

■ H Y P O T H E S I S

Does activation of the Fc γ R1a play a role in the pathogenesis of the acute lung injury/acute respiratory distress syndrome?

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A B S T R A C T

ALI (acute lung injury) and its more severe form ARDS (acute respiratory distress syndrome) are inflammatory diseases of the lung characterized by hypoxaemia and diffuse bilateral infiltrates. Disruption of epithelial integrity and injury to endothelium are contributing factors of the development of ALI/ARDS, and alveolar damage is the most pronounced feature of ALI/ARDS. The resulting increase in lung microvascular permeability promotes influx of inflammatory cells to the alveolar spaces. Oedema fluid contains pro-inflammatory mediators and plasma proteins, including Igs (immunoglobulins). Moreover, several reports describe the presence of autoantibodies and immune complexes [anti-IL-8 (interleukin-8) autoantibody/IL-8 complexes] in lung fluids (oedema and bronchoalveolar lavage fluids) from patients with ALI/ARDS. These immune complexes associate with Fc γ R1a (Fc γ IIa receptor) in lungs of patients with ARDS. Furthermore, the expression of Fc γ R1a is substantially elevated in lungs of these patients. Fc γ R1a appears on virtually all myeloid cells, platelets and endothelial cells. It is a low-affinity receptor for IgG that preferentially binds aggregated immunoglobulins and immune complexes. Fc γ Rs regulate phagocytosis and cell-mediated cytotoxicity, and initiate the release of inflammatory mediators. It should be noted that immune complexes formed between either anti-neutrophil autoantibodies and their specific antigens or anti-HLA (human leucocyte antigen) antibodies and target antigens are implicated in the pathogenesis of TRALI (transfusion-related acute lung injury), and importantly, animal studies indicate that Fc γ Rs are essential for these complexes to cause damage to the lungs. Therefore, we hypothesize that Fc γ Rs such as Fc γ R1a could contribute to the pathogenesis of ALI/ARDS.

ALI (ACUTE LUNG INJURY)/ARDS (ACUTE RESPIRATORY DISTRESS SYNDROME)

ALI and the ARDS were first described in 1967 and represent a severe form of diffuse lung disease. Alternations of lung function in these pathological

entities include rapid onset of dyspnoea, hypoxaemia and respiratory failure. The alveolar-capillary barrier becomes disrupted allowing massive influx of oedema fluid and inflammatory cells. The formation of pulmonary oedema is a consequence of both endothelial injury and increased vascular permeability. In accordance with the

Key words: acute lung injury, Fc γ R1a, IgG receptor, lung, signal transduction.

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; Fc γ R, Fc γ receptor; IL, interleukin; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; KC, keratinocyte-derived chemokine; LIX, lipopolysaccharide-induced CXC chemokine; LPS, lipopolysaccharide; MIP-2, macrophage inflammatory protein 2; TLR4, Toll-like receptor 4; TRALI, transfusion-related acute lung injury.

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recommendations of the American–European Consensus Conference Committee (the consensus definition of 1994), patients are classified as having ALI when characteristic changes in lung compliance and residual capacity of the lungs are found. Diagnosis of ALI is given when an individual rapidly (in less than 7 days) develops severe hypoxaemia [a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (P_{aO_2}/F_{iO_2}) is 300 or less], and the frontal radiograph is abnormal (visible infiltrate). In fact, the presence of diffuse bilateral infiltrates on chest radiograph is indicative of pulmonary oedema in patients with ALI. More severe hypoxaemia (P_{aO_2}/F_{iO_2} of 200 or less) warrants diagnosis of ARDS [1–4].

Clinical disorders associated with the progression to ALI/ARDS can be divided into two groups, i.e. diseases directly affecting the lungs such as pneumonia or gastric aspiration and conditions that can indirectly lead to lung injury such as sepsis or severe trauma [1,3]. Significantly, the highest risk of developing ALI/ARDS is attributed to sepsis [1,4]. There are also additional factors that can predispose individuals for development of ALI/ARDS, as for example, alcoholism or polymorphism of certain genes [1,3]. Nevertheless, the disease course is similar between pulmonary and extrapulmonary ALI/ARDS [3].

INCIDENCE OF AND THERAPY FOR ALI/ARDS

The incidence of ALI and ARDS in the United States is 79 cases and 59 cases per 100000 persons per year respectively. Because of lack of effective treatment modalities for ALI/ARDS, the mortality remains at about 40% [1,4].

