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Evaluation of Platelet Responses in Transfusion-Related Acute Lung Injury (TRALI)

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ABSTRACT

Platelets are versatile cells which are capable of eliciting nonhemostatic immune functions, especially under inflammatory conditions. Depending on the specific setting, platelets may be either protective or pathogenic in acute lung injury and acute respiratory distress syndrome (ARDS). Their role in transfusion-related acute lung injury (TRALI) is less well defined; however, it has been hypothesized that recipient platelets and transfused platelets both play a pathogenic role in TRALI. Overall, despite conflicting findings, it appears that recipient platelets may play a pathogenic role in antibody-mediated TRALI; however, their contribution appears to be limited. It is imperative to first validate the involvement of recipient platelets by standardizing the animal models, methods, reagents, and readouts for lung injury and taking the animal housing environment into consideration. For the involvement of transfused platelets in TRALI, it appears that predominantly lipids such as ceramide in stored platelets are able to induce TRALI in animal models. These studies will also need to be validated, and moreover, the platelet-derived lipid-mediated mechanisms leading to TRALI will need to be investigated.

Contents

Transfusion-related acute lung injury (TRALI) is a leading cause of transfusion-associated fatalities and is characterized by the onset of acute respiratory distress within 6 hours of a blood transfusion [1,2]. The pathogenesis has proven to be challenging to decipher, and consequently, specific therapies are not yet available [1,3]. Overall, the pathogenesis of TRALI can be viewed as a 2-hit phenomenon, where both hits are required for induction. The first hit is conveyed by preexisting clinical risk factors present in the transfused recipient, often signified by a state of inflammation, whereas the second hit is represented by factors in the transfusion product [1]. These factors may be antileukocyte antibodies, such as anti–human leukocyte antigen class I/II antibodies or anti–human neutrophil antigen antibodies, or biological response modifiers such as accumulated lipids in aged platelets [1,4-6]. The role of recipient leukocytes in the pathogenesis of antibody-mediated TRALI has been investigated, with CD4+ T regulatory cells and dendritic cells being protective via interleukin (IL)-10 [7] and neutrophils (polymorphonuclear cells [PMNs]), monocytes, and macrophages being pathogenic cells [1,8-10]. The role of recipient platelets, however, has

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Table 1
Recipient platelet involvement in TRALI as investigated using 34-1-2S–based mouse models

