Activity of pulmonary edema fluid interleukin-8 bound to α2-macroglobulin in patients with acute lung injury

ANNA K. KURDOWSKA, THOMAS K. GEBER, SUSANNE M. ALDEN, BOZENA R. DZIADEK, JAMES M. NOBLE, THOMAS J. NUCKTON, AND MICHAEL A. MATTHAY

1Department of Biochemistry, University of Texas Health Center, Tyler, Texas 75708; and 2School of Medicine, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0130

Received 24 September 2001; accepted in final form 15 December 2001

Kurdowska, Anna K., Thomas K. Geiser, Susanne M. Alden, Bozena R. Dziadek, James M. Noble, Thomas J. Nuckton, and Michael A. Matthay. Activity of pulmonary edema fluid interleukin-8 bound to α2-macroglobulin in patients with acute lung injury. Am J Physiol Lung Cell Mol Physiol 282: L1092–L1098, 2002.First published December 21, 2001; 10.1152/ajplung.00378.2001.—The formation of α2-macroglobulin (α2-M)/interleukin-8 (IL-8) complexes may influence the biological activity of IL-8 and the quantitative assessment of IL-8 activity. Therefore, in this study, concentrations of free IL-8 and IL-8 complexes with α2-M were measured in pulmonary edema fluid samples from patients with acute lung injury/acute respiratory distress syndrome (ALI/ARDS) and compared with control patients who are ventilated and critically ill but do not have hydrostatic pulmonary edema. Patients with ALI/ARDS had significantly higher concentrations of α2-M (P < 0.05) as well as α2-M/IL-8 complexes (P < 0.05). Because a substantial amount of IL-8 is complexed to α2-M, standard assays of free IL-8 may significantly underestimate the concentration of biologically active IL-8 in the distal air spaces of patients with ALI/ARDS. Furthermore