Therapies available for ALI/ARDS are nonspecific and generally supportive, including mechanical ventilation [1–3]. Since hyperactive inflammation is one well-recognized feature of ALI/ARDS [5,6] (see below), several trials of steroid effectiveness in patients with ALI/ARDS have been conducted. Prolonged treatment with low doses of corticosteroids has been shown to improve both lung function and survival of patients with ALI/ARDS [7]. These findings are consistent with a causal role for the host inflammatory response in the early stages of the disease that ultimately drives activation of the coagulation cascade and inhibition of fibrinolysis. However, therapies to restore homeostasis in the coagulation or fibrinolytic systems are either partially effective or not effective at all and are associated with serious complications [8,9].

PATHOBIOLOGY OF ALI/ARDS

Early stage of ALI/ARDS (exudative phase) is associated with diffuse alveolar damage, neutrophil infiltrate, haemorrhage and the accumulation of a protein-rich

pulmonary oedema (Figure 1). Other histopathological changes that are characteristic of the exudative phase include disruption of the epithelial barrier and injury to the capillary endothelium. Some patients with ALI/ARDS progress to a fibroproliferative phase, during which chronic inflammation, fibrosis and neovascularization take place. The final stage of ALI/ARDS, in surviving patients, is the recovery phase that results in a variable degree of restoration of lung function in these patients [1–3]. During the exudative phase, cytokines and other pro-inflammatory molecules initiate, amplify and perpetuate inflammatory responses in the lungs. Increased oxidative stress and the appearance of proteolytic enzymes in the alveolar spaces and interstitium results in a substantial decrease in the surfactant level in the lungs and thereby promotes atelectasis. Elastolytic proteases disrupt the structural framework of the lung. In addition, damage to the epithelial barrier contributes to and exacerbates the alveolar flooding, and also delays recovery by impairing fluid clearance. Finally, capillary thrombosis is most probably brought about by an impairment in the balance between pro-/anti-coagulant and pro-/anti-fibrinolytic proteins [2,3].

The increase in lung microvascular permeability and the resultant formation of pulmonary oedema are causative of alveolar flooding. Lung fluids from patients with ALI/ARDS contain an abundance of plasma proteins and inflammatory mediators. Studies from several laboratories have described the presence of various autoantibodies in these fluids [10–13]. Our findings indicate that autoantibodies directed against IL-8 (interleukin-8) form immune complexes (anti-IL-8 autoantibody/IL-8 immune complexes) in lung fluids from patients with ALI/ARDS [10,11]. Furthermore, anti-IL-8 autoantibody/IL-8 immune complexes are found in association with Fc γ RIIa (Fc γ receptor IIa) in pulmonary tissues from these patients [14].

IgG/IMMUNE COMPLEX RECEPTORS (Fc γ Rs)

Fc γ Rs interact with the Fc portion of IgG. To date, three different classes of Fc γ Rs have been identified in humans, i.e. Fc γ RI, Fc γ RII and Fc γ RIII. The Fc γ RI family contains four different isoforms (Ia, Ib1, Ib2 and Ic), the Fc γ RII group comprises six proteins (IIa1, IIa2, IIb1, IIb2, IIb3 and IIc), and the Fc γ RIII group two (IIIa and IIIb). Fc γ Rs consist of two (Fc γ RII and Fc γ RIII) or three (Fc γ RI) N-terminal extracellular immunoglobulin-like domains (ligand binding domains), a transmembrane region and an intracellular tail with a C terminus. Fc γ RI exhibits high affinity for monomeric IgG, whereas Fc γ RII and Fc γ RIII bind immune complexes more efficiently than uncomplexed IgG. The binding affinities of Fc γ Rs are influenced additionally by variations in