Study	Mouse strains	First hit	Second hit	Methods platelet targeting	Readouts lung injury	Recipient platelet involvement in TRALI
Looney et al, 2009 [29]	• BALB/c Male, 8-12 wk	• LPS (*Escherichia coli*, O55:B5) 0.1 mg/kg, ip*/# or it♦, 24 h prior to 34-1-2S	• 34-1-2S 0.5*, 1# or 4.5♦mg/kg, iv (jugular vein), 24 h post LPS, analysis after 2 h	Depletion • Rabbit antimouse platelet serum #/♦ 25 μg, iv, 2 h prior to 34-1-2S Functional • Aspirin* 100 mg/kg, ip, 24.5 h prior to 34-1-2S, repeated 2 h prior to 34-1-2S • Anti–P-selectin mAb (clone RB40.34)# 30 μg, iv, ≥24 h prior to 34-1-2S, repeated immediately prior to 34-1-2S • Anti-M2 Ab# (blocks GPIbα-binding site on MAC-1) 100 μg, iv, immediately prior to 34-1-2S	• Bloodless extravascular lung water • EVPE • Lung MPO activity	Pathogenic, critically involved Depletion • Rabbit antimouse platelet serum: prevents TRALI Functional • Aspirin: prevents TRALI • Anti–P-selectin mAb: does not prevent TRALI • Anti-M2 Ab: does not prevent TRALI
Strait et al, 2011 [38]	• BALB/c Male, 8-20 wk	• No first hit applied	• 34-1-2S 125 μg, iv (tail vein), analysis after 30 min	Depletion • Rabbit antimouse platelet serum 200 μL of a 1:40 vol/vol dilution in saline, iv, 2 d prior to 34-1-2S, repeated 1 d prior to 34-1-2S Functional • Neuraminidase 0.1 U, ip, 24 h prior to 34-1-2S	• Penh analysis • Percentage water weight of lungs	Dispensable, not required for TRALI Depletion • Rabbit antimouse platelet serum: does not inhibit TRALI Functional • Neuraminidase: does not inhibit TRALI
Caudrillier et al, 2012 [37]	• BALB/c Male, 8-12 wk	• LPS (origin not specified) 0.1 mg/kg, ip, 24 h prior to 34-1-2S	• 34-1-2S 0.5 mg/kg, iv (jugular vein), 24 h post LPS, analysis after 2 h	Functional • Tirofiban (GPIIb/IIIa inhibitor) 0.5 μg/g, iv, immediately prior to 34-1-2S • Aspirin 100 mg/kg, ip, 24.5 h prior to 34-1-2S, repeated 2 h prior to 34-1-2S	• Bloodless extravascular lung water • EVPE • NET formation in plasma and lung microcirculation	Pathogenic, induces NETs in TRALI Functional • Tirofiban: decreases NET formation and associated platelet sequestration; protects against TRALI • Aspirin: decreases NET formation associated platelet sequestration; lung injury not analyzed
Thomas et al, 2012 [39]	• BALB/c Male, 8-10 wk	• LPS (origin not specified) 0.1 or 0.5 mg/kg, ip, 24 h prior to 34-1-2S	• 34-1-2S 1 mg/kg 34-1-2S, iv (retro-orbitally), 24 h post LPS, analysis after 2 h	Functional • Neuraminidase (details not specified)	• NET formation in lung alveoli	Dispensable, not required for NET formation in TRALI Functional • Neuraminidase: does not prevent NET formation (data not shown)
Hechler et al, 2016 [30]	• BALB/cByJ* Male, 8-12 wk • PF4-Cre/iDTR# Male, 8-12 wk (BALB/cBy.B6)	• LPS (*E coli*, 055:B5) 0.1 mg/kg, ip, 24 h prior to 34-1-2S	34-1-2S 0.5* or 0.8# mg/kg, iv (retro-orbitally), 24 h post LPS, analysis after 10 min or 2 h	Depletion • Rat anti-GPIbα mAb (clone RAM.6)* 2 mg/kg, iv, 2 d prior to 34-1-2S (inefficient depletion) • DT# 100 ng/d ip, repeated daily, 5 to 2 d prior to 34-1-2S Functional • Rat anti-GPVI mAb (clone JAQ1)* 2.5 mg/kg, sc, 4 d prior to 34-1-2S (depletion of GPVI on circulating platelets) • Aspirin* 10 or 100 mg/kg, ip, 24.5 h prior to 34-1-2S, repeated 2 h prior to 34-1-2S • Clopidogrel* ($P2Y_{12}$ receptor inhibitor) 50 mg/kg, orally, repeatedly at 40, 26, 16, and 2 h prior to 34-1-2S • GPIIb/IIIa-deficient platelet transfusion post-DT treatment# 3.5×10^6 platelets/μL, 200 μL, iv (retro-orbitally), 25 h prior to 34-1-2S, repeated 24 h prior to 34-1-2S (immediately prior to LPS)	• Blood oxygen levels • BAL protein levels • Lung histology	Dispensable, but prevents lung hemorrhages Depletion • Rat anti-GPIbα mAb*: inconclusive because of inefficient platelet depletion • DT#: does not prevent TRALI but delays lung hemorrhages Functional • Rat anti-GPVI mAb*: does not prevent TRALI but inhibits lung hemorrhages • Aspirin*: does not protect against TRALI • Clopidogrel*: does not protect against TRALI • GPIIb/IIIa-deficient platelet transfusion #: does not prevent TRALI but prevents lung hemorrhages
Cognasse et al, 2020 [31]	• BALB/c Male, 8-12 wk	• LPS (*E coli*, 0111) 0.1 mg/kg, ip, 24 h prior to 34-1-2S	• 34-1-2S 1 mg/kg, iv, 24 h post LPS, analysis after 2 h	Depletion • Rat anti-GPIbα polyclonal Ab 2 mg/kg, iv, 30 min prior to 34-1-2S Functional • ML354 (anti–PAR4-receptor inhibitor)	• BAL protein levels • Wet lung weight/body weight ratio • Lung histology	Dispensable, but reduces TRALI severity Depletion • Rat anti-GPIbα Ab: does not prevent TRALI but limits the

Table 1 *(continued)*

Study	Mouse strains	First hit	Second hit	Methods platelet targeting	Readouts lung injury	Recipient platelet involvement in TRALI
				4 mg/kg, ip, 30 min prior to 34-1-2S	(macroscopic lung damage score)	severity Functional • ML354: does not prevent TRALI but limits the severity

