Anti-interleukin-8 autoantibody:interleukin-8 immune complexes in acute lung injury/acute respiratory distress syndrome

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ABSTRACT

ALI/ARDS (acute lung injury/acute respiratory distress syndrome) is a severe inflammatory lung disease associated with very high mortality. Importantly, no effective therapy has been developed to date for ALI/ARDS. Neutrophils have been implicated in the pathogenesis of ALI/ARDS, and IL-8 (interleukin-8) has been identified as the main chemotactic factor for neutrophils in lung fluids of patients with ALI/ARDS. Significantly, studies from our laboratory have revealed the presence of anti-IL-8 autoantibody:IL-8 immune complexes in lung fluids from patients with ALI/ARDS. Autoantibodies to several cytokines, including IL-8, have been found in human plasma and other tissues. The function of anticytokine autoantibodies is far from clear; however, in some instances, it has been suggested that such autoantibodies may contribute to the pathogenesis of variety of human diseases. In addition, many of these autoantibodies can form immune complexes with target cytokines. Furthermore, immune complexes consisting of anti-IL-8 autoantibodies and IL-8 are very stable due to the high affinity of autoantibodies against IL-8. These complexes are present in various human tissues, including the lung, as they have been detected in lung fluids from patients with ALI/ARDS. In this review, the significance of the latter findings are explored, and the possible involvement of anti-IL-8 autoantibody:IL-8 immune complexes in pathogenesis of ALI/ARDS is discussed.

INTRODUCTION

ARDS (acute respiratory distress syndrome) was first described in 1967 by Ashbaugh and co-workers [1] in patients presenting with diffuse alveolar infiltrates clearly visible on chest radiographs. Since then, criteria for identifying patients with ALI (acute lung injury) and ARDS have been defined, and it was proposed to distinguish patients with ARDS on the basis of the severity of hypoxaemia. Patients are classified as having ALI if the $P_{aO_2}/F_{iO_2}$ ratio (partial pressure of arterial oxygen to the fraction of inspired oxygen) is 300 or less. A

Key words: acute lung injury (ALI), acute respiratory distress syndrome (ARDS), autoantibody, cytokine, IgG receptor (FcγR), interleukin-8 (IL-8), lung.

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; ERK, extracellular-signal-regulated kinase; $F_{iO_2}$, fraction of inspired oxygen; GM-CSF, granulocyte/macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; anti-IL-8:IL-8 complex, anti-IL-8 autoantibody:IL-8 immune complex; ITAM, immunoreceptor tyrosine-based activation motif; LPS, lipopolysaccharide; KC, keratinocyte-derived chemokine; anti-KC:KC complex, anti-KC autoantibody:KC immune complex; MAPK, mitogen-activated protein kinase; $P_{aO_2}$, partial pressure of arterial oxygen; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLCγ, phospholipase Cγ; PLD, phospholipase D; TNF-α, tumour necrosis factor-α.

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Table 1  Definition of ALI and ARDS
The definitions are based on those provided in [2,4].

<table>
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<th>Definition</th>
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<tr>
<td><strong>Onset</strong></td>
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<td>Acute (less than 7 days)</td>
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<td><strong>Severity of hypoxaemia</strong></td>
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<tr>
<td>ALI, PaO2/FiO2 ratio of 300 or less; ARDS, PaO2/FiO2 ratio of 200 or less</td>
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<tr>
<td><strong>Radiological findings</strong></td>
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<td>Diffuse bilateral infiltrates indicative of pulmonary oedema</td>
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<tr>
<td><strong>Exclusion criterium</strong></td>
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<td>Presence of left atrial hypertension (pulmonary-artery wedge pressure 18 mmHg or more)</td>
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$\text{PaO}_2/\text{FiO}_2$ ratio of 200 or less indicates that a patient suffers from ARDS [2–4]. The current definition of ALI/ARDS is shown in Table 1. This definition, apart from oxygenation and radiographic criteria, includes exclusion criterium for patients with cardiogenic pulmonary oedema [2,4].