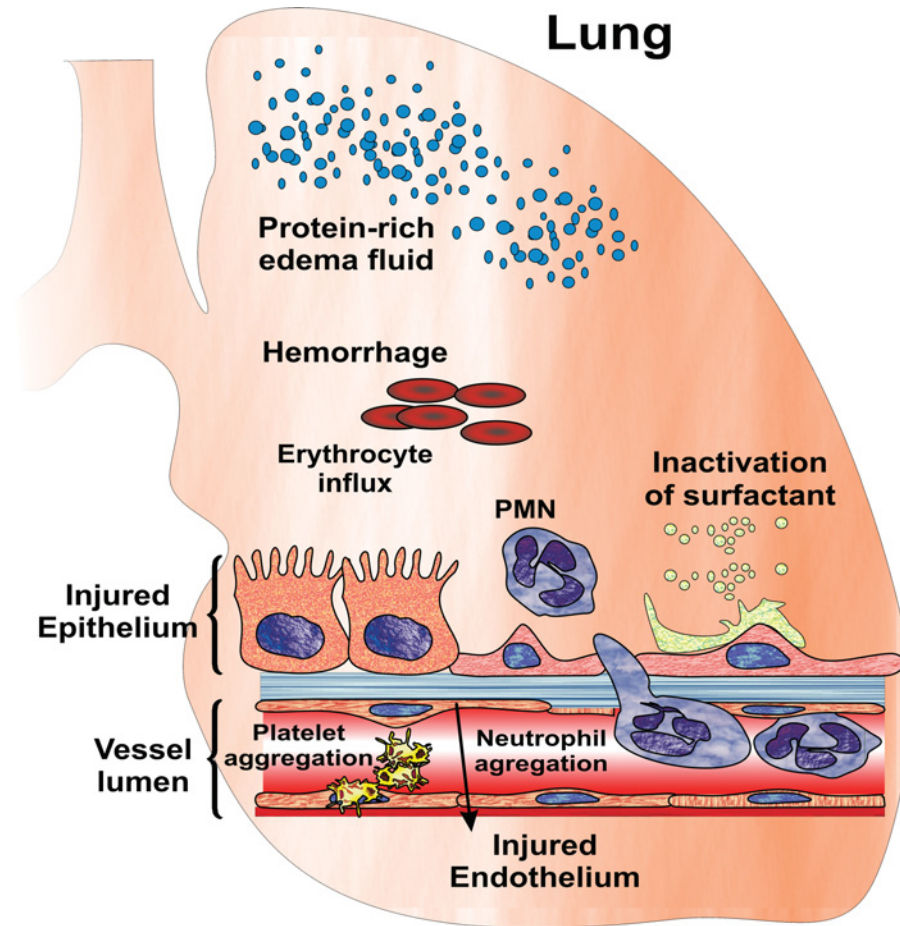


Figure 1 Events characterizing early stage of lung damage in patients with ALI/ARDS

See text for details.

glycosylation patterns of both the receptors and IgG [15,16].

DIVERSE FUNCTIONS OF Fc γ Rs

Functionally, Fc γ Rs can be divided into two categories: activating and inhibitory receptors. Activating Fc γ Rs contain ITAM (immunoreceptor tyrosine-based activation motif), while inhibitory Fc γ Rs contain ITIM (immunoreceptor tyrosine-based inhibitory motif). Cross-linking of the former receptors is followed by phosphorylation of tyrosine residues within the ITAM by kinases of the Src [v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homologue] family. This in turn initiates the Fc γ R signalling cascade [15,16] (Figure 2).

ITAM-bearing Fc γ Rs mediate a pleiotropy of functions in various cell types. These functions include facilitating phagocytosis of diverse pathogens, induction of antibody dependent cytotoxicity, modulation of cell activation, regulation of apoptosis and triggering of the release of pro-inflammatory mediators [15,16].

Fc γ RIIb, an ITIM-containing receptor, is the only inhibitory Fc γ R. It is expressed on B cells, mast cells, macrophages, monocytes and neutrophils, and inhibits pro-inflammatory functions of these cells [15,16].

POLYMORPHISM OF Fc γ Rs

Two allelic forms of Fc γ RIIa have been described in humans: Fc γ RIIa-R131 and Fc γ RIIa-H131. Fc γ RIIa-R131 has an arginine (R) residue, while Fc γ RIIa-H131 has a histidine (H) residue at position 131. The first allotype binds all human IgG subclasses, particularly IgG₂, with low affinity, whereas the second one binds IgG₂ and IgG₃ with higher affinity. Only one variant of the Fc γ RIIb gene exists, in which isoleucine (I) at position 232 is replaced by a threonine (T) residue. Fc γ RIIb-T232 displays enhanced ability to inhibit B-cell receptor signalling compared with Fc γ RIIb-I232 [15].