Bloodless extravascular lung water = [wet lung weight/(dry lung weight, corrected for the dry weight of blood remaining in the lung using hemoglobin levels)]. EVPE = ^{125}I-albumin radioactivity in the lung homogenate − (^{125}I-albumin concentration in plasma sample × calculated plasma volume in lungs) in ^{125}I-albumin–instilled mice. Pehn = changes in breathing pattern shown by barometric plethysmography as "enhanced pause." Percentage water weight of lungs = difference between the pre- and postlyophilization lung weight/prelyophillization lung weight × 100%. PF4-cre/iDTR = inducible DT receptor expression in PF4-positive cell populations (megakaryocytes and platelets). Indicated symbols (*, #, ♦) should only be compared within each row. MAC-1, macrophage antigen 1.

sparked a debate [11]. In this article, we will review the evidence for the involvement of recipient platelets as well as the involvement of transfused platelets in the pathogenesis of TRALI.

1. Immune Functions of Platelets

Platelets are classically known for their role in hemostasis [12]; however, in the last decade, it has become clear that platelets can also function as immune cells [13-15]. This includes immune-sensing functions for antimicrobial host defense and their ability to communicate with and regulate a variety of immune cells. An example of their antimicrobial defense is illustrated by studies showing that platelets are able to inhibit bacterial growth through encapsulating the bacteria and secreting the antimicrobial peptide β-defensin-1 as well as via signaling of PMNs to extrude neutrophil extracellular traps (NETs) [16]. Bacterial trapping by platelets has also been described to occur via the platelet adhesion receptor glycoprotein (GP)Ib [17] or during sepsis via platelet toll-like receptor 4 resulting in NET formation [18]. More recently, migrating platelets were shown to be mechanoscavengers that collect bacteria deposited at the vascular surface [19]. Furthermore, platelets can communicate with other immune cells by releasing CD40L, secreting several cytokines and chemokines, or shedding of platelet microparticles or via their major histocompatibility complex class I molecules [13-15]. These immunomodulatory functions of platelets are discussed in more detail in a companion article in this issue of *Transfusion Medicine Reviews* [20]. In the next section of this article, we will first briefly discuss how platelets may play a role in a setting of acute lung injury and acute respiratory distress syndrome (ARDS).

2. Platelets in Acute Lung Injury and ARDS

The versatility of the immune functions of platelets is evident from the fact that platelets have the ability to influence both physiological as well as pathological responses in the lungs [21]. Platelets may enter the pulmonary circulation after being formed in the bone marrow from megakaryocytes, but it has also been described in mice that platelets may be produced by megakaryocytes in the lungs, although this needs to be verified in humans [22]. In the lungs, platelets may mediate protective or injurious pathogenic responses depending on the specific setting of ARDS or the type of acute lung injury. One study showed that platelets have an important role in the host defense to pneumonia-derived sepsis caused by the gram-negative *Klebsiella pneumoniae* in mice [23]. Antibody-induced thrombocytopenia was correlated with impaired survival proportional to the degree of platelet depletion [23]. Thrombocytopenia was also accompanied by increased bacterial growth in lungs, in distant organs, and in the circulation, and increased proinflammatory cytokines were noted but did not affect lung inflammation. Similarly, in the setting of pneumonia by the gram-positive *Streptococcus pneumoniae*, induction of thrombocytopenia in mice by administration of antimouse thrombocyte serum also reduced survival and resulted in higher bacterial burden in lungs, spleen, and blood but did not affect lung inflammation despite increases in plasma proinflammatory cytokine levels [24]. Treatment with the platelet P2Y12 receptor inhibitor clopidogrel prolonged the bleeding time but had no effect on the bacterial loads [24]. Collectively, these studies indicate that platelets are protective in a setting of gram-negative or gram-positive pneumonia by inhibiting bacterial growth, a function which no longer occurs when the platelets are depleted in vivo. In contrast, a pathogenic role of platelets was found in influenza A–induced pneumonia and acute lung injury [25]. Mice infected with influenza A demonstrated a massive increase of aggregates of activated platelets in the lungs and activation of platelet protease-activated receptor 4 (PAR4), exacerbated acute lung injury, and increased mortality in mice. On the other hand, deficiency of platelet receptor GPIIIa or administration of antiplatelet agents such as the GPIIb/IIIa antagonist eptifibatide, the PAR4 antagonist MRS 2179, or clopidogrel protected mice from influenza virus–induced lung injury and mortality [25]. This study underlines the ability of platelets to regulate pathogenic responses in influenza-induced acute lung injury. In a different murine model of acid-induced acute lung injury (modeling acute lung injury due to pulmonary aspiration of gastric content, which may damage the alveolar-capillary membrane), it was found that platelet P-selectin–dependent platelet-PMN interactions occurred in blood and lung vessels [26]. The onset of acid-induced acute lung injury could be prevented by reducing the number of circulating platelets or by blocking P-selectin. Furthermore, activated platelets induced endothelial ICAM-1 expression and increased the adhesion of PMNs to endothelial cells. Targeting the platelet-PMN aggregation, using a specific thromboxane receptor antagonist (platelet-PMN adhesion to untreated human pulmonary microvascular endothelial cells was shown to require endothelial cell thromboxane receptors), reduced the recruitment of PMNs and prevented endothelial permeability, improving survival [26]. This study demonstrates another pathogenic role of platelets which occurs via interaction with PMNs. The multifactorial roles of platelets and their protective or pathogenic aspects in acute lung injury and ARDS appear to be dependent on the specific setting, and the readers are referred to a recent comprehensive review [21]. In this article, the focus will be directed more on the involvement of platelets in a setting of blood transfusion–triggered acute lung injury or TRALI.