The incidence of ALI/ARDS has been difficult to estimate for a variety of reasons, including the lack of a clear definition until the mid-1990s. According to some studies, the annual rate is between 1.5 and 13.5 cases per 100,000 of the population; however, the study conducted by the NIH (National Institutes of Health) reported as many as 75 cases per 100,000 [2,5]. Most investigators agree that higher, rather than lower, numbers are more accurate, especially as the latter estimate is based on screening a large number of patients [2,5]. Rubenfeld et al. [6] estimated that there are approx. 190,600 cases of ARDS each year in the U.S.A., which are associated with 74,500 deaths and 3.6 million hospital days. The number of deaths from ARDS is comparable with that attributed to breast cancer or HIV disease [6]. Even with intensive therapy, patients typically follow a rapid clinical course, resulting in death in between 50–75% of patients. Mortality in patients with accompanying sepsis approaches 90% [6a,7]. Indeed, although a variety of potential treatments continue to be explored, no effective pharmacological interventions exist [3].

ALI/ARDS is a complex disease process arising in a variety of clinical settings and is characterized by severe respiratory failure. ALI/ARDS is usually associated with an underlying catastrophic medical or surgical illness, and clinical disorders commonly linked to ALI/ARDS include sepsis, pneumonia, traumatic injury and major surgery. Sepsis, in particular, is one of the most common risk factors and accounts for approx. 40% of cases of ARDS [2–5a,7]. ALI/ARDS can be regarded as severe inflammatory lung disease in which the initial acute systemic inflammatory response leads to microvascular damage and, subsequently, to increased pulmonary vascular and epithelial permeability. This phase is characterized by the influx of protein-rich oedema fluid into the air spaces and acute respiratory failure [2–5a,7]. The acute phase of ALI and ARDS is a consequence of severe damage to the pulmonary alveolar capillary membrane. Owing to the increased permeability of the membrane, the alveolar spaces become flooded with inflammatory cells and plasma proteins and, subsequently, pulmonary blood flows through non-ventilated or poorly ventilated alveoli. The initial inflammatory response is called the exudative phase, during which extravasation of protein-rich intravascular fluid and inflammatory cell infiltration take place (Figure 1). Damage to the capillary endothelium and the alveolar epithelium are early events, and are accompanied by interstitial and intra-alveolar oedema, and haemorrhage. During this phase, large numbers of neutrophils enter the lungs. Various pro-inflammatory mediators are also released, including cytokines, reactive oxygen and nitrogen species, and proteases. As the disease progresses, intravascular coagulation, inhibition of fibrinolysis, formation of fibrin-rich hyaline membranes, inactivation of surfactants, platelet aggregation and, finally, capillary thrombosis are frequently observed. The changes characteristic for the exudative phase are summarized in Figure 1. The subsequent phase, called the fibroproliferative phase, is characterized by chronic inflammation, neovascularization and fibrosis. The final phase of ARDS, the recovery phase, leads to an improvement in oxygenation and lung compliance [2–5a].

Diffuse ALI that is characteristic of ALI/ARDS could be propagated by multiple processes [2,4,8]. One of the possible mechanisms underlying injury to the lung is the influx of neutrophils observed in the majority of patients with ALI/ARDS. There is a significant increase in both the total number of neutrophils and the proportion of neutrophils occurring in the alveolar spaces. For example, neutrophils constitute 70–80% of the cells in BAL (bronchoalveolar lavage) fluid from patients with ARDS compared with approx. 0.8–3% in normal subjects [9,10]. Activated neutrophils that release metalloproteinases, myeloperoxidase, collagenases, and reactive oxygen and nitrogen species during migration into alveolar spaces may contribute to the endothelial and epithelial injury that is characteristic of ARDS [8,9,11]. Moreover, IL (interleukin)-8, a potent neutrophil attractant and activator, has been implicated in the neutrophil recruitment in lungs of patients with ARDS [12,13].