Fc γ RIIIa was shown to have two allelic forms, i.e. Fc γ RIIIa-V158, which has a valine (V) residue at position

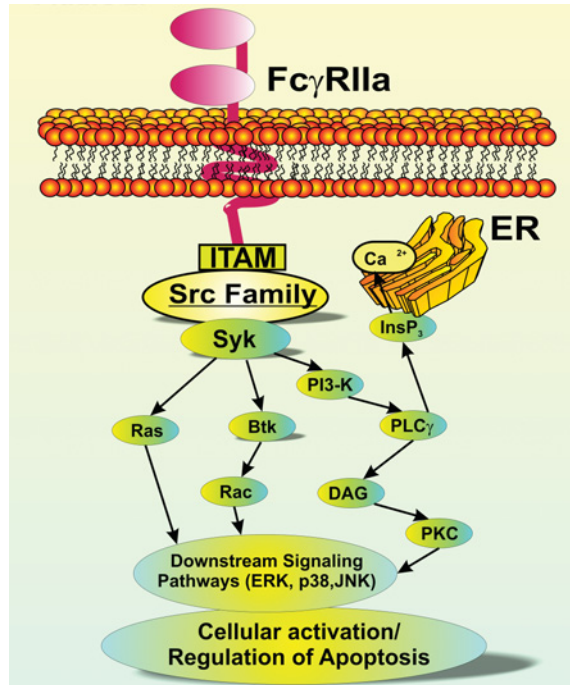


Figure 2 Fc γ RIIa signalling cascade

Activation of the Src family leads to the recruitment of a kinase from the Syk family followed by phosphorylation of a series of downstream proteins such as phosphoinositide 3-kinase (PI3-K). Subsequent events include production of PtdIns(3,4,5)P₃ and recruitment of Bruton's tyrosine kinase (Btk) and phospholipase C γ (PLC γ). This leads to increased calcium mobilization and also triggers additional downstream signalling events as activation of the Ras/Raf/MAPK (mitogen-activated protein kinase) pathways [15,16]. DAG, diacylglycerol; ERK, extracellular-signal-regulated kinase; InsP₃, Ins(1,4,5)P₃; JNK, c-Jun N-terminal kinase; PKC, protein kinase C.

158, and Fc γ RIIIa-F158 that has a phenylalanine (F) residue in that position. The former allelic variant of Fc γ RIIIa binds IgG₁, IgG₃ and IgG₄ with higher affinity than the Fc γ RIIIa-F158 variant. Three alleles of the Fc γ RIIIb gene are known (NA1, NA2 and SH). The NA1 type mediates phagocytosis of IgG₁- and IgG₃-opsonized particles more efficiently than the NA2 variant [15].

Polymorphisms in Fc γ R genes influence susceptibility of individuals to the development of many autoimmune diseases and to acquiring of various infections. Patient responses to treatment with intravenous immunoglobulins and monoclonal antibodies are also affected [15].

HYPOTHESIS: Fc γ Rs IN ALI/ARDS

Our hypothesis is that immune and inflammatory responses triggered in the host during the development of ALI/ARDS lead to an increase in expression and functionality of Fc γ Rs, specifically Fc γ RIIa, in the lung. In support of this hypothesis, we have shown that the

level of Fc γ RIIa is higher in lung tissues from patients with ARDS than in normal lung tissues [14] (Figure 3). Moreover, Hietbrink et al. [17] and Kanters et al. [18] reported that lung fluid neutrophils from three patients with ALI were in a highly primed state characterized by elevated expression of an epitope that the authors later classified as 'activated' Fc γ RIIa.

Additional indirect supportive data for our hypothesis comes from two recently published studies [19,20]. In the first one, Endeman et al. described the presence of the association between the Fc γ RIIa-R131 genotype and severe sepsis in community-acquired pneumonia [19]. The authors of the second manuscript summarized the results of multiple clinical trials of patients with sepsis, severe sepsis or septic shock who received polyclonal intravenous immunoglobulin therapy. They concluded that there was a survival benefit of this therapy in patients with sepsis [20]. This finding indicates that Fc γ Rs could contribute to the pathogenesis of sepsis (see below), which constitutes the major risk factor for development of ALI/ARDS [1,4].