3. Recipient Platelet Involvement in TRALI

Animal models of TRALI have significantly contributed to obtaining insights into the pathophysiology of TRALI [1]. This is especially true for the clinically relevant TRALI model based on injection of the anti–major histocompatibility complex class I antibody 34-1-2S [1,27]. Like in humans, this model features the 2 hits of TRALI pathophysiology with the first hit being represented by, for example, low levels of IL-10 (IL-10 KO mice or via depletion of CD4+ T regulatory cells or dendritic cells) [7], infusion of C-reactive protein (CRP) [28], or priming with low-dose lipopolysaccharide (LPS) [9,29-32]. The second hit is conveyed by the 34-1-2S antibody infusion. Furthermore, this murine TRALI model displays other characteristics observed in human TRALI patients such as the induction by antileukocyte antibodies, the onset of TRALI

Table 2
Studies investigating the involvement of transfused platelets as a trigger for TRALI development

Study	Study type	First hit	Second hit	Readouts TRALI	Transfused platelet involvement in TRALI
Silliman et al, 2003 [43]	Ex vivo • Sprague-Dawley rats Male, 300 g	• LPS (*Salmonella enteritides*, strain not specified) 2 mg/kg, ip, 2 h prior to lung isolation, 165 min prior to human platelet plasma, lipid extracts, HPLC-purified lipids, or lyso-PCs	• Human platelet plasma (stored 5 d, apheresis- and whole blood-derived, heat-treated) lung perfusion, 165 min post LPS, analysis after 30 min • Lipid extracts (from human platelet plasma) lung perfusion, 165 min post LPS, analysis after 30 min • HPLC-purified lipids (from human platelet plasma) lung perfusion, 165 min post LPS, analysis after 30 min • Purified lyso-PCs (commercial) 5 or 10 μmol/L, lung perfusion, 165 min post LPS, analysis after 30 min	• PA pressure • Pulmonary edema index • LTB_4 levels in lung perfusate • Lung histology	Pathogenic, lipids in stored platelets induce TRALI •No effects on PA pressure • All second hits increase pulmonary edema index • Human platelet plasma increases LTB_4 levels • Lyso-PCs induce pulmonary PMN sequestration, septal damage, edema, and hyaline membrane formation
Gelderman et al, 2011 [44]	In vivo • SCID mice Sex not specified, 6-8 wk	• LPS (*E coli*, 0111:B4) 3 mg/kg, iv, 2 h prior to UVB-irradiated human platelets	• UVB-irradiated human platelets (stored 1 d prior to irradiation, apheresis-derived, leukoreduced) 10^9 platelets, iv, 2 h post LPS, analysis after 1 or 2 h	• Lung W/D weight ratio • BAL protein levels • Lung histology	Pathogenic tendency, UVB-irradiated human platelets may have the tendency to increase TRALI severity compared to unradiated platelets • No effect on lung W/D weight ratio • Increases BAL protein levels • Induces severe loss of alveolar structure, cellular infiltration, and protein-rich alveolar exudate (no scoring of lung damage)
Tung et al, 2011 [45]	In vivo • Merino sheep Female, 4 to 7 y (30-54 kg)	• LPS (*E coli*, O55:B5) 15 μg/kg, 30-90 min prior to human platelets	• Supernatant of aged human platelets (stored 5 d, whole blood-derived, heat-treated) 10% of total blood volume being 65 mL/kg, analysis after ≥2 h	• ABG • Lung histology (microscopic lung damage score)	Pathogenic, aged human platelet supernatant induces TRALI • Decreases end-tidal CO_2, decreases pulmonary compliance, and increases arterial partial pressure of CO_2 • Causes pulmonary edema, cellular infiltration, thickening of alveolar wall, and loss of alveolar structure
