Transfusion-Related Acute Lung Injury: The Work of DAMPs*

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Summary

Current notions in immunology hold that not only pathogen-mediated tissue injury but any injury activates the innate immune system. In principle, this evolutionarily highly conserved, rapid first-line defense system responds to pathogen-induced injury with the creation of infectious inflammation, and non-pathogen-induced tissue injury with 'sterile' tissue inflammation. In this review, evidence has been collected in support of the notion that the transfusion-related acute lung injury induces a 'sterile' inflammation in the lung of transfused patients in terms of an acute innate inflammatory disease. The inflammatory response is mediated by the patient's innate immune cells including lung-passing neutrophils and pulmonary endothelial cells, which are equipped with pattern recognition receptors. These receptors are able to sense injury-induced, damage-associated molecular patterns (DAMPs) generated during collection, processing, and storage of blood/blood components. The recognition process leads to activation of these innate cells. A critical role for a protein complex known as the NLRP3 inflammasome has been suggested to be at the center of such a scenario. This complex undergoes an initial 'priming' step mediated by 1 class of DAMPs and then an 'activating' step mediated by another class of DAMPs to activate interleukin-1 β and interleukin-18. These 2 cytokines then promote, via transactivation, the formation of lung inflammation.

Introduction

Amongst the numerous possible complications, transfusion-related acute lung injury (TRALI) has emerged as the most important cause of morbidity and mortality resulting from transfusion of blood or blood components [1, 2]. In fact, TRALI is currently being regarded as the number 1 cause of severe morbidity and mortality related to blood transfusion therapy [3]. TRALI is defined as new acute lung injury (ALI) that develops during or within 6 h of receiving transfusion of any blood product, and, in its severe form, leads to death in 5–10% of cases [1, 2, 4]. Although the pathogenesis of TRALI is not completely understood and a complete mechanistic understanding of the underlying pathophysiology of TRALI has yet to be elucidated, 2 major theories have evolved to explain its pathogenesis: i) antibody-mediated TRALI and ii) the 2-hit model. More recently, a growing amount of evidence has also been provided suggesting a pathogenetical role of innate immune events in TRALI that has been shown to be strongly associated with recipient inflammatory responses induced by transfusion [5]. However, how this inflammatory response becomes activated in TRALI is still a matter of debate.

In this brief review, evidence has been collected in support of the notion that the TRALI-associated inflammatory response of the host is initiated by damage-associated molecular patterns (DAMPs) generated during collection, processing, and storage of blood/blood components. These DAMPs, after transfusion, are able to activate host pulmonary neutrophils, endothelial cells, and platelets, i.e. the cells of the host innate immune system equipped with pattern recognition receptors (PRRs).

Innate Immunity, Inflammation and the DAMPs

During the past decade, incredible progress has been made in our understanding of how microbial pathogens are sensed by innate immune pathways that represent the first line of host

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Fig. 1. Function of the injury-activated innate immune system – the 2 sides of the coin: Host innate immune defense-induced infectious or sterile inflammatory responses generally resolve the initial insult, leading to tissue and wound repair, and restoring and maintaining homeostasis. However, when uncontrolled and exaggerated, the innate immune inflammatory response can be detrimental to the host and may lead to pathologies associated with manifestation of acute and/or chronic diseases. DAMPs = damage-associated molecular patterns, PAMPs = pathogen-associated molecular patterns, PRRs = pattern recognition receptors.

defense. A limited number of highly conserved microbial motifs, known as pathogen-associated molecular patterns (PAMPs), are recognized by germ line-encoded PRRs, which are expressed in both mobile ‘classical’ immune cells, such as dendritic cells, macrophages and neutrophils, and sessile non-immune cells, such as vascular cells, epithelial cells and fibroblasts.

Of note, some of these receptors, such as the Toll-like receptors (TLRs, e.g. TLR2, TLR4) and the nucleotide oligomerization domain(NOD)-like receptor family (NLR), pyrin domain containing 3 (NLRP3), are also able to sense various endogenous molecules that arise during any cell or tissue injury, either as single entities or after forming complexes. We originally coined these ‘signals of any dangerous injury’ as DAMPs [6], as these endogenous host-derived non-microbial molecules are released following tissue injury or cell death, and have functions similar to those of PAMPs in terms of their ability to activate proinflammatory pathways. A prototypical DAMP is the ‘high mobility group box 1’ (HMGB1), a highly conserved nuclear protein, which acts as an architectural chromatin-binding factor that bends DNA and promotes protein assembly at specific DNA targets. DAMPs are normally hidden molecules and are either actively secreted by immune cells or actively released from dying cells (for review see [7]). In fact, during tissue damage, cells or nuclei lose their structural integrity, and endogenous DAMPs that are sequestered under normal conditions, such as lipids, proteins, or nucleic acids, gain access to membrane-bound and cytosolic innate immune receptors.