LIGANDS FOR Fc γ Rs IN PATIENTS WITH ALI/ARDS

A considerable subpopulation of Fc γ RIIa in lungs of patients with ALI/ARDS associate with anti-IL-8 autoantibody/IL-8 immune complexes [14,21]. At present, it is not known what the pathophysiological significance of this observation is. However, our group reported that anti-IL-8 autoantibody/IL-8 immune complexes in lung fluids from patients with ALI/ARDS are important prognostic indicators for the development and ultimate outcome of ALI/ARDS [10,11,22]. Presence of elevated concentrations of these complexes was associated with poor outcome in patients with ALI/ARDS [11]. We also detected anti-IL-8/IL-8 complexes (at increased levels) in lung fluids of patients at risk of ALI/ARDS who later developed ALI/ARDS. These patients were evaluated immediately after admission to intensive care before influx of neutrophils and lung injury occurred, indicating that the complexes are formed locally in the lung prior to the development of ALI/ARDS [22].

We have purified anti-IL-8 autoantibodies from lung fluids of ALI/ARDS patients. These autoantibodies bind IL-8 with high affinity (about 10⁻¹² M) and inhibit the activity of IL-8 *in vitro* when experiments are performed using a mixture of IL-8 and autoantibodies (in excess) [10]. However, complexes between these antibodies and IL-8 purified from lung fluids of ALI/ARDS patients display pro-inflammatory activity via Fc γ RIIa [21,23-25]. Moreover, blocking of Fc γ RIIa suppresses the biological effects of these complexes *in vitro* [21,23-25].

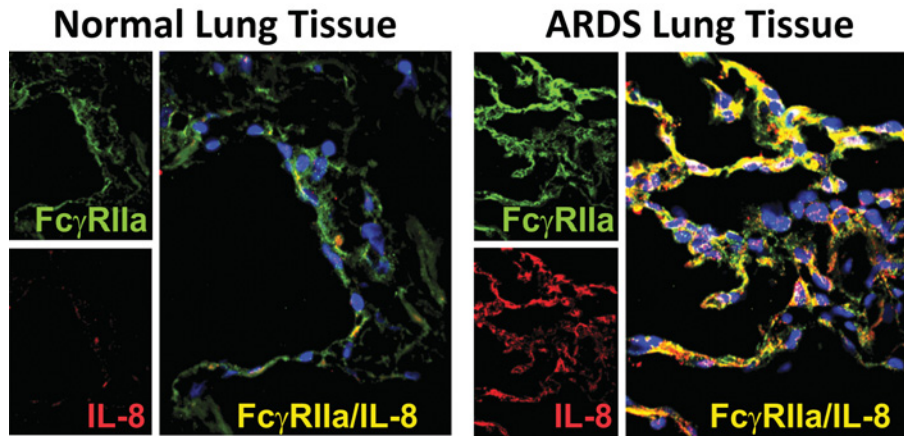


Figure 3 Expression of FcγRIIa and association of IL-8 in anti-IL-8 autoantibody/IL-8 immune complexes with FcγRIIa in normal lung tissues and tissues from ARDS patients

Left panels from the top: FcγRIIa (green)/IL-8 (red) $\times 60$. Big left panel: co-localization (yellow) of FcγRIIa (green) and IL-8 (red). Nuclei are stained blue. Right panels from the top: FcγRIIa (green)/IL-8 (red) $\times 60$. Big right panel: co-localization (yellow) of FcγRIIa (green) and IL-8 (red). Nuclei are stained blue. Acquisition settings for the images were UV/567/647, and specific parameters for the fluorophores were Cy3 (red) excitation at 567 nm and emission at 600 nm, Alexa 647 (pseudo-colour green) excitation at 647 nm and emission at 700 nm. The sections were evaluated using a PerkinElmer Ultra VIEW LCI confocal imaging system with a Nikon TE2000-S fluorescence microscope and a PlanApo'60 immersion oil objective. Ultra VIEW Imaging Suite software (version 5.5.0.4) was used for image processing.

Our studies were the first to show that purified anti-IL-8 autoantibody/IL-8 immune complexes trigger chemotaxis of human blood neutrophils, induce neutrophil activation and modulate survival of these cells. Anti-IL-8/IL-8 complexes also have the ability to damage human epithelial cells, promoting cell dysfunction and loss of integrity. Moreover, these complexes display pro-inflammatory activity towards human endothelial cells. Finally, FcγRIIa are the principal receptors that mediate the biological activities of the anti-IL-8/IL-8 complexes *in vitro* [21,23–25].