The increase in endothelial and epithelial permeability that occurs in ARDS allows higher molecular-mass proteins, such as IgG and IgM, to enter the airspaces [11]. Studies from our laboratory have shown that a significant portion of IL-8 in lung fluids from patients with ARDS is associated with anti-IL-8 autoantibodies [14]. Autoantibodies against IL-8 have been found in human plasma, which contains anti-IL-8 IgG as well as IgG:IL-8 complexes [14,15], and in gastric mucosa where IgA binds IL-8 [16], and it has been suggested
that anti-IL-8 autoantibodies may be involved in the pathogenesis of human diseases [16].

**ANTI-IL-8 AUTOANTIBODIES**

The total protein content of BAL fluid is significantly increased in patients with ALI/ARDS in comparison with normal subjects. Furthermore, in ALI/ARDS, there is loss of size selectivity for proteins that pass through the alveolar epithelium and the fraction of high-molecular-mass proteins increases. The plasma proteins in BAL fluid from patients with ARDS are in similar proportions to those in plasma. IgM, for example, is practically undetectable in normal BAL fluid, but it is a major component of ARDS lavage fluid [11]. Consequently, levels of high-molecular-mass proteins, including immunoglobulins, are increased in the lungs of patients with ALI/ARDS.

Autoantibodies to several cytokines, including IL-1α, IL-2, TNF-α (tumour necrosis factor-α), IFN (interferon)-γ, IL-6, IL-8 and MCP-1 (monocyte chemo-attractant protein-1) have been identified in normal human plasma [15,17–22]. Autoantibodies have also been detected in patients treated with recombinant or natural IFN-α and IFN-β [23]. An increase in the concentrations of anticytokine autoantibodies, including anti-IL-8 autoantibodies, has been observed in certain inflammatory diseases [19–21,24,25]. The concentration of gastric anti-IL-8 autoantibodies was increased in gastritis and correlated with IL-8 production [16]. Similarly, plasma concentrations of autoantibodies against IL-8 were elevated in rheumatoid arthritis [25]. Most of anticytokine autoantibodies are of the IgG class [17,18,20–22], except for anti-TNF-α and anti-IL-8 autoantibodies [14–16,19]. The former include both IgG and IgM classes, and the latter include IgA, IgG and IgM classes [14–16,19]. Most anti-IL-1α autoantibodies are of the IgG4 isotype [24], whereas anti-TNF-α autoantibodies are predominantly IgG1 and IgG2 [19]. Other common human autoantibodies are primarily IgM, IgG1 and IgG2 [26]. The plasma anti-IL-8 autoantibodies are polyclonal, since all samples have both κ and λ light chains [15]. IgG2 and IgG3 are the primary IgG subclasses according to one report [15]. Our
studies have shown that anti-IL-8 autoantibodies in BAL fluid from patients with ALI/ARDS and normal plasma belong to IgG, IgM and IgA classes, and IgG4 and IgG2 are the predominant IgG subclasses (IgG1 > IgG2 > IgG1 and IgG2) [14]. Sera from healthy donors contain autoantibodies against IL-1α with a frequency of 24%, TNF-α autoantibodies with a frequency of 48%, IL-6 autoantibodies with a frequency of 1.9% and IL-8 autoantibodies with a frequency of 35% [15,21,24,27]. We have detected anti-IL-8 autoantibodies in five out of 38 normal human plasmas (13%), and four out of nine ALI/ARDS plasmas (44%) [14], whereas Watanabe et al. [28] were not able to find any autoantibodies against IL-8 in plasma from healthy donors (15 individuals were tested). The discrepancies between findings relating to the frequency of anti-IL-8 autoantibodies may be due to differences in the populations tested and assays used to detect these autoantibodies [14,15,28].