The innate immune system has evolved at least 5 major PRR families that cooperatively operate to recognize these exogenous PAMPs or endogenous DAMPs: the TLRs, the NLRs, the RIG-I-like receptors (RLRs), the C-type lectin receptors (CLRs), and the recently discovered AIM2-like re-

ceptors (ALRs) [8–10]. In addition, atypical PRRs, like the advanced glycation end product (AGE) receptor (RAGE) and their ligands have been identified. RAGE was initially discovered as a receptor for AGEs, such as carboxymethyl lysine (CML), but has also been found to interact with non-AGE ligands, such as HMGB1 and S100/calgranulins [11].

Although originally thought to be directed only against pathogen-induced tissue injury, the innate immune defense system is now regarded as being directed against any macro- or microtraumatic injury, including physically, chemically or environmentally induced injuries. After recognizing a PAMP or DAMP, the cells of the innate immune system react with an infectious or sterile inflammatory response. This is marked by the recruitment of inflammatory cells, particularly innate immune cells such as neutrophils and macrophages, and the production of proinflammatory cytokines, chemokines and adhesion molecules. In addition, platelets and vascular cells, e.g. endothelial cells and vascular macrophages, are integrally involved in an innate immune host inflammatory response largely because of their direct crosstalk with neutrophils.

The infectious or sterile inflammatory responses generally resolve the initial insult, leading to tissue and wound repair, and restoring and maintaining homeostasis (fig. 1). Thus, inflammation is vital for host defense, not only against pathogen-induced injury but also against any injury jeopardizing a human body. However, there is another side of the coin: An innate immune inflammatory response – when uncontrolled and exaggerated – can be detrimental to the host, and may lead to pathologies associated with the manifestation of acute and/or chronic diseases, such as sepsis [12], atherosclerosis [13], arthritis (gout) [14], or Alzheimer’s disease [15]. There is increasing evidence suggesting that transfusion-related acute injuries, in particular TRALI, have to be added to those innate immunity-induced pathologies [5, 16, 17].

The Inflammasomes

Whereas PRRs engage different signaling cascades that lead to proinflammatory gene expression via transcriptional processes, transcription-independent events such as activation of proteases and/or phagocytosis are also initiated at the same time. These processes take place at molecular platforms called inflammasomes. The inflammasomes make a considerable contribution to the establishment of tissue inflammation. These platforms consist of intracellular multiprotein complexes whose essential components are a sensing recognition receptor, the adapter protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), and the inflammatory protease caspase-1. Activation of the inflammasome by different types of tissue damage (DAMPs) or by pathogen-associated motifs (PAMPs) results in the autocatalytic cleavage of caspase-1. Once activated, caspase-1 cleaves the biologically inactive precursors (pro-forms) of interleukin-1 β (IL-1 β) and IL-18, generating the biologically active cytokines [18, 19].

The prototypical NLRP3 inflammasome has gained much attention due to its various and prominent functions not only in antimicrobial responses but also in sterile inflammatory responses. It is predominantly located in macrophages but has also been found in neutrophils [20] and endothelial cells [21]. In fact, NLRP3 is the most extensively investigated NLR family member and is believed to be a general sensor of a large variety of PAMPs and DAMPs, including high concentrations of endogenous extracellular adenosine triphosphate (eATP) and certain (exogenous and endogenous) microparticles.

Current notions hold that full activation of the NLRP3 inflammasome requires 2 steps, an initial priming step and then an activating step [18, 19]. The priming step leads to the up-regulation of NLRP3 expression and also induces pro-IL-1 β expression, thus controlling the threshold of inflammasome activation. Of note, the initial stimuli/signals priming NLRP3 include all ligands (i.e. PAMPs of bacterial or viral origin, and DAMPs) for PRRs, such as TLRs, RLRs, and NLRs, which – via PRR-triggered transcriptional processes (i.e. NF- κ B activation) – lead to enhanced up-regulation of NLRP3 expression and induction of proIL-1 β mRNA/proIL-1 β levels prior to the inflammasome activation. For example, numerous microbe-derived PAMPs, in particular lipopolysaccharide (LPS) but also single- and double-stranded RNA, peptidoglycans, and CpG DNA, have been reported to activate the NLRP3 inflammasome [22, 23]. In fact, stimuli signaling via either the adapter protein myeloid differentiation factor 88 (MyD88) or Toll/IL-1 receptor (TIR)-domain-containing adapter-inducing interferon- β (TRIF) can induce expression of NLRP3 [24, 25]. Besides certain PAMPs, such as LPS, these stimuli would include the DAMPs HMGB1 and HSP70 known to trigger transcriptional pathways through TLR4-recruited MyD88 and TRIF.

