Experimental strategies directed at inflammation and coagulation in ARDS and TRALI
Tuinman, P.R.

Citation for published version (APA):
Tuinman, P. R. (2013). Experimental strategies directed at inflammation and coagulation in ARDS and TRALI

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
General Introduction and Outline of this Thesis

Pieter R. Tuinman
Marcus J. Schultz
Nicole P. Juffermans
Part I - General introduction and Outline of this Thesis

This thesis investigates pharmacological therapeutic strategies in ARDS and in particular TRALI. The syndromes are related, but there are also distinct differences. In this introduction, the pathogenesis of both syndromes and pharmacological therapies are described, pointing out similarities and dissimilarities, thereby outlining the rationale of the therapeutic interventions which were chosen.

Acute respiratory distress syndrome

Introduction

Acute lung injury (ALI) and its more severe form acute respiratory distress syndrome (ARDS) contribute substantially to the expanding burden of critical illness within our intensive care units. Since the first description of ARDS in 1967, considerable progress has been made in understanding the pathophysiology of acute lung injury [1-4].

Pathogenesis: an extensive cross-talk between inflammation and coagulation

The pathogenesis of ARDS is characterized by uncontrolled inflammation, inappropriate activation and accumulation of leukocytes and platelets, activation of coagulation and impaired fibrinolysis, as well as altered permeability of alveolar endothelial and epithelial barriers [1,5]. Normally, inflammation and coagulation are two important host defense mechanisms against injury and infection. Inflammation and coagulation, obviously, require strict regulation, i.e., after an insult the host response must be counterbalanced by inhibition or deactivation in order to restore homeostasis. In ARDS however, these regulatory mechanisms may fail, resulting in exaggerated and sustained inflammation and coagulation. Pulmonary inflammation in ARDS patients causes remarkably similar haemostatic disturbances to those observed in systemic inflammation, as seen in septic patients.

Inflammation

Upon insult, immune cells are immediately directed to the site of injury or infection to initiate a pro-inflammatory response. Activation of the innate immune response by binding of cell injury-associated endogenous molecules (danger-associated molecular patterns, DAMPs) or of microbial products to pattern recognition receptors on the lung epithelium and alveolar macrophages, is now recognized as a potent driving force for acute lung inflammation [5,6]. An example of such a receptor is the receptor for advanced glycation end products (RAGE), which is highly expressed on alveolar epithelial type 1 cells [7]. In patients with ARDS, plasma levels of soluble (s)RAGE are associated with severity of lung injury [8].

The pulmonary endothelium is responsible for a number of physiologic functions,
including the control of vasomotor tone and the trafficking of leukocytes as a cornerstone of the innate immune system [9-11]. The quiescent state of the endothelium expresses an anti-adhesive, anticoagulant and vasodilatory phenotype, whereas endothelial activation results in a pro-adhesive, procoagulant and vasoconstrictive phenotype [9]. The interaction with neutrophils is crucial for maintaining homeostasis and eradicating bacterial and fungal pathogens [12]. The pulmonary circulation contains about 28% of the blood neutrophil pool that is available on demand for host defense [13]. The main site of neutrophil migration in the lung is the capillary bed, where, in contrast to other organs, neutrophil recruitment from blood into inflamed tissue occurs in the post-capillary venules [11,14]. The neutrophils are larger than the diameter of pulmonary capillaries and as such, their transit time in the lung is reduced, allowing for more interaction time with the vessel wall [15]. In ARDS, neutrophils adhere to the activated capillary endothelium, also referred to as capture, and marginate through the interstitium into the air space. In the airspace, alveolar macrophages secrete cytokines, e.g. interleukin (IL)-1, -6, -8 and -10 and tumor necrosis factor α, which act locally to stimulate chemotaxis and activate neutrophils [1]. It is within this inflammatory response that the lung barrier breaks down and allows transit of a protein-rich edema fluid into air spaces [1]. Furthermore, damage to epithelial cells involves the basal membrane and reduces the amount and function of surfactant. This increases alveolar surface tension, decreases lung compliance and causes atelectasis [1,16].