Chi et al, 2014 [46]	In vivo • SCID mice* Male, 6-8 wk • SCID/LYS-eGFP mice# Sex and age not specified	• LPS (*E coli*, 0111:B4) 3 mg/kg, ip, 2 h prior to Mirasol-treated human platelets	• Mirasol-treated human platelets (stored 2-20 h prior to Mirasol treatment, storage time prior to transfusion not specified, apheresis-derived, leukoreduced) 10^9 platelets, iv, 2 h post LPS, analysis after 2 h	• Lung histology* • Confocal microscopy lungs#	Pathogenic, human platelets mediate TRALI, regardless of Mirasol treatment • Induces pulmonary PMN sequestration in mice*/#, regardless of Mirasol treatment
Caudrillier et al, 2015 [47]	In vivo • NOD/SCID mice Sex not specified, 6-10 wk	• LPS (*E coli*, O55:B5) 0.1 mg/kg, ip, 24 h prior to Mirasol-treated human platelets	• Mirasol-treated human platelets (stored overnight prior to Mirasol treatment, stored 1 or 5 d prior to transfusion, apheresis-derived) 10^8 platelets, iv,24 h post LPS, analysis after 4 h	• Bloodless extravascular lung water • EVPE	Dispensable, Mirasol-treated human platelets do not induce TRALI
Xie et al, 2015 [48]	In vitro • HMVEC-L >90% confluence on 96-well plates	• LPS (*Salmonella enteritides*, strain not specified) 200 ng/mL, 6 h prior to PMP or sCD40L • PMNs 10:1 PMN to HMVEC-L ratio, post-LPS, allowed to settle to HMVEC-L (period of time not specified) prior to PMP or sCD40L	• Human PMPs (stored 3 d, apheresis-derived) 6 h post LPS, post PMN settling, analysis after 30 min • sCD40L (recombinant) 25 μg/mL, 6 h post LPS, post-PMN settling, analysis after 30 min	• HMVEC-L damage measured as reduction of viable cells/mm^2	Pathogenic, PMPs and sCD40L activate PMN-mediated damage in LPS-primed HMVEC-L
McVey et al, 2017 [6]	In vivo • BALB/c Male, 8-12 wk	• LPS (*E coli*, 0111:B4) 2 mg/kg, ip, 2 h prior to murine platelets	• Aged murine (C57BL/6) platelets (stored up to 7 d, whole blood-derived) 10 mL/kg of 10^9 platelets/mL, iv, 2 h post LPS, analysis after 6 h • ARC39-treated aged murine (C57BL/6) platelets (stored up to 5 d in the presence of the ASM inhibitor ARC39 (10 μmol/L), whole blood-derived) 10 mL/kg of 10^9 platelets/mL, iv, 2 h post LPS, analysis after 6 h • Murine ASM-deficient (*smpd1*$^{-/-}$) platelets (stored up to 7 d, whole blood-derived) 10 mL/kg of 10^9	• BAL protein levels • Lung W/D weight ratio • Lung MPO activity • Lung histology (microscopic lung damage score)	Pathogenic, ceramide accumulates in platelets and mediates TRALI • Aged WT platelets mediate TRALI, whereas ARC39-treated platelets or ASM-deficient platelets alleviate TRALI

Table 2 *(continued)*

Study	Study type	First hit	Second hit	Readouts TRALI	Transfused platelet involvement in TRALI
			platelets/mL, 2 h post LPS, iv, analysis after 6 h		

Indicated symbols (*, #) should only be compared within each row. Bloodless extravascular lung water = [wet lung weight/(dry lung weight, corrected for the dry weight of blood remaining in the lung using hemoglobin levels)]. EVPE = ^{125}I-albumin radioactivity in the lung homogenate – (^{125}I-albumin concentration in plasma sample × calculated plasma volume in lungs) in ^{125}I-albumin–instilled mice. Pulmonary edema index = lung weight in grams/rat weight in kilograms. LYS-eGFP = enhanced GFP gene expression controlled by the lysozyme M promoter. ABG, arterial blood gasses; LTB_4, leukotriene B_4; PA, pulmonary artery.

within 2 hours postinduction, the presence of pulmonary PMN infiltration, and the occurrence of pulmonary edema. Also, IL-10 levels have been described to be low [33,34] and CRP levels have been described to be increased [35,36] in human TRALI patients.