The activation of NLRP3 itself is distinct from this initial priming step. However, despite the precise nature of the inflammasome complex, immunologists know very little about how it is activated. Although the leucine-rich repeats (LRRs) of other PRRs have been shown to directly bind their cognate activators, this is unlikely to be true for NLRP3, given the sheer number of physically and chemically diverse stimuli that have been shown to induce NLRP3-dependent IL-1 β secretion [19].

Nevertheless, a number of studies have suggested 3 possible means by which the inflammasome is activated. In reality, however, these factors are probably non-exclusive. First, efflux of potassium, which is initiated by pore-forming toxins, membrane disruption, or ligand-triggered channels, has been shown to lead to NLRP3 inflammasome assembly. For example, as often seen during cell death, high extracellular concentrations of ATP, a known potent activator of the NLRP3 inflammasome, reduce intracellular K⁺ concentration by approximately 50% [26]. NLRP3 activation by this mechanism is thought to occur via binding of eATP to the 2-transmembrane ionotropic purinergic receptor P2X7 leading to K⁺ efflux mediated by the P2X7-receptor-associated cation hemi-channel pannexin-1 [27, 28].

Phagosomal microparticles (crystals, crystalline material particles, protein aggregates) are another class of stimulating DAMPs that activate NLRP3, although they can also provoke inflammation through inflammasome-independent pathways [29]. The demonstration of NLRP3 activation and IL-1 β release by monosodium urate (MSU) crystals and calcium pyrophosphate dehydrate (CPPD) crystals was a milestone in inflammasome research [30]. Sensing of MSU and other crystalline particles such as silica and asbestos by NLRP3 seems to be a general phenomenon and is not restricted to exogenous inorganic material [19, 20]. It has been hypothesized that lysosomal rupture in response to crystals activates NLRP3. In fact, NLRP3 activation by microparticles requires binding and uptake by phagocytosis. Several sets of data suggest a common mechanism of crystal-induced activation of the NLRP3 inflammasome whereby lysosomal perturbation, not the crystal structure itself, is sensed. According to this model, particles such as silica or MSU induce lysosomal damage and leakage that is perceived as a DAMP by the innate immune system. Cathepsin B may gain access to the cytosol to induce the cleavage of an as-yet-unidentified substrate, which in turn leads to NLRP3 activation [31].

Lastly, it has been demonstrated that production of reactive oxygen species (ROS), which are highly evolutionarily conserved danger signals that are triggered by numerous means, leads to indirect or even direct activation of NLRP3. Indeed, elevated ROS production has been observed upon treatment with many NLRP3 activators tested to date. Thus, it has been shown that phagocytosis of microparticles by macrophages results in generation of ROS [32], and ATP-induced inflammasome activation has also been linked to ROS [33].

Pathogenesis of TRALI

Recently competent review articles have been published that are devoted to current insights into mechanisms of pathogenesis of TRALI [5, 17, 34–41]. Quoting briefly from these articles, TRALI primarily consists of acute bilateral pulmonary edema occurring within 6 h of a transfusion in the absence of cardiac failure or intravascular volume overload. The typical clinical presentation is respiratory distress, hypoxia/hypoxemia, and hypotension 1–6 h after transfusion initiation, and is normally associated with fever. Pathohistologically, the pulmonary microvasculature appears to be the site of TRALI, as plausibly explained by the fact that the capillary network of the lungs is the first to be reached by transfused blood and blood components.

Both neutrophils passing the lung and the pulmonary endothelium appear to play a central role in the development of the TRALI reaction. Indeed, several studies have lent support to the notion that neutrophil accumulation/activation or endothelial activation, or both, induced by multiple mechanisms in the lung leads to an inflammatory state of the pulmonary microvasculature, resulting in capillary leaks that, in turn, lead to pulmonary edema. Neutrophilic aggregates in the pulmonary vasculature are the most significant histopathology findings both in deceased patients and in experimental lung sections. Inflammation of the microvasculature of the lung is thought to be mediated by both activated endothelial cells and activated neutrophils via multiple damaging events including secretion of cytokines and other proinflammatory mediator substances, production of ROS, and secretion of proteases. In addition, some recent studies have suggested that platelets sequestered in the lungs may also be involved in neutrophil/endothelial cell-mediated vascular inflammation.

As generally quoted in the literature, the exact activation mechanisms of TRALI induction are still poorly understood. An immunity-oriented, antibody-mediated mechanism has been implicated in most cases of TRALI. In a minority of reported cases, however, an antibody could not be identified and, therefore, an alternative ‘2-hit’ concept has been postulated and is still being discussed. According to current knowledge, both mechanisms presumably occur and, basically, TRALI is thought to just represent the final common pathway of neutrophil priming/ activation, endothelial injury/inflammation and capillary leakage, i.e. a phenomenon that can be triggered either by antibodies or other biological response modifiers, or even both, in patients with or without underlying risk factors.