Little is known about the biological activity of cytokine autoantibodies. It has been suggested that autoantibodies to some cytokines function as inhibitors and/or immunoregulators of cytokine activity [27,29]. Anti-IL-1α autoantibodies purified from sera of patients with rheumatoid arthritis inhibited both IL-1α-induced thymocyte proliferation and the binding of 125I-IL-1α to receptors on rheumatoid synovial cells. Patients whose sera contained anti-IL-1α antibodies had lower concentrations of rheumatoid factor than patients with undetectable antibodies [30]. In addition, anti-IL-1α antibodies from normal human plasma blocked 125I-IL-1α binding to its receptor on murine thymoma EL4-6.1 cells [24]. Moreover, antimicrobial functions of normal human blood neutrophils were impaired when anti-GM-CSF (granulocyte/macrophage colony-stimulating factor) autoantibodies were included in the cell preparations used to evaluate these functions [31]. Anti-GM-CSF autoantibodies are the most prevalent anticytokine autoantibodies in pooled human IgG samples and are common in patients with pulmonary proteinosis [31,32]. Autoantibodies to cytokines may also act as transporters of cytokines, protect cytokines from urinary excretion and proteolytic degradation, or control the activity of cytokines [27]. Anti-IL-1α autoantibodies, for example, bind to IL-1α with a KD lower than 5 × 10⁻¹¹ mol/l. However, the KD for IL-1α binding to target cells is either approx. 5 × 10⁻¹² mol/l (lower affinity) or 1 × 10⁻¹²–1 × 10⁻¹¹ mol/l (higher affinity), indicating that anti-IL-1α antibodies may regulate the binding of IL-1α to its cellular receptors [27]. In addition, anti-IL-1α antibodies from some healthy donors did not inhibit IL-1α activity, even though they were of the same class (IgG), subclass (IgG1) and concentration as the ‘blocking’ antibodies. These findings suggest that some anti-IL-1α antibodies recognize epitopes on the IL-1α molecule not involved in receptor binding. We have found [14] that the KD for anti-IL-8 autoantibodies from lung fluids of patients with ALI/ARDS was 6.4 × 10⁻¹² mol/l. In comparison, the KD for anti-IL-8 autoantibodies from human plasma was 4.6 × 10⁻¹² mol/l. Furthermore, we are the first group to study the function of anti-IL-8 autoantibodies. Anti-IL-8 autoantibodies inhibited binding of 125I-IL-8 to human neutrophils and suppressed the chemotactic activity of IL-8. Our findings also indicate that one molecule of anti-IL-8 autoantibody binds one molecule of IL-8, and the autoantibody combines with an epitope contained within residues 35–72 of the IL-8 molecule [14].

**ANTI-IL-8:IL-8 [ANTI-IL-8 AUTOANTIBODY:IL-8 IMMUNE] COMPLEXES**

Autoantibodies may not always suppress the biological activity of cytokines. In fact, it has been reported that anti-IL-1α antibodies prolonged the plasma half-life of 125I-IL-1α and altered its tissue distribution when antibody:IL-1α complexes were injected into rats. IL-1α autoantibodies might modulate IL-1α activity by acting as IL-1α carriers [24]. Similar results were obtained for IL-3, IL-4, IL-7 and IL-6 [33,34]. For example, neutralizing antibodies against murine IL-6 given to mice before an IL-6 inducer reduced the effect of IL-6, whereas the same antibody complexed with murine IL-6 elicited an enhanced response [34].

In agreement with the concept that anticytokine:cytokine complexes could enhance cytokine activity, studies from our laboratory revealed that anti-IL-8:IL-8 complexes may contribute to the pathogenesis of ARDS [14,35,36]. Patients at risk of ARDS who had high concentrations of anti-IL-8:IL-8 complexes in their lung fluids were more likely to develop ARDS [35,36], and the level of anti-IL-8:IL-8 complexes also correlated with mortality in patients with well-established ARDS [35]. It should be noted that the occurrence of anti-IL-8:IL-8 complexes is quite high, since they are present in 55–70% of normal human plasma [14,15]. Although we have not specifically studied the frequency of plasma anti-IL-8:IL-8 complexes in patients with ARDS, we have evaluated plasma from eight patients with ARDS for the presence of the complexes. All of these plasma contained detectable levels of anti-IL-8:IL-8 complexes [14]. We have also demonstrated that anti-IL-8:IL-8 complexes purified from oedema fluids from patients with ALI possess pro-inflammatory activity in vitro and have the ability to prolong neutrophil life [37–39]. Moreover, our findings indicate that anti-IL-8:IL-8 complexes are deposited in the lungs of patients with ARDS [40]. Other clinical studies have also shown that anti-IL-8:IL-8 complexes are related to disease activity. For example, Shute et al. [41] reported that increased concentrations of these complexes in the bronchial mucosa are related to disease activity in patients with asthma.
The complexed autoantibody enhanced eosinophil responses to IL-8, including the release of GM-CSF and chemotaxis [42]. Elevated concentrations of the complexes were also correlated with the clinical severity of rheumatoid arthritis [43] and with disease development in patients with heparin-induced thrombocytopenia and thrombotic syndrome, life-threatening conditions usually accompanied by serious complications, including stroke and myocardial infarction [44–46].