Platelets, coagulation and fibrinolysis

Interaction between inflammatory cells and haemostatic effector cells, such as platelet-neutrophil interaction, is important in ARDS [5,17-19]. Long considered innocent bystanders, evidence is emerging that platelets are important mediators of lung injury [20,21]. First, there is anatomic evidence that platelets are deposited in the acutely injured lung [22]. In addition, besides the release of a variety of cytokines, activated platelets display adhesion molecules and other factors with signalling properties on their surface, enabling them to interact not only with the pulmonary endothelium, but also with one another and with leukocytes in the pulmonary vessels. Platelets triggering neutrophils leading to vascular damage has been shown to be a plausible mechanism in the pathogenesis of acute lung injury [23-25]. A key molecule involved in this interaction is thromboxane-A2 [24], which is released by platelet-neutrophil aggregates [26]. Other possible mediators involved are e.g. β₂-integrin, macrophage antigen-1 (Mac-1) and soluble CD40 ligand [26-30]. In line with a role for activated platelets in ARDS, platelet inhibition, for example by aspirin, has been found to attenuate lung injury in animal models of ARDS [17,31,32].

Normally, the coagulation system is locally activated in order to limit blood loss or to prevent invading pathogens from spreading beyond initial infection. Pulmonary coagulopathy is intrinsic to ARDS. Indeed, both microvascular thrombi and alveolar fibrin deposits are hallmarks of ARDS, irrespective of its cause [1,33-36], and are much more outspoken than the
fibrin deposition associated with severe inflammation in other organs. In addition, the extent of pulmonary coagulopathy depends on the severity of lung injury [34] and is clearly linked to clinical outcome [37-39]. Changes in pulmonary coagulation and fibrinolysis in ARDS resemble those found in the systemic circulation with sepsis [36]. Pulmonary coagulopathy with lung injury is characterized by activated coagulation via activation of the tissue factor (TF)–pathway, attenuation of fibrinolysis, and enhanced breakdown and/or decreased production of the natural anticoagulants activated protein C (APC), antithrombin (AT) and tissue–factor pathway inhibitor (TFPI) (Figure 1) [33,35,40,41]. All three processes may lead to excessive alveolar fibrin depositions. In addition, the extensive cross-talk between coagulation and inflammation may further inflame the lungs [36]. Activated coagulation factors may initiate or exaggerate injury [2,42,43], impair alveolar aeration and perfusion [44] and promote fibrosis [45].

The delicate balance between protective and injurious innate and adaptive immune responses and haemostatic pathways may determine whether alveolar injury continues or is repaired and resolved [5].

**Figure 1.** Schematic and simplified presentation of coagulation, fibrinolysis and anticoagulant pathways.

The coagulation cascade is started through activation of tissue factor (TF)-factor VII (FVIIa) complex. Several coagulation factors accelerate the conversion of prothrombin to thrombin. Activated protein C (APC) can inactivate coagulation factors Va and VIIIa. Antithrombin (AT) serves to block the action of multiple coagulation factors (e.g., Xa and IIa). Tissue factor pathway inhibitor (TFPI) inhibits stepwise the activation of coagulation factors. The fibrinolytic system is designed to degrade clots and fibrin degradation products (FDP) are formed. The main inhibitor of the plasminogen activators is plasminogen activator inhibitor type (PAI)–1. +: stimulating effect; -: inhibiting effect.
Pharmacological treatments of ARDS

Pharmacological treatments

Due to the broad spectrum of mechanisms driving the development of ARDS, many clinical trials have assessed different types of pharmacologic interventions [3,16,46,47]. Whereas most drug trials in ARDS have been negative, the use of neuromuscular blockade is one therapy which has proven to be clinically effective [48]. This is likely due to a reduction in barotrauma caused by ventilator-patient dyssynchrony. Other studies suggest that targeted therapies at specific subsets of patients may be successful.