Antibody-Mediated Model of TRALI Induction

In view of the early observation that the plasma-rich components, such as fresh frozen plasma and apheresis platelets, have been most frequently implicated in TRALI induction, as well as on the basis of the frequent detection of anti-leukocyte antibodies in implicated units and the increased risk of previ-

ously alloimmunized donors, an antibody-mediated model of TRALI induction was postulated. This concept suggests that passive transfer of existing donor antibodies such as leukoagglutinating antibodies via transfusion of plasma containing blood components results in binding to cognate antigens (including HLA class I and II and non-HLA neutrophil-specific antigens) expressed on the patient’s (host’s) neutrophils. As a consequence, antibody-bound neutrophils are activated, aggregated and sequestered in the pulmonary microvasculature where complement activation and release of neutrophilic ROS and other neutrophil bioactive products causes endothelial damage, resulting in inflammation. The inflamed endothelium would then allow leakage of fluids into the pulmonary space producing the clinical symptoms of TRALI. In fact, leukocyte antibodies had been identified in the implicated donor in 65–90% of reported clinical cases of TRALI [42–46].

‘2-Hit’ Theory

The clinical observation that not all patients transfused with a cognate antibody to neutrophil antigens develop TRALI led to another theory of its pathogenesis, the ‘2-hit’ theory, a term originally coined by Silliman et al. [42]. Subsequent studies [44–46] lent further support to the assumption that TRALI may be caused by a ‘2-event’ mechanism. The model postulates that the first hit, a priming step, consists of an initial injury to the pulmonary endothelium that leads to endothelial activation, resulting in the activation, adherence and sequestration of the patient’s own neutrophils in the pulmonary capillary beds. Pre-existing clinical conditions that could cause the initial proinflammatory endothelial activation have been identified, and include severe infection and sepsis, recent surgery, trauma or mechanical ventilation. The second hit then is mediated by transfusion of blood or blood components, and may comprise the introduction of ‘biological response modifiers’, such as reactive lipid molecules or leukocyte antibodies, that accumulate during storage. This second event is then thought to further activate primed neutrophils, resulting in extended injury to the pulmonary microvasculature, i.e. in a process that ultimately leads to capillary leakage of fluids into the pulmonary bed that produces the well-known clinical symptoms of TRALI.

Of interest in this context are studies on ‘2-hit’ animal models showing that stored blood products are able to induce pulmonary injury after priming of the lungs with LPS [47–49]. In the experimental models used, pretreatment of rats with intraperitoneal LPS was observed to increase neutrophil sequestration in the lung microvasculature and to augment neutrophil ROS production, elastase release, and endothelial intercellular adhesion molecule expression [50]. In another set of studies, LPS infusion into mice caused endothelial activation and accumulation of neutrophils on lung endothelium both in vitro and in vivo. The adherent neutrophils were only stimulated to become fully activated and to mediate lung damage if the LPS was presented by platelets via TLR4 [51].

Table 1. Demonstration of DAMPs (and their known PRRs) that are potentially generated/released during collection, processing and storage of blood/blood components and known to activate the innate immune system

Potential DAMPs (and their corresponding PRRs)	Stored RBCs	Stored platelets	Stored FFP	References ^a
Heme (TLR4)	X	Ø	Ø	56, 57
eATP (NLRP3)	X	(X)	not determined	60, 75–77
HMGB1 (TLR2/4, RAGE)	X	(X)	not determined	62, 63
Lipid peroxidation products, e.g. oxLDL (TLR4)	X	not determined	not determined	67, 68
AGE-N-CML (RAGE)	X	not determined	not determined	61
Microparticles (NLRP3)	X	X	X	58, 59, 72–81

DAMPs = damage-associated molecular patterns, PRRs = pattern recognition receptors, RBC = red blood cell, FFP = fresh frozen plasma, AGE = advanced glycation end products, eATP = extracellular adenosine triphosphate, TLR2/4 = Toll-like receptor 2/4, NLRP3 = NOD-like receptor family, pyrin domain containing protein 3, oxLDL = oxidized low density lipoproteins, CML = carboxymethyl lysine, RAGE = receptor for AGEs, X = proven, (X) = indirectly assumed, Ø = not valid.

^aThe references are related to the potential DAMPs only.

In conclusion, as also similarly stated elsewhere [40], recent developments in understanding the pathogenesis of TRALI include specification of the role of different leukocyte antibodies involved as well as the design of models referring to multipliers and attenuators of TRALI. In this regard, the ‘2-hit’ model represents a promising and currently popular notion about TRALI pathogenesis and is supported by evidence derived from various experimental models.