**CONNECTION OF ANTI-IL-8:IL-8 COMPLEXES WITH ALI/ARDS**

To define the function of anti-IL-8:IL-8 complexes in ARDS, we measured the concentrations of free and complexed IL-8 in BAL fluids from 19 patients at risk of ARDS and 40 more patients with well-defined ARDS (1, 3, 7, 14 and 21 days after the onset of ARDS) [35]. In agreement with our previous observations [14], we found that the amount of complexed IL-8 was significantly decreased in survivors of ARDS over time. In contrast, the concentration of complexed IL-8 did not decline with time in those individuals who died. Furthermore, the presence of increased amounts of anti-IL-8:IL-8 complexes correlated with the development of ARDS. The increase in endothelial and epithelial permeability that occurs in ARDS allows higher molecular-mass proteins, such as IgG and IgM, to enter the airspaces [11]. To provide an insight into whether anti-IL-8 autoantibodies move passively into the lungs as protein permeability increases following endothelial and epithelial injury, or whether it might be produced in the lungs, we normalized anti-IL-8:IL-8 complex concentrations for BAL fluid total protein. The ratio of anti-IL-8:IL-8 complexes to total protein was significantly higher at the onset of ARDS than in patients at risk for ARDS. In addition, patients with ARDS with an elevated anti-IL-8:IL-8 complex/protein ratio were approx. 4.5 times more likely to die than patients with lower concentrations of anti-IL-8:IL-8 complexes. Although this suggested a strong relationship between the complex/protein ratio and both onset and outcome of ARDS, it also suggests that the formation of anti-IL-8:IL-8 complexes is not a simple consequence of increased protein permeability in the lungs. An increase in the complex/protein ratio could be explained by the local production of anti-IL-8 autoantibodies within the lungs. Lymphocyte concentrations in BAL fluid do not increase above normal at the onset of ARDS [35], so the production of specific anti-IL-8 autoantibodies would have to occur either from the small number of existing B-cells in the alveolar spaces, or from B-cells in the interstitium or other lung lymphoid tissue [35]. Moreover, anti-IL-8 autoantibodies appear in plasma during infection in patients who did not have detectable amounts of these antibodies before the infection (A.K. Kurdowska, unpublished work), and plasma levels of anti-IL-8 autoantibodies are significantly higher in patients with ARDS than normal subjects [14]. These last two observations suggest that the production of anti-IL-8 autoantibodies is triggered in the inflammatory conditions. Patients at risk of ARDS usually have an underlying severe inflammatory disease. An increase in the concentration of autoantibodies to IL-8 could occur in these patients. If the ratio of the antibody to the antigen favours complex formation, such complexes appear in the alveolar compartment. Moreover, escalating inflammation could lead to the production of higher quantities of IL-8 in patients whose immune system is already primed to produce anti-IL-8 autoantibodies. Therefore more complexes may be formed, adding to the severity of the disease. In support of our hypothesis, Sylvester et al. [22] have demonstrated that, after LPS (lipopolysaccharide) administration to humans, the plasma concentration of IL-8 dramatically increased and promoted the formation anti-IL-8:IL-8 complexes. Alternatively, anti-IL-8:IL-8 complexes could be formed in close proximity to endothelial cells, due to IL-8 binding to syndecans and/or other matrix proteins [47]. In this way, the local concentration of anti-IL-8:IL-8 complexes would be increased. In agreement with this concept, we have detected complexes associated with the endothelium in lung tissues from patients with ARDS (R. Fudala, A. Krupa and A.K. Kurdowska, unpublished work).