The 34-1-2S TRALI models have been extensively used to investigate the contribution of recipient platelets in the pathogenesis of TRALI, a notion that seems reasonable based on the capability of platelets to elicit a wide range of immune functions. Strikingly, several discrepancies have arisen regarding the role of recipient platelets in affecting TRALI responses by 34-1-2S [11]. Some studies have found recipient platelets to be pathogenic [29,37], whereas other studies found recipient platelets to be dispensable for the onset of TRALI [38,39] or not completely dispensable but with limited pathogenic involvement [30,31]. An early study by Looney et al found that, in LPS-primed mice, in vivo platelet depletion using a rabbit antimouse platelet serum administered 2 hours before TRALI induction with 34-1-2s protected against TRALI [29]. In addition, treatment with aspirin also prevented the onset of TRALI; however, blocking of P-selectin with an anti–P-selectin antibody or inhibition of macrophage-1–dependent platelet-PMN interactions had no effect on the prevention of TRALI [29]. Strait et al also used a similar rabbit antimouse serum to deplete platelets before TRALI induction and confirmed these findings [38], however, instead of just antibody-induced thrombocytopenia, they hypothesized that the antiplatelet antibodies blocked the development of TRALI by either depleting complement or desensitizing effector cells [38] . When they administered the rabbit antimouse serum 2 days and 1 day prior to 34-1-2S infusion, they did not find an effect of platelet depletion on the occurrence of TRALI [38]. In addition, they also depleted platelets in vivo using a non-immune mechanism through treatment with neuraminidase and found that this also did not affect the onset of TRALI [38]. There were remarkable differences noted between the methodologies used by Looney et al and Strait et al for these platelet-specific experiments. Strait et al did not apply a first hit and the duration of TRALI in the model was 30 minutes, whereas Looney et al primed mice with a low dose of LPS as a first hit and analyzed the data after a TRALI duration of 2 hours. In addition, both studies also used different readouts for lung injury. The study of Looney et al used bloodless extravascular lung water, extravascular plasma equivalents (EVPE), and lung myeloperoxidase (MPO) activity (indicating PMN infiltration) [29], whereas Strait et al used Penh analysis and the percentage water weight of the lungs [38]. Interestingly, the laboratory of Looney (Caudrillier et al) also found an important role for platelets as inducers of NETs in murine TRALI [37]. Platelet functions were targeted in vivo using tirofiban and aspirin, and both treatments decreased NET formation and associated platelet sequestration. In the case of tirofiban, the link with lung injury was also investigated, and this was found to protect against TRALI. Another study, by Thomas et al, also reported the presence of NETs in murine TRALI; however, depletion of platelets with neuraminidase did not prevent NET formation in the lungs [39]. This discrepancy between the studies by Caudrillier et al and Thomas et al is particularly challenging to analyze, as the data of the neuraminidase experiment by Thomas et al were not shown and no details were provided. Furthermore, Hechler et al thoroughly investigated the involvement of recipient platelets in LPS-primed and 34-1-2S–induced murine TRALI by using a wide variety of platelet-targeting approaches [30]. In vivo platelet depletion was efficiently achieved by administrating diphtheria toxin (DT) to platelet factor 4 (PF4)-Cre/inducible DT receptor (iDTR) mice. The authors also administered a rat anti-GPVI monoclonal antibody (clone JAQ1) to mice to induce specific depletion of the GPVI receptor [40]. In addition, the mice were also treated with aspirin or clopidogrel or transfused with GPIIb/IIIa-deficient platelets post-DT treatment. They consistently found that all these platelet-targeting strategies did not prevent the occurrence of TRALI, suggesting that recipient platelets are dispensable for TRALI induction [30]. The authors did, however, observe that the development of lung hemorrhages was inhibited in the 3 experimental regimens to deplete platelets: DT treatment of the PF4-Cre/iDTR mice, anti-GPVI treatment, or upon GPIIb/IIIa-deficient platelet transfusions [30]. This suggested that recipient platelets may play a limited pathogenic role in the development of TRALI. In addition, Hechler et al [30] were unable to confirm that aspirin treatment protected against TRALI, in contrast to the studies of Looney et al [29] and Caudrillier et al [37]. LPS priming was performed in both studies; however, the readouts for lung injury were also different. Hechler et al used blood oxygen levels, bronchoalveolar lavage (BAL) protein levels, and lung histology (without using a damage score), whereas Looney et al used bloodless extravascular lung water, EVPE, and lung MPO activity. It cannot be excluded that the use of different readouts, at least to some extent, could significantly affect the assessment and conclusion of the occurrence of significant lung injury. A recent study by Cognasse et al also examined the involvement of recipient platelets in 34-1-2S–mediated TRALI [31]. This study depleted platelets in vivo using a rat anti-GPIbα polyclonal Ab, or they inhibited platelet activation by pretreating mice with the PAR4-pathway inhibitor ML354 [31]. Both platelet-targeting interventions did not prevent the onset of TRALI; however, they did reduce the severity of the disease [31]. All these studies investigating the involvement of recipient platelets in 34-1-2S–mediated TRALI are summarized in Table 1.