TRALI Pathogenesis in Light of the Innate Immune System

Introduction

At the center of modern knowledge about TRALI pathogenesis is the recognition that TRALI is strongly associated with patient (host) inflammatory responses induced by transfusions of blood products, and is mediated, in particular, by the patient’s own activated neutrophils that are passing lung and pulmonary endothelial cells. However, how neutrophils and endothelial cells become activated in TRALI is still a matter of debate. Here, I add a further contribution to this on-going debate by postulating that TRALI reflects an acute autoinflammatory, innate immunity-mediated disease of the lung caused by the patient’s own PRR-bearing neutrophils, endothelial cells, platelets and macrophages that are activated after recognition of DAMPs transmitted via transfusion of donor blood or blood components. Evidence supporting this working hypothesis is explored below.

RBC Storage Lesions and Generation of DAMPs

As reviewed previously [52, 53], red cell storage lesions are characterized by changes in red blood cells (RBCs) during storage, which can be considerably aggravated by contaminating white blood cells.

Over time, glucose in stored blood is consumed and levels of 2,3-diphosphoglycerate and ATP decrease, while potassium levels increase. As a result, there is loss of RBC mem-

brane during storage that leads to substantial changes in rheological properties. Accordingly, stored RBCs become more fragile as they age. This fragility associated with loss of RBC integrity leads to hemolysis, which is well known to occur during processing and storage of RBCs [54]. Of note, abnormal hemolysis in individual RBC units has been observed and may be caused by several factors including inappropriate handling during processing of blood, inappropriate storage conditions, bacterial hemolysins, antibodies that cause complement lysis, defects in the RBC membrane, or an abnormality in the blood donor [55]. Moreover, upon transfusion, it is likely that additional hemolysis and microparticle formation occur due to breakdown of fragile RBCs.

Consequently, hemolysis is associated with the generation of potential DAMPs that are either released, e.g. heme, ATP and HMGB1, expressed, e.g. N^ε-CML, or newly formed, e.g. distinct microparticles (table 1). Heme is a derivate of free hemoglobin (Hb) of the cell that is released with increasing RBC storage time [56]. Interestingly, on circulation in vivo, heme is normally scavenged by scavenging systems led by hemopexin, which bind to free heme and deliver it to liver, where it is degraded by the enzyme heme oxygenase. However, the scavenging of free heme by hemopexin in cases of moderate to intense hemolysis collapses, resulting in the accumulation of free heme in circulation [57]. By analogy, during RBC storage, scavenging of free heme can be assumed to fail due to a lack of hemopexin.

Microparticles are also generated with increasing hemolysis during prolonged RBC storage (and probably upon transfusion). Fragmentation and formation of 50- to 100-nm microparticles containing a substantial amount of Hb (Hb-containing microparticles) have been found to occur [58, 59].

eATP is an important DAMP that has been shown to be released in substantial quantities during lysis of erythrocytes [60]. This interesting study provided evidence showing that massive lysis of erythrocytes can result in the release of sufficient quantities of ATP to activate the P2X7 receptor on T cells. Further potential DAMPs generated during RBC stor-

age include AGEs, the RAGE ligand, and N^e-CML, a molecule on the surface of stored RBCs that was described to be 1 functional consequence of the storage lesion [61].

Another DAMP generated during RBC storage is HMGB1 that could be demonstrated in studies on packed RBCs [62]. In this study, HMGB1 levels were shown to be elevated in both leuko-reduced and non-leuko-reduced packed RBCs. HMGB1 levels increased with duration of RBC storage, and leuko-reduced procedures attenuated HMGB1 levels in packed RBCs by approximately 55%. By analogy, HMGB1 levels can be assumed to be elevated in stored platelet units since studies have revealed that HMGB1 is an endogenous protein in human platelets [63].

Of interest in this context is also a family of DAMPs (recognized by different PRRs) that encompasses oxidation-specific epitopes, such as phosphocholine of oxidized phospholipids and malondialdehyde, which form oxidized low-density lipoproteins (oxLDL) [64, 65]. These DAMPs are generated as a consequence of lipid peroxidation, known to occur in stored blood and blood components [42, 66–68].

Apheresis Platelet Concentrates, Fresh Frozen Plasma and the Generation of DAMPs

Apart from RBC storage, the generation of DAMPs can also take place in plasma-rich components, such as apheresis platelet concentrates and fresh frozen plasma. First of all, any freshly drawn blood sample is already damaged during early stages of collection and processing. For example, slight changes in collection or centrifugation procedure, e.g. the use of serum samples instead of EDTA plasma or a delay of centrifugation of whole blood, may lead to generation of DAMPs, as demonstrated for soluble HMGB1 [69].