The fact that the higher levels of anti-IL-8:IL-8 complexes were present in BAL fluid samples from patients with ARDS compared with patients at risk of ARDS may indicate a link between complex concentration and the development of ARDS. To explore this hypothesis further, we have evaluated distinct populations of patients at risk of ARDS [36]. Concentrations of free and complexed IL-8 were measured in BAL fluids from 26 patients at risk of ARDS. These patients fell into three groups: group 1 patients developed ARDS, but group 2 and 3 patients did not. IL-8 concentrations were high in patients from group 1 and 2, and low in group 3 patients. However, only group 1 (patients who developed ARDS) had substantially higher levels of anti-IL-8:IL-8 complexes. This study indicates that these complexes correlate with the observed early enhanced intrapulmonary inflammatory response in patients at risk who progress to ARDS.

Finally, we evaluated lung tissues from patients with ARDS for the presence of anti-IL-8:IL-8 complexes. We detected IL-8 associated with IgG in tissues of patients with ARDS but not in normal tissues, indicating that anti-IL-8:IL-8 complexes are deposited in the lungs of patients with ARDS [40]. The actual diagnostic and therapeutic significance of our observations remain to be established; however, since lung deposition of immune complexes causes severe lung inflammation leading to injury in animal models [48–50], anti-IL-8:IL-8 complexes could potentially contribute to the pathogenesis of ARDS. Accordingly, our animal studies have confirmed that rabbit
anti-IL-8:IL-8 complexes or analogous mouse complexes [anti-KC:KC complexes (anti-KC autoantibody:KC immune complexes), where KC is keratinocyte-derived chemokine, also known as CXCL1 (chemokine ligand 1)] have the ability to induce severe lung inflammation [51,52]. We have demonstrated that instillation of purified rabbit anti-IL-8:IL-8 complexes into the lungs of rabbits stimulates an increase in lung fluid concentrations of IL-8 and neutrophils [51]. Furthermore, we have developed a mouse model to evaluate the contribution of anti-KC:KC complexes generated in situ in lung inflammation and injury (immune-complex-induced lung inflammation). Although mice do not express IL-8, murine KC (CXCL1/KC) is functionally equivalent to human IL-8 [53]. In this model, autoantibodies to KC (in plasma and the alveolar compartment) are first induced by immunization with KC, and then KC is administered intratracheally to generate anti-KC:KC complexes in the lung. In these animals we found increased transalveolar influx of neutrophils, increased permeability and alveolar haemorrhage, together with histological evidence of increased infiltration of inflammatory cells, interstitial thickening and the presence of alveolar exudate. All of these findings indicate the presence of severe pulmonary inflammation and alveolar damage [52].

The possibility that autologous immune complexes, such as anti-IL-8:IL-8 complexes or anti-KC:KC complexes in mice, may be involved in the pathogenesis of lung inflammation/injury has not been considered before. It is known that the deposition of heterologous immune complexes (reverse passive Arthus reaction) can trigger a localized inflammatory response in different tissues, including the lung [50]. The models of immune-complex-induced alveolitis are based on the local formation of heterologous immune complexes, which then trigger the alveolar inflammatory response. A foreign antigen is given intravenously and, immediately after that, an antibody against this antigen (usually a rabbit antibody) is administered intratracheally. We have developed a model in which mice are immunized with murine antigen (KC) for several weeks. After autoantibodies develop, the antigen (KC) is administered intratracheally and autologous immune complexes (anti-KC:KC complexes) form in the lungs. This model mimics very well the situation observed in patients with ARDS who have anti-IL-8 autoantibody complexes in their lung fluids [52].