Although whether anti-IL-8/IL-8 complexes are causative of disease progression *in vivo* remains to be established, autoantibodies/immune complexes can trigger the development of ALI/ARDS in humans. Patients with the ‘catastrophic’ variant of the antiphospholipid syndrome can succumb to ALI/ARDS as do patients receiving blood transfusions. TRALI (transfusion-related acute lung injury) is believed to be a result of stimulatory activity of immune complexes formed between either anti-neutrophil autoantibodies and their specific antigens or anti-HLA (human leucocyte antigen) antibodies and target antigens [26–28]. In addition, animal studies indicate that FcγRs are essential for the latter complexes to cause damage to the lungs [29].

ADDITIONAL EVIDENCE: ANIMAL MODELS OF LUNG INJURY AND FcγRs

There is a substantial body of literature describing regulation of lung inflammation in animal models of immune complex-induced lung injury. Most of

these models rely on the reverse passive Arthus reaction, a localized alveolitis triggered by deposition of heterologous immune complexes. The local formation of heterologous immune complexes is achieved by intratracheal administration of IgG antibody against a foreign antigen, such as BSA, followed by the intravenous injection of this antigen [30]. We have developed a mouse model of ALI triggered by anti-KC (keratinocyte-derived chemokine) autoantibody/KC immune complexes and concluded that these complexes are injurious for lungs based on our investigations in mice [31]. Murine KC (CXCL1/KC) is a functional homologue of IL-8 [32], so this model provides excellent means for studying the mode of action of immune complexes containing autoantibodies to chemokines [31]. Other murine CXC chemokines that promote neutrophil migration include MIP-2 (macrophage inflammatory protein 2) (CXCL2), LIX (lipopolysaccharide-induced CXC chemokine) (CXCL5) and lungkine (CXCL15) [33–35]. KC, however, shares more common properties with human IL-8 than MIP-2, LIX or lungkine. KC is an early response chemokine and is responsible for the initial influx of neutrophils to the alveolar compartment [36]. Since platelet–neutrophil interactions have been shown to contribute to lung injury in murine models of acid-induced ALI, sepsis-associated ALI and TRALI, and since FcγRIIa is expressed by both platelets and neutrophils, it is very likely that this receptor facilitates neutrophil capture by circulating platelets [15,16,37,38].

Furthermore, mice deficient in all FcγRs or FcγRI are protected from lung injury [30,31,39]. Importantly, the same holds true for mice lacking FcγRIII, a murine homologue of human FcγRIIa [16,30,39].

Perhaps even more relevant are findings of attenuated inflammatory response in the lungs of LPS (lipopolysaccharide)-treated mice or rats in the absence of Fc γ Rs [31,40]. Moreover, studies from our laboratory as well as those of others indicate that there is cross-talk between Fc γ Rs, including Fc γ RIIa and TLR4 (toll-like receptor 4) in human neutrophils [41] and in mouse models of LPS-induced lung injury [42,43]. Finally, there are reports suggesting a regulatory role of Fc γ Rs in *Escherichia coli* sepsis and *Pseudomonas aeruginosa* pneumonia in mice [44,45].

It should be noted that anti-IL-8/IL-8 complexes consist of one IgG and one IL-8 molecule [10] and are capable of triggering complement activation (A. Krupa and A.K. Kurdowska, unpublished work). However, our *in vitro* experiments are performed in the absence of serum or other sources of complement, and in such conditions, complement activation cannot occur. This is different from the situation *in vivo* where complement activation plays an important role. It has been demonstrated, for example, that blocking of complement activation protects against lung injury triggered by the administration of heterologous immune complexes. Furthermore, C5a mediates activity of immune complexes by regulating expression of Fc γ Rs [30,39]. Therefore, both complement and the Fc γ R cascade are likely to be involved in orchestrating the inflammatory events in ALI/ARDS.