Moreover, generation of DAMPs in the form of microparticles in plasma-rich components is substantial, and their levels can be affected by many sample-processing steps, ranging from collection of blood and centrifugation of samples to storage of the various components [70, 71]. Thus, microparticles have been reported to be released in stored platelets [72, 73] and have recently been impressively demonstrated in apheresis platelets [74]. In addition, in stored platelets, indirect evidence suggests the release of ATP [75–77]. Microparticles have also been detected in fresh frozen/thawed plasma [78–80] (table 1). Of note, in a recent study, microparticles were found in stored fresh frozen plasma, peaking at 14 days after onset of storage at –80 °C, with a subsequent decline over the next 2 months [81].

DAMPs-Induced Inflammatory Responses Potentially Operating in TRALI

Overwhelming evidence from research work in the field of innate immunity clearly indicates that PAMPs and DAMPs induce and maintain infectious and sterile tissue inflammation via activation of a large variety of PRR-bearing cells of the innate immune system [7, 82, 83]. Most PRRs respond to ex-

ogenous PAMPs or endogenous DAMPs by triggering activation of proinflammatory transcription factors, including NF- κ B, activating protein 1 (AP1), cAMP response element-binding (CREB), and interferon regulatory factors (IRFs). Induction of proinflammatory genes encoding cytokines, chemokines, adhesion molecules, ROS-producing enzyme systems, and regulators of the extracellular matrix promotes the recruitment, attraction and activation of neutrophils, which are critical for eliminating microorganisms and other foreign particles as well as host cell debris. As outlined above, a subset of PRRs forming the core of inflammasomes activates the protease caspase-1, which causes maturation of the cytokines IL-1 β and IL-18. Cell adhesion molecules and chemokines expressed and secreted by both activated PRR-bearing leukocytes and endothelial cells facilitate leukocyte extravasation from the circulation to the affected site in response to tissue injury. In fact, leukocyte extravasation within injured tissue can be regarded a highly dynamic, interactive, and coordinated process that plays a central role during the inflammatory response of the innate immune system. The interaction of activated neutrophils with the activated endothelium under shear forces is comprised of many sequential events, each involving specific leukocyte and endothelial recognition receptors, as well as adaptor and signaling molecules within PRR-triggered innate immune pathways leading to activation of those proinflammatory transcription factors.

Platelets, which are anucleate but complex and versatile cells that are also equipped with PRRs, operate via intercellular interactions as key effectors and amplifiers in innate immunity-mediated inflammation [84]. Platelets have been shown to store and release a wide range of biologically active substances including cytokines, chemokines and adhesion molecules, and are a major source of proinflammatory molecules [85]. In addition, both interstitial and vascular macrophages, the master cells in inflammation expressing a large variety of PRRs, play a crucial role in innate immunity-mediated tissue inflammation including vascular wall inflammation [86, 87].

In TRALI, such a global scenario of innate immunity-induced inflammation may take place in the region of the pulmonary microvasculature through the involvement of distinct DAMPs generated during collection, processing and storage of blood/blood components and which interact with PRRs expressed on neutrophils and platelets that are passing the lung as well as pulmonary endothelial cells and macrophages (fig. 2).

For example, the DAMP heme, released during hemolysis of stored RBCs and recognized by TLR4 [7, 88, 89] possesses proinflammatory capabilities and plays a central role during the onset and/or persistence of inflammation, in part via activation of neutrophils [90, 91]. Experimental studies in rats also indirectly support the notion that heme may contribute to a transient inflammatory reaction in the lung during blood transfusion [92].

Fig. 2. Working hypothesis of the pathogenesis of TRALI. DAMPs, generated during processing and storage of blood-blood components and administered via blood product transfusion, are sensed by pulmonary PRR-bearing neutrophils (PMN) and probably also platelets, endothelial cells (ECs) and macrophages (MØ) (see also text).

Similarly, microparticles, basically considered to reflect a broad primitive response to stress, have been found to exert proinflammatory effects [93–95]. In fact, circulating microparticles promote cellular crosstalk in various pathological settings such as inflammation by conveying a broad spectrum of bioactive molecules. In particular, microparticles derived from stored RBCs have been shown to contribute to neutrophil priming and activation, thereby enhancing the inflammatory response observed in patients who receive older RBC units during transfusion [96].

eATP, when defined as a proinflammatory DAMP generated during blood storage, is of particular importance. There is increasing evidence suggesting a role in inflammation of the purinergic signaling system, which includes the action of eATP and P2 receptors [97]. In fact, recent animal studies have provided convincing evidence indicating that ATP is present in inflamed tissues *in vivo* at extracellular concentrations sufficient for P2 receptor activation. Increased eATP levels amplify inflammation *in vivo* by promoting leukocyte recruitment and, as mentioned above, by NLRP3-inflammasome activation via P2X7 [97].