Inflammation

Because inflammation predominates in ARDS, corticosteroids have been extensively examined. Early treatment with hydrocortisone in patients with severe community acquired pneumonia prevented progression to ARDS [49]. In addition, corticosteroids have been found to have beneficial effects when given at low or moderate doses sooner than two weeks in patients with ARDS, but appear to be harmful if started later. Of interest, their use is of unclear benefit if a lung protective ventilation strategy is used [5,47,50]. Except for corticosteroids, no other anti-inflammatory therapy has been shown to conclusively impact long-term survival, although some of these agents have improved physiologic variables [3,5,16,47,50]. Other pharmacologic interventions studied are for example N-acetylcysteine, surfactant, prostaglandin E1, neutrophil elastase inhibitor and ketoconazole [3,16].

Platelets, coagulation and fibrinolysis

Platelet depletion was found to abrogate lung injury in ARDS models inflicted by acid instillation [26] and in transfusion-related acute lung injury (TRALI) [51]. Of more clinical relevance, antiplatelet agents have been shown to have anti-inflammatory effects [52]. Inhibition of platelets by acetylsalicylic acid (ASA) was also found to be protective in ARDS models [32,53]. Observational studies have shown an association between ASA and other antiplatelet drugs and reduced organ failure and mortality in the critically ill [54]. However, data on the effect of ASA on ARDS are conflicting. Whereas use of ASA was associated with a reduced incidence of ARDS in a retrospective analysis in medical ICU patients, this benefit could not be confirmed in a large multicenter observational study, when adjusted for the propensity score [55,56]. The difference between animal and human data may be due to differences in dosage used.

Activation of coagulation is both a consequence and a contributor to ongoing lung injury. In line with this, pulmonary coagulopathy has become a potential target for therapeutic interventions in patients with lung injury. The coagulation disturbances in ARDS are alike those seen in sepsis and the interest for anticoagulant strategies in ARDS is mainly derived from studies in patients with sepsis. Clinical trials inconsistently suggest beneficial effects of the systemic anticoagulants APC, inactivated recombinant factor VIIa and TFPI in patients
general introduction

17

with ARDS [57-61]. Severe systemic bleeding due to anticoagulation may have offset the possible positive effects. To our knowledge, no clinical trials have studied the effects of systemic AT, heparin or danaparoid in ARDS.

Of note, the results of clinical trials are often in contrast to very promising results in animal studies. While such a contrast can be expected in translational research, the lack of efficacy of many of these agents does raise questions, for example, whether or not these treatments may perform better when applied earlier in the time course of the disease or locally instead of systemically.

Transfusion-related acute lung injury

Introduction

Blood transfusion is one of the most frequent interventions in the intensive care unit (ICU). Up to 50% of ICU patients receive a blood transfusion [62,63]. Although lifesaving at times, blood transfusion is associated with increased morbidity and mortality in the critically ill [64,65]. One of the mechanisms underlying adverse outcome may be transfusion-related acute lung injury (TRALI) [66,67]. Observational studies in the critically ill patient population report a high mortality of TRALI, which equals that of ARDS with a staggering 45% [68-70]. Furthermore, TRALI patients had a longer length of ICU-stay and were longer mechanically ventilated [69].

Pathogenesis of TRALI: are there differences compared to ARDS?

The two-hit hypothesis

This hypothesis implicates two separate hits. First, the pulmonary vascular endothelium is activated resulting in priming of neutrophils by one or more endogenous stimuli (e.g. trauma or sepsis), which are considered the first event [71]. These primed neutrophils are hyper-reactive. Agents that do not normally activate polymorphonuclear leukocytes, can cause release of the microbicidal arsenal by these primed and sequestered neutrophils [72]. The second event is the blood transfusion. It is thought that HLA- or HNA-antibodies or bioactive lipids which have accumulated during prolonged storage of the blood product can cause activation and neutrophil-mediated toxicity of the vascular endothelium resulting in capillary leak and lung injury. Neutrophils can be repeatedly primed, so that they reach a threshold, where a second hit (e.g. transfusion) results in neutrophil activation and capillary leak [71,72]. This threshold model is supported by the clinical observations that patients with a different first event have very different risks of developing TRALI [68,69]. However, a first hit is not a requirement per se. An antibody-antigen interaction can induce lung injury in a relatively healthy recipient [71]. This antibody mediated reaction is the main difference in pathogenesis between TRALI and other forms of ARDS. Although the particular mechanism involved
(antibody-antigen complex vs. activation by bioactive lipids) may be different, all pathways result in the same downstream event, namely neutrophil mediated lung injury [71-73].

