not bind to autologous endothelial cells to commence the pathological cascade reported by Looney *et al* (2006). Rather, these antibodies bind to the cognate alloantigen on the surface of the transfused neutrophils where they induce neutrophil priming, sequestration within the lungs, and development of TRALI. *In vivo* studies performed with 111-indium labelled neutrophils that have been transfused to patients with neutrophil agglutinins showed similar findings, as these cells

were abnormally sequestered in the lungs (McCullough *et al*, 1986). Most likely, after antibody-dependent neutrophil priming, 'stiff' neutrophils were trapped in the pulmonary capillaries.

Viable neutrophils may still be present in other blood components; Popovsky and Moore (1985) reported that 6% of all TRALI cases were a result of antibodies present in the recipient. However, as general leucocyte depletion is introduced in more and more countries, antibody binding to contaminating leucocytes in platelet and erythrocyte concentrates will not be of importance in the future.

Prevention of TRALI

The donor of the implicated blood component in antibody-mediated TRALI is usually a multiparous woman who had several exposures to paternal leucocyte antigens from the foetus during pregnancy. The clinical significance of plasma from multiparous donors was confirmed by Palfi *et al* (2001). They showed that, in a prospective randomised controlled trial of 100 intensive care patients receiving a unit of control plasma and, 4-h later, a plasma unit from a multiparous donor or vice versa, transfusion of plasma from multiparous donors was associated with significantly lower oxygen saturation and higher TNF- α concentrations than transfusion of control plasma. Therefore, in late 2003 the UK National Blood Service introduced a policy of using male donors whenever possible to produce fresh frozen plasma (Chapman *et al*, 2006). However, the exclusion of multiparous female donors would result in the loss of approximately of 30% of donors (Densmore *et al*, 1999; Webert & Blajchman, 2003). For apheresis donors, the disqualification of all multiparous donors seems to be disproportionate and initiating screening of parous female donors for the presence of leucocyte antibodies appears to be more reasonable. Such a practice will cause a loss of approximately 6–8% of apheresis platelets, for which blood centres should be able to compensate (Insunza *et al*, 2004). The use of leucocyte-depleted cellular blood components protects against the very rare cases of TRALI because of leucocyte antibodies in the recipient and possibly also against other neutrophil priming agent-mediated TRALI reactions. For the prevention of the latter, it has been suggested that either fresh components should be used for patients at risk or that neutrophil-priming agents should be removed by washing the components before transfusion (Silliman *et al*, 2005). However, the patients at risk still need to be characterised in more detail and the impact of different preparation methods, including leucocyte depletion, on neutrophil-priming agent release as well as the critical storage time, need to be determined. Finally, the risk of bacterial contamination, quality impairments and delays in the provision of either fresh or washed cellular blood components must be weighed against the potential benefits of preventing mainly milder cases of neutrophil priming agent-mediated TRALI.

Conclusion: a threshold model of TRALI

Neutrophils contribute essentially to the pathogenesis of TRALI: without neutrophils, TRALI does not occur. As summarised in this article, a broad range of substances is capable of priming or activating neutrophils, either directly or via an activated pulmonary endothelium, or both. These substances are, by definition, present in the transfused blood component, but often are also endogenously present in the transfusion recipient, because of surgery, infection, or other inflammatory responses. Thus, pathophysiology of TRALI makes it difficult to exactly define which and how many substances the neutrophil has to encounter before lung damage occurs. We therefore suggest that TRALI evolves once the neutrophil has overcome a threshold (Fig. 3). Compared with a quiescent neutrophil, a primed neutrophil has reached a certain level of activation, still below the threshold; how strong the neutrophil has been activated depends on the number of priming agents and their (cumulative) potency. In the event of transfusion, the agents present in the transfused compound may activate the neutrophil further, which leads to TRALI. However, it is also possible that the stimuli present in the transfused blood component are not strong enough to overcome the threshold – which would explain why blood components obtained from a single immunised donor and transfused to different patients do not always elicit a TRALI reaction (Kopko *et al*, 2002; Toy *et al*, 2004): the potency of this stimulus is too weak to overcome the threshold if the level of preactivation is too low. From epidemiological observations, it seems also reasonable to state that a mild TRALI reaction (in

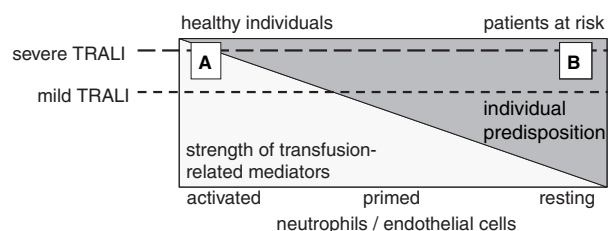


Fig 3. Threshold model of transfusion-related acute lung injury (TRALI). The TRALI threshold model proposes that a certain threshold must be overcome to induce a TRALI reaction. The threshold of mild TRALI, in which oxygen supply is sufficient, is lower than that of severe TRALI, where patients require mechanical ventilation (horizontal lines). In order to overcome these thresholds, numerous factors must act together. These factors can be summarised as the strength of transfusion-related mediators (light box) and as the individual predisposition of the patient (grey box). The strength of transfused mediators depends on their neutrophil/endothelial priming or activating capacity. The individual predisposition covers both constitutive (genetic) and co-morbidity-related factors. A transfused strong neutrophil antibody can induce a TRALI reaction even if the individual predisposition is low, e.g. in an otherwise healthy recipient (A). In contrast, in an individual 'at risk', such as a patient with a co-morbidity-related activation of the pulmonary endothelium, the transfusion of mediators with a relatively low neutrophil-priming activity will be sufficient to overcome the threshold (B).

which oxygen support is sufficient) needs a lower level of activation than does a severe TRALI reaction (where the patient requires mechanical ventilation). However, a single transfusion of an antibody-containing blood component to a healthy volunteer might be powerful enough to overcome the threshold straight away (Brittingham, 1957; Dooren *et al*, 1998).

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