The AGE N^ε-CML – interacting with RAGE – may represent another example of a DAMP able to induce inflammation of the pulmonary microvasculature potentially mediated by transfused RBCs. Evidence has been provided suggesting that stored RBCs can induce inflammation-promoting ROS in pulmonary endothelial cells through interaction with RAGE, leading to (inflammation-mediated) endothelial injury [61].

The DAMP HMGB1, also found to be generated during RBC storage, may represent another candidate contributing to TRALI. During the past decade, a panoply of reports referred to the fact that HMGB1 mediates the activation of innate immune responses by playing a critical role at the intersection of the host inflammatory response to sterile and infectious insults [98]. HMGB1 is actively secreted by stimulation of the innate immune system with exogenous pathogen-de-

rived molecules, and is passively released by severe cell injury in the absence of pathogen invasion. Established molecular mechanisms of HMGB1 binding and signaling through TLR4 and/or TLR2 have revealed signaling pathways that – via activation of proinflammatory transcription factors – mediate the release of a range of proinflammatory mediator substances including cytokine and chemokines.

Last but not least, the oxidation-specific epitopes of DAMPs, also known to be generated in stored blood components, may play a role in TRALI pathogenesis. For example, oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (oxPAPC) was shown to induce an inflammatory response in alveolar macrophages via TLR4 [99]. Furthermore, the binding of oxLDL to CD36 has been shown to trigger an inflammatory response through the assembly of a TLR4/TLR6 heterodimer [100].

Linking the ‘2-Hit’ Model of TRALI to the ‘2-Step’ Activation of the NLRP3 Inflammasome

As mentioned above, the ‘2-hit’ pathogenic model for the development of TRALI postulates an initial ‘priming hit’ through a pre-existing clinical conditions, e.g. sepsis, recent surgery or mechanical lung ventilation, that causes initial proinflammatory leukocyte/endothelial activation, and a ‘second hit’, mediated by transfusion of stored blood or blood components, that introduces ‘biological response modifiers’, ultimately leading to clinical manifestation of the acute disease.

On the other hand, as noted above, full activation of the NLRP3 inflammasome requires 2 steps, an ‘initial priming step’ mediated by DAMPs interacting with PRRs, such as TLR2 and TLR4, and a ‘second activating step’ mediated by another class of DAMPs interacting with the NLRP3 receptor.

On the level of innate immune events, a ‘2-hit’ scenario is already known from experimental studies in mice: a primary burn injury primes the innate immune system as indicated by enhanced TLR4 responsiveness (‘first hit’) for a lethal (other-

Fig. 3. Scenario model: The 2-hit-hypothesis of TRALI in light of the NLRP3 inflammasome activation in pulmonary neutrophils and endothelial cells: collaboration between PAMPs and DAMPs or between 2 classes of DAMPs (see also text, section *Linking the '2-Hit' Model of TRALI to the '2-Step' Activation of the NLRP3 Inflammasome*). AP-1 = activating protein-1, eATP, extracellular adenosine triphosphate, HMGB1 = high mobility group box 1, IL-1R = interleukin-1 receptor, IL-1 β = interleukin-1 beta, IRF3/7 = interferon regulatory factor 3/7, NLRP3 = NOD-like receptor family, pyrin domain containing 3, PAMPs = pathogen-associated molecular patterns, TIR = Toll/Interleukin-1 receptor, TLR2/4 = Toll-like receptor 2/4.

wise nonlethal) inflammatory response to LPS challenge after 7 days ('second hit') [101]. These experimental findings suggest that the clinically observed propensity of some seriously injured burn patients to respond with excessive inflammation to further innate immune activation, such as a secondary infection, resulting in multiple organ failure, might be explained in part by initial injury-induced priming of TLR responses [102]. We have suggested that a '2 (multiple)-hit' mechanism also occurs in the development of chronic allograft dysfunction, which can be regarded as a model disease of innate immunity [103].

In view of these scenarios, it is tempting to speculate that there may even be a link between the '2-hit' model of TRALI and the '2-step' activation of the NLRP3 inflammasome in the different types of innate immune cells in the lung, i.e. neutrophils passing the lung as well as pulmonary endothelial cells and macrophages (fig. 3). Clinical conditions identified as priming conditions for TRALI (severe infection/sepsis, trauma, recent surgery or mechanical ventilation) are associated with the generation of PAMPs and/or DAMPs that may stimulate PRRs (e.g. TLR2 and/or TLR4) by initiating the priming step of the NLRP3 inflammasome. For example, the PAMP LPS and the DAMP HMGB1 have been measured in the plasma of patients suffering from severe sepsis [104, 105]. In addition, elevated plasma levels of HMGB1 have been found in severe trauma patients [106] as well as after major elective general surgery, and have been observed to be related to surgical stress such as operative time and blood loss. The level remained high over time in patients with postoperative complications, suggesting progression of the complication [107]. Elevated HMGB1 levels were also found in bronchoalveolar lavage fluid of patients during mechanical ventilation and ventilator-associated pneumonia [108]. It is conceivable that the DAMP heme transmitted by blood transfusion may also initiate the priming step of the NLRP3 inflammasome.