**Inflammation**

The pulmonary inflammatory response in TRALI resembles the response seen in ARDS. Both entities involve interaction of activated neutrophils, platelets and pulmonary endothelium. The role of the endothelium, as well as the priming and activation of neutrophils, has well been described in TRALI [72,73]. In short, neutrophils are attracted to the lung by release of chemokines and cytokines. Adhesion to the endothelium of the lung capillaries is realised. The adhered neutrophils are then activated by the blood transfusion, containing HLA/HNA antibodies or bioactive lipids. This neutrophil-mediated inflammatory response results in capillary damage and leakage of fluid into the alveolar space [64,72]. Concerning effector cells, obviously, anti-neutrophil antibody-mediated organ injury is not a mechanism of action in ARDS.

**Platelets, coagulation and fibrinolysis**

Also, there are some differences in platelet activation between the two syndromes. Similar to TRALI, platelets roll along and firmly adhere to lung endothelial cells during endotoxemia, as shown by intravital microscopy [74]. This interaction is mainly mediated by platelet P-selectin. However, whereas blocking of P-selectin was protective in acid-induced ALI by reducing platelet-neutrophil aggregates [18], a role for P-selectin was not established so far in TRALI. The bioactive lipids can accumulate during storage of blood products and consist of a mixture of lysophosphatidylcholines (lyso-PCs). These compounds are capable of activating primed neutrophils as mentioned before [72]. A possible mechanism is priming of the respiratory burst reaction through the cells platelet-activating factor receptor [75,76]. Furthermore, platelet-derived CD40L has been implicated as cofactor or even the cause of antibody negative TRALI. CD40L has been shown to accumulate during storage of blood products and in 8 out of 12 TRALI patients plasma CD40L levels were increased compared to their pre-transfusion levels [77].

Thrombocytopenia can be observed during a TRALI reaction and is also a prominent feature in an animal model of TRALI, suggesting that platelets are involved in TRALI pathogenesis [78]. Platelets roll along and firmly adhere to the lung endothelial cells [78] and in a murine TRALI model, it has been shown that development of TRALI is critically dependent on platelets [51]. Among other mechanisms, activated platelets are reported to induce the formation of neutrophil extracellular traps (NETs) and these NETs have been detected in the lungs and plasma of TRALI patients [19,79].

Although less well studied in TRALI, coagulopathy seems to be also an imported factor. For example, in animals, massive transfusion results in extensive numbers of microthrombi in the pulmonary vasculature [80]. In addition, in other animal models is shown that transfusion
of aged blood products results in lung injury, which is characterized by activation of pulmonary coagulation and fibrinolysis, alike ARDS [81,82].

Besides differences in host response between ARDS and TRALI, there are possible differences in pathology findings. Post mortem findings in lungs of ARDS patients classically show diffuse alveolar damage (DAD), pulmonary oedema, hyaline membrane formation and extensive neutrophil aggregation in alveoli. Of interest, autopsy findings in two patients that developed fatal TRALI after receiving plasma of donors who were positive for HLA antibodies corresponding to the patients' phenotype, did not show DAD nor hyaline membrane formation [83]. Instead, alveolar oedema and pleural effusion were prominent. However, it should be noted that the presence of hyaline membranes and DAD on autopsy may have more to do with how long the patient lived after experiencing a TRALI reaction. Taken together, although TRALI can be considered part of the ALI spectrum, some clinical and pathologic differences exist.