**PRO-INFLAMMATORY ACTIVITY OF THE COMPLEXES**

Our clinical and animal studies suggest that anti-IL-8:IL-8 complexes could contribute to the pathogenesis of ALI/ARDS. Therefore we examined whether anti-IL-8:IL-8 complexes exhibit pro-inflammatory activity in vitro. As IL-8 has been implicated in neutrophil recruitment in the lungs of patients with ARDS and there is a significant influx of neutrophils to the lungs of these patients [9–13], we evaluated the activity of the complexes using human blood neutrophils. Essential functions of human neutrophils include directed migration (chemotaxis), ‘respiratory burst’ (generation of superoxide) and degranulation (enzyme release) [54,55]. The complexes were purified from pulmonary oedema fluid samples obtained from patients with ALI. First, we found that IL-8 bound to the autoantibody retained its ability to trigger chemotaxis of neutrophils. Next, we examined the ability of anti-IL-8:IL-8 complexes to induce neutrophil activation, i.e. neutrophil respiratory burst and degranulation. Anti-IL-8:IL-8 complexes triggered superoxide and myeloperoxidase release from human neutrophils [37]. We also examined the role of anti-IL-8:IL-8 complexes in modulating spontaneous apoptosis of normal human neutrophils. Elimination of inflammatory cells by programmed cell death, or apoptosis, plays an important role in the resolution of the inflammatory process. Inhibition of apoptosis could therefore result in the continuous presence of increased numbers of inflammatory cells, some of them injured, that can prolong the recovery process [56]. In recent years, apoptosis in ARDS has been extensively studied [56,57]. Consequently, it was established that apoptosis of neutrophils is delayed in patients with ARDS [57]. There have been several reports suggesting the involvement of members of the Bcl-2 family in the regulation of neutrophil apoptosis. The Bcl-2 family of apoptosis-associated proteins can be divided into two groups: anti-apoptotic proteins (such as Bcl-2, Bcl-XL, Mcl-1 and A1/Bfl-1) and pro-apoptotic proteins (such as Bax, Bak, Bad, Bik and Bid). Neutrophils have been reported to express proteins of both groups, i.e. Mcl-1, A1/Bfl-1 and Bcl-XL, and Bax, Bad and Bak. Anti-apoptotic proteins down-regulate apoptosis by controlling the activity of caspases and by blocking the apoptotic cascade at the level of the mitochondria [58,59]. Neutrophils express the caspases 1, 3, 8 and 9 [60,61]. The family of caspases can be divided into two functional subgroups: the initiator and the executioner caspases. Apoptotic responses in neutrophils are initiated by activation of caspase 8 or caspase 9 that can result in activation of executioner caspases, such as caspase 3, leading eventually to apoptosis [61]. We found that anti-IL-8:IL-8 complexes inhibited apoptosis of neutrophils [39]. Furthermore, anti-IL-8:IL-8 complexes induced an increase in the level of the anti-apoptotic protein Bcl-XL. In contrast, levels of the pro-apoptotic proteins Bax and Bak were decreased in the same conditions. Anti-IL-8:IL-8 complexes also suppressed the activity of caspase 3 and caspase 9 [39]. In summary, our findings indicate that the complexes enhance IL-8 activity in the lung because of their ability to attract and activate neutrophils, and modulate apoptosis of these cells [37,39].
Activity of immune complexes is mediated by receptors for IgG (FcγRs). Two families of stimulatory receptors that interact with immune complexes, FcγRII (CD32) and FcγRIII (CD16), have been identified in humans [62]. The first family consists of two activating receptors, namely FcγRIIa and FcγRIII, and the second also consists of two receptors, namely FcγRIIia and FcγRIIic. These receptors elicit cell activation, endocytosis and phagocytosis [62]. FcγRIIa is the most widespread human FcγR, being present on most inflammatory cells. It acts together with monomeric IgG, but binds avidly to complexes of IgG. FcγRIIa, as the activating FcγR, initiates phagocytosis, antibody-dependent cellular cytotoxicity, transcription of cytokines genes and the release of inflammatory mediators. FcγRIIa is thus a potent activator of inflammation. In addition, recombinant soluble FcγRIIa has been shown to totally inhibit immune-complex-induced activation of inflammatory cells, suggesting that FcγRIIa plays a critical role in the earliest phases of immune-complex-induced inflammation [63].