Overall, discrepancies are present in these studies, which all used 34-1-2S to induce murine TRALI. Not all studies primed mice with LPS, and the studies that utilized LPS did not all use the same type of LPS (Table 1). In addition, most studies had a TRALI duration of 2 hours, but there was also a study which only monitored TRALI up to 30 minutes (Table 1). Remarkably, there was a very high degree of variation in the readouts that were used to assess the lung injury (Table 1). Another important factor which may also be contributing to the heterogeneity may be the gastrointestinal microbiota. It was demonstrated that mice housed in a barrier-free animal facility were susceptible to 34-1-2S–dependent TRALI induction, whereas mice housed in a more sterile specific pathogenic-free (SPF) animal facility were resistant to antibody-mediated TRALI [32]. This resistance in SPF-housed mice could be overcome by priming the mice with LPS or by conducting fecal transfer using fecal matter from susceptible barrier-free housed mice [32]. It is also likely that one SPF animal facility may harbor a different microbial environment than another SPF animal facility, which may possibly contribute to the observed heterogeneity of TRALI results. This may perhaps be reflected by the fact that there are several examples of mortality occurring in 34-1-2S–based TRALI models in certain SPF animal facilities (eg, [31]), whereas in other SPF animal facilities, mortality does generally not occur (eg, [9]). The nature of the recipient platelet response in TRALI may therefore be dependent on the composition of the gastrointestinal microbiota.

Despite these conflicting findings, it appears conceivable that recipient platelets have a pathogenic involvement in TRALI, but the extent of this involvement may be limited.

An interesting recent study found an important role for platelets in preventing endothelial cell leakage during leukocyte diapedesis [41]. Platelets were found to dock to endothelial von Willebrand factor, preventing leakage during leukocyte extravasation by stimulating endothelial Tie-2. How this mechanism relates to a TRALI setting will be interesting to investigate.

Regarding TRALI in humans, a mild thrombocytopenia occurs in TRALI patients; thus, this supports that platelets are at least involved to some extent in the pathogenesis of TRALI [1]. Moreover, it has not been described that a low platelet count in humans is a protective factor for the onset of TRALI, indirectly indicating that the recipient platelet involvement in the induction of TRALI may be minor.