TESTING OF THE HYPOTHESIS

The published data supporting our hypothesis is primarily indirect evidence ('by association'), predominantly from *in vitro* studies and animal models of ALI. Despite the inquisitory value of this observation, more studies utilizing specimens from patients with ALI/ARDS are certainly needed. Lung tissues from these patients should be rigorously analysed for the presence of different types of Fc γ Rs. The levels and expression patterns of Fc γ Rs should also be evaluated. Our studies, for example, indicate that Fc γ RIIa is expressed in injured lungs and is present on endothelial cells in patients with ARDS [14,21]. Inflammatory cells in lung fluids of patients with ALI/ARDS should be similarly tested. Moreover, it would be useful to know which proteins from the Fc γ RIIa-signalling cascade are up- or down-regulated in the lungs of these patients. Whether Fc γ RIIa is the only or the most abundant Fc γ R in lungs in ALI/ARDS is at present not known. Because of the limited availability of clinical samples, fluorescence-based methods, such as laser confocal microscopy or fluorescence resonance energy transfer, are preferable.

Studies using well-established animal models of ALI/ARDS and mice transgenic for Fc γ RIIa would also be very valuable. Such mice have been generated

by other investigators [46,47]. When considering a model of ALI/ARDS, one should take into account the complexity of the disease in ALI/ARDS. Therefore, two hit models are probably the most appropriate [48]. Nevertheless, to study the role of Fc γ RIIa in the pathogenesis of ALI/ARDS, a model of immune-complex-triggered lung injury should be used initially [30,31,39], then, perhaps treatment with LPS could be added to create a two-hit model [31,40]. Contribution of human autoantibodies/immune complexes such as anti-IL-8/IL-8 complexes to the pathogenesis of lung injury could also be studied using Fc γ R-deficient mice that are transgenic for Fc γ RIIa. These mice should be treated with the immune complexes formed between IL-8 and fully human anti-IL-8 monoclonal antibodies [49]. To study the role of Fc γ RIIa in different populations of cells, adaptive transfer experiments could be performed. Cells from mice lacking murine Fc γ Rs and expressing human Fc γ RIIa could be injected into Fc γ R-knockout mice [47]. In addition, other common models of ALI/ARDS, including ventilator-induced lung injury and hyperoxia, ought to be evaluated using mice deficient in Fc γ Rs that are transgenic for human Fc γ RIIa [48]. Moreover, Fc γ RIIa may be inhibited in the above-described mouse models employing specific Fc γ RIIa blockers [50–52]. Finally, the role of Fc γ R signalling could be explored in these models taking advantage of the availability of small inhibitors of signalling proteins and silencing RNAs targeting these proteins [53–55].

CONCLUSIONS

Studies with animal models of ALI/ARDS and Fc γ RIII (homologue of human Fc γ RIIa)-deficient mice support our hypothesis [30,31,39,40,43–45]. Our findings of increased levels of Fc γ RIIa and its association with anti-IL-8/IL-8 immune complexes in lungs of patients with ARDS provide direct evidence of expression of functional Fc γ RIIa in the lungs of these patients [14,21]. The relevance of these observations is substantiated by the several facts described by us and other researchers. First of all, concentrations of anti-IL-8/IL-8 immune complexes in lung fluids from patients with ALI/ARDS were predictive of disease progression and the eventual outcome of ALI/ARDS [10,11,22]. Secondly, purified complexes exhibit pro-inflammatory activities *in vitro* [21,23–25]. Thirdly, other autoantibodies have been detected in patients with ALI/ARDS, and also could be correlated with disease activity [12,13]. Finally, autoantibodies/immune complexes at large are known to induce ALI/ARDS in humans [26–28].

At present, involvement of Fc γ RIIa in the pathophysiology of ALI/ARDS is not understood, but the many basic and clinical studies place this receptor at the 'heart' of the inflammatory milieu in the

lung. It is certainly conceivable that FcγRIIIa could contribute to the pathogenesis of ALI/ARDS. Keeping in mind the association of FcγRIIIa polymorphism with the development of sepsis (a major risk factor for ALI/ARDS) in community-acquired pneumonia, one can envision that this receptor could play an important regulatory role during the course of this illness, as evident from animal models of sepsis and pneumonia [19,44,45].

Comprehensive research into the function and molecular mechanisms of FcγRIIIa activity could lead to development of novel and more effective therapies for ALI/ARDS. It is important to note that specific inhibitory molecules, i.e. FcγRIIIa blockers, have already been tested in animal models of inflammation [50–52]. Signalling proteins from the FcγRIIIa cascade could serve as therapeutic targets as well. The progress made in the fields of designing and utilizing small inhibitors of signalling proteins and pulmonary delivery of inhibitory entities makes this possibility very attractive [53–55].

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