It has been established that the engagement of FcγRIIa initiates a tyrosine kinase cascade dependent upon the cytoplasmic tail of this receptor, which contains one copy of ITAM (immunoreceptor tyrosine-based activation motif), a substrate for phosphorylation by members of the Src tyrosine-kinase family. Phosphorylated ITAM can bind to and activate Syk or Lyn tyrosine kinases, which subsequently activate a number of downstream signals. Recent studies show that phosphorylation of tyrosine residues within ITAM can activate Syk and Lyn, both of which play a critical role in immune complex-mediated cell activation. Phosphorylation of ITAM also results in the recruitment and activation of the Src tyrosine kinase, which subsequently activates a number of downstream signals.

One of the downstream signals activated by FcγRIIa is PLCγ, a phospholipase that hydrolyzes PtdIns(4,5)P2 to DAG and IP3. DAG and IP3 act as second messengers in the regulation of intracellular calcium and protein kinase C (PKC) activation. The activation of PKC leads to the translocation of actin filaments and the assembly of the actin cytoskeleton, which is essential for phagocytosis and the formation of phagocytic vacuoles. PKC activation also results in the activation of other downstream signals, such as MAPKs (mitogen-activated protein kinases) and mTOR (mammalian target of rapamycin), which are involved in the regulation of cellular proliferation, differentiation, and survival.

FcγRIIa is a potent activator of MAPKs, including ERK, JNK, and p38. These kinases play a critical role in the regulation of cell survival, proliferation, and differentiation. For example, ERK activation results in the transcription of inflammatory cytokines, such as TNF-α and IL-1β, and the expression of adhesion molecules, which are involved in the recruitment of leukocytes to sites of inflammation. JNK activation results in the induction of pro-apoptotic genes, such as Bax and Bak, and the inhibition of anti-apoptotic genes, such as Bcl-2 and Bcl-xL, which are involved in the regulation of cell death.

The exact role of FcγRIIa in the pathogenesis of ARDS is not fully understood. However, it has been shown that FcγRIIa is upregulated in the lungs of patients with ARDS and that the expression of FcγRIIa is correlated with the severity of the disease. Furthermore, the administration of anti-FcγRIIa antibodies has been shown to improve the survival of mice with ARDS, suggesting that FcγRIIa plays a critical role in the pathogenesis of ARDS.

In conclusion, FcγRIIa is a critical component of the immune system and plays a crucial role in the regulation of immune complex-mediated cell activation. The targeting of FcγRIIa may represent a novel therapeutic strategy for the treatment of immune complex-mediated diseases, such as ARDS.
and their specific antigens or anti-HLA antibodies and target antigens are implicated in the pathogenesis of TRALI (transfusion-related ALI) strengthens our hypothesis [75,76], in particular that the latter immune complexes activate neutrophils via FcγRs in a mouse model of TRALI [77].

CONCLUDING REMARKS

ALI/ARDS is a complex clinical syndrome consisting of acute respiratory failure with hypoxaemia and diffuse infiltrates. It is frequently associated with serious clinical disorders, such as sepsis or trauma [2–8]. Dysregulation of the lung inflammatory response is often identified as a hallmark of ALI/ARDS [2]. Multiple experimental and clinical studies have indicated that neutrophil migration into the lung tissue plays a crucial role in the pathogenesis of ALI/ARDS [9,10,54,55,78], and IL-8 mediates this influx of neutrophils [2,12,79]. Our findings have shown that a significant portion of IL-8 in lung fluids from patients with ALI/ARDS is associated with anti-IL-8 autoantibodies (anti-IL-8:IL-8 complexes) [14,35,37]. Several observations made by our laboratory support the hypothesis [75,76], in particular that the latter immune complexes retain functional activity germane to alveolitis [133]. Our findings have shown that anti-IL-8:IL-8 complexes are deposited in the lungs in vitro [157], and their specific antigens or anti-HLA antibodies and target antigens are implicated in the pathogenesis of TRALI (transfusion-related ALI) strengthens our hypothesis [75,76], in particular that the latter immune complexes activate neutrophils via FcγRs in a mouse model of TRALI [77].

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