4. Transfused Platelet Involvement in TRALI

Platelet transfusions can elicit fatal TRALI reactions in humans [42]. Several studies have researched the contribution of transfused platelets to the development of TRALI using in vivo/ex vivo animal models or by in vitro analysis [6,43-48]. The first hit in all these studies was priming with LPS, and the second hit was the transfusion of either platelets (stored or treated) or their derived products such as supernatants, lipids, or platelet microparticles. An important early study of Silliman et al demonstrated that lipids in stored platelets could induce TRALI in an ex vivo rat model [43]. In this study, transfusion of 5-day stored human platelet plasma, lipid extracts from human plasma, high-performance liquid chromatography (HPLC)–purified lipids from human platelet plasma, or purified lysophosphatidylcholines (lyso-PCs) all increased the pulmonary edema index, thereby contributing to the development of TRALI. An elegant study by McVey and colleagues demonstrated that ceramide accumulates in aged platelets (stored up to 7 days) and that transfusion of these aged platelets induced TRALI in mice [6]. Mice were also transfused with stored platelets (up to 5 days) which were treated with the acid sphingomyelinase (ASM) inhibitor ARC39 or with stored platelets (up to 7 days) from ASM-deficient mice, which both diminish the formation of ceramide. Compared with the wild-type aged platelets, transfusion of stored ARC39-treated platelets or stored ASM-deficient platelets alleviated the development of TRALI [6]. Using a sheep model of TRALI, Tung et al also demonstrated that transfusion of supernatant from 5-day aged human platelets induced TRALI. The factor in the supernatant responsible for this effect was not investigated, but it may be plausible that accumulated lipids were responsible as in the Silliman et al and McVey et al studies [6,43]. Furthermore, Gelderman et al found that ultraviolet B (UVB)–irradiated human platelets transfused into LPS-primed severe combined immunodeficient (SCID) mice had increased severity of TRALI (based on increased BAL protein levels and damage observed on lung histology) compared to nonirradiated platelets [44]. Remarkably, however, there was no effect on the lung wet to dry (W/D) weight ratio, which is a direct measure for the degree of pulmonary edema. Because BAL protein levels can also be increased in inflammatory settings without significant injury and the lung histology was not scored for lung damage, a more appropriate conclusion may be that UVB-irradiated platelets only have a slight tendency to increase TRALI severity. Chi et al [46] and Caudrillier et al [47] both investigated the effect of Mirasol-treated human platelets transfused into LPS-primed mice. Chi et al found that transfusion of human platelets mediated TRALI, regardless of Mirasol treatment [46], whereas Caudrillier et al concluded that Mirasol-treated human platelets did not induce TRALI [47]. Despite the apparent conflicting outcome of these studies, perhaps in part based on the use of different strains and doses of LPS, it seems that there is no direct contribution of Mirasol treatment to the development of murine TRALI. Lastly, Xie et al used an in vitro system consisting of human pulmonary microvascular endothelial cells (HMVEC-L) primed with LPS and supplemented with PMNs, and investigated the contribution of human platelet concentrate-derived microparticles (PMPs) and sCD40 in the development of TRALI [48]. TRALI was assessed by measuring HMVEC-L viability (damage), and it was found that PMPs and CD40L (which is present in high levels in PMPs) could activate PMN-mediated damage in the LPS-primed HMVEC-L [48]. All these studies investigating the involvement of transfused platelets in TRALI are summarized in Table 2.

Overall, with respect to transfused platelet involvement in TRALI, it seems that the supernatant of stored platelets and, more specifically, lipids such as ceramide can mediate TRALI in animal models (Table 2). UVB-irradiated human platelets may have increased tendency to induce murine TRALI, whereas Mirasol treatment of platelets does not directly appear to contribute to murine TRALI (Table 2). PMPs and soluble CD40 ligand (sCD40L) can activate PMN-mediated damage of pulmonary endothelial cells, but this was not further investigated in vivo (Table 2).

For the human setting, it is important to keep in mind that transfusion of lipids in stored platelets may not always elicit a TRALI reaction, as this is also dependent on the tightly regulated interplay with recipient first-hit factors. Also within the same patient, any change in inflammatory parameters may influence the ability of the accumulated lipids to trigger TRALI.

5. The Potential Link Between Human TRALI, Platelet Transfusions, and COVID-19

There are several similarities between TRALI and COVID-19. Hospitalized COVID-19 patients also develop shortness of breath and life-threatening hypoxemic respiratory failure due to pulmonary edema, also presumed to be caused by leaking pulmonary vasculature and damaged endothelium. In addition, it appears that plasma IL-6, IL-8, CRP, and NETs are also increased (eg, [49-51]), and also complement has been described to be involved in COVID-19 (eg,[52]). The contribution of platelet transfusions to the occurrence of TRALI in COVID-19 patients has, to best of our knowledge, not been investigated. Notably, however, transfusion of convalescent plasma has been shown to induce cases of TRALI in COVID-19 patients (eg,[53,54]). Further research may clarify the similarities between human TRALI and COVID-19, including the contribution of transfused platelets.

6. Conclusions

Platelets have nonhemostatic immune functions which may translate into a pathogenic or protective effect in acute lung injury or ARDS, depending on the specific triggers and the nature of the inflammatory environment. In TRALI, their involvement is less clear, as conflicting outcomes have been reported by studies investigating the contribution of recipient platelets. This heterogeneity could be due to variations in the used animal models, methods, reagents, readouts for acute lung injury, or animal housing conditions despite the fact that all these studies used the antibody 34-1-2S to induce murine TRALI. It seems plausible, however, that platelets are pathogenic in TRALI but that their involvement is minor. In vivo targeting of platelets may not prevent the onset of TRALI but limit its severity, and thus, targeting recipient platelets does not seem to be an attractive approach to search for potential therapies. It may be more important, however, to first further investigate the contribution of recipient platelets in TRALI by standardizing the experimental design, parameters, and conditions. Regarding the involvement of transfused platelets in TRALI, lipids, particularly ceramide, in stored platelets have been shown to contribute to TRALI as demonstrated in animal models. This will also need to be further validated, and the mechanisms leading to lung injury will need to be researched.

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Declaration of competing interest

None.

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