Pharmacologic treatment of TRALI

Clinical trials of pharmacological treatment strategies in TRALI are lacking to date. Several case reports have reported on the use of glucocorticoids in TRALI treatment, but no randomized controlled trials have studied this therapy in TRALI [84]. Aspirin is a therapy of particular interest, since high dose aspirin improved outcome in an animal model of TRALI.

Taken together, the amount of research in TRALI medicine bears no relation to the large quantity of research in ARDS. Considering the similarities between these life-threatening diseases it is conceivable that strategies aimed at limiting ARDS may also apply to TRALI.

Outline of the thesis

The need for the development of therapeutic strategies for ARDS and in particular TRALI is the starting point of this thesis. This dissertation focuses on several potential therapeutic pathways targeting inflammation, platelets and coagulation. Knowledge from studies in ARDS is used for studies in TRALI. We chose a translational approach, in which both preclinical and clinical studies are used. This thesis starts with studies on inflammation, followed by studies on platelets, coagulation and fibrinolysis.

Part II of this thesis describes studies on inflammation. In chapter 2, we tested the hypothesis that blood transfusion is associated with changes in pulmonary and systemic inflammation and coagulation in patients after cardiac surgery, in a prospective case-control study in two university hospital ICUs, comparing cardiac surgery patients who received no, limited (1-2 units) or multiple transfusions (≥ 5 units). In chapter 3, we tested the hypothesis that sRAGE is elevated following cardiac surgery and associated with increased pulmonary vascular leakage, in a prospective case-control study in two university hospital ICUs, measuring
circulating sRAGE together with gallium-transferrin pulmonary leak index in 60 consecutive cardiac surgery patients stratified by the amount of transfusion. Additionally, we investigated the role of blood transfusion as a determinant of plasma sRAGE. In chapter 4, we tested the hypothesis that the HMGB1, S100A12 and sRAGE are associated with TRALI, in a prospective case-control study in cardiac surgery patients, comparing 14 TRALI cases with controls (transfused and non-transfused) without lung injury. In chapter 5, we tested the hypothesis that methylprednisolone attenuates pulmonary inflammation in TRALI, in a murine “two-hit” model of antibody mediated TRALI.

Part III describes studies on platelets, coagulation and fibrinolysis. In chapter 6, we tested the hypothesis that aspirin in a high dose is superior to low dose aspirin or clopidogrel in preventing acute lung injury, in a murine model of LPS-induced ALI. In Chapter 7, we tested the hypothesis that aspirin use before admission to the ICU would protect against TRALI development, in a post-hoc analysis of a nested case-control study, comparing TRALI cases with matched controls (transfused patients not developing lung injury). In chapter 8, we tested the hypothesis that binding of platelet CD40L to endothelial CD40 is essential in the onset of TRALI, in a murine model of antibody mediated TRALI and in a case-control study in cardiac surgery patients comparing TRALI cases with transfused patients not developing lung injury. In chapter 9, we tested the hypothesis whether nebulization of anticoagulants attenuates pulmonary coagulation without having systemic side-effects, by performing a systematic review of preclinical studies and human trials of nebulized anticoagulants in the setting of lung injury in animals and ALI/ARDS in humans. Chapter 10 is a narrative review, with an outlook on future areas of interest for research on therapy in TRALI. We first summarize the evidence for a role of enhanced pulmonary coagulation and impaired fibrinolysis in the pathophysiology of TRALI, also focusing on the role of platelets. Furthermore, potential therapeutic interventions targeting coagulation are discussed.

In chapter 11, the results from all chapters are summarized and discussed.
Reference List

2. Ware LB: **Pathophysiology of acute lung injury and the acute respiratory distress syndrome.** *Semin Respir Crit Care Med* 2006, **27**: 337-349.
10. Aird WC: **Endothelium as an organ system.** *Crit Care Med* 2004, **32**: S271-S279.
13. Peters AM: **Just how big is the pulmonary granulocyte pool?** *Clin Sci (Lond)* 1998, **94**: 7-19.
20. Zarbock A, Ley K: **The role of platelets in acute lung injury (ALI).** *Front Biosci* 2009, **14**: 150-158.