Full activation of the NLRP3 inflammasome in pulmonary neutrophils, endothelial cells and macrophages then is achieved via transfused DAMPs such as microparticles and/or eATP, which are known to activate the NLRP3 receptor as outlined above. Subsequent production of IL-1 β and IL-18 – together with other proinflammatory mediator substances produced via transactivation processes – contributes to the creation of an inflammatory milieu in the pulmonary microvasculature, leading to lung edema.

Conclusions

The selected findings discussed briefly here support the notion that the release/formation of biologically active DAMPs in stored blood/blood components are capable of activating cells expressing PRRs (neutrophils, platelets, endothelial cells, and macrophages) in the lung of the patient during transfusion, thereby inducing inflammation in the pulmonary microvasculature leading to TRALI (fig. 2). Potential DAMPs generated in different stored blood components may operate through distinct patterns in the various clinical manifestation of TRALI, with regard to time of onset, duration and intensity of the acute life-threatening disease. Considering a presumed antibody-mediated TRALI, anti-leukocyte antibodies present in blood/blood components may elicit the expression/release of DAMPs from damaged leukocytes that are passing through the lung. In this case, these secondarily in vivo-generated DAMPs, similar to DAMPs generated in vitro during storage, may induce the same pathogenetical scenario of TRALI without DAMPs from transfused blood products actually being present.

Finally, it is tempting to speculate a linkage between the '2-hit' model of TRALI and the '2-step' activation of the NLRP3 inflammasome (fig. 3). An initial 'priming step' of the NLRP3 activation is provided in patients suffering from se-

vere infection, sepsis or major surgery and/or polytrauma, i.e. conditions associated with the generation of PAMPs or DAMPs able to stimulate PRRs such as TLRs ('first hit'). The real 'activating step' of NLRP3 activation is provided by transfusion of stored blood products containing another class of DAMPs, e.g. microparticles and/or eATP. It is the transmission of these activating DAMPs that leads to full activation of the NLRP3 inflammasome associated with an intense, morbid, and sometimes lethal pulmonary inflammatory response. Moreover, it is conceivable that the 'priming step' as well as the 'activating step' of NLRP3 inflammasome activation are exclusively achieved by transfusion of stored blood products when these contain both 'priming DAMPs' (e.g. heme, HMGB1, oxLDL) and 'activating DAMPs' (e.g. microparticles and eATP).

Outlook

The concept that TRALI may reflect an acute pulmonary disease caused by an exaggerated uncontrolled innate immune response is in agreement with other recent reports on an expanding spectrum of acute and chronic inflammatory diseases considered to be 'autoinflammatory' diseases. Autoinflammatory diseases are uniquely due to the NLRP3 inflammasome activated by PAMPs and different classes of DAMPs, and are associated with a dysfunctional caspase-1 activity and secretion of IL-1 β and IL-18. The number of areas within medicine for which a role for innate immunity-mediated inflammatory responses is definitely considered is ever increasing. Therefore, it is not surprising that new paradigms for therapeutic interventions are emerging that have recently been reviewed [109]. In principle, and in brief, blocking of the DAMPs and/or PRRs concerned, as well as inhibiting efferent innate im-

mune molecules (e.g. IL-1 β as a product of the activated NLRP3 inflammasome), have to be on the list of future research work to develop innovative therapeutic strategies.

To gain a more precise understanding of the potential role of innate immune events in TRALI, studies would first have to be devoted to searching for potential DAMPs in different blood products in relation to processing methods and storage time. The aim here is to discover certain 'DAMPs' able to elicit TRALI. Subsequent studies would then be aimed at interfering with these DAMPs, using monoclonal antibodies or specific molecule inhibitors, e.g. blockade of HMGB1 using a monoclonal antibody. Lowering eATP levels, for instance by stimulating its breakdown, would be another therapeutic approach. In fact, in view of its important role in inflammation, the purinergic signaling system may open new therapeutic avenues for the treatment of inflammatory diseases such as TRALI. With regard to effective inhibition of the efferent arc of an exaggerated innate immune response, blockade of the activity of IL-1 β using anakinra or canakinumab may be considered.

Consideration of a '2-hit' model of TRALI may help investigators design and implement future treatment advances toward controlling the potential detrimental consequences of excessive innate immune stimulation of pulmonary neutrophils, platelets, endothelial cells and macrophages via PAMPs and/or DAMPs generated during severe clinical conditions preceding transfusion of stored blood/blood components.

Disclosure Statement

The author declares no competing financial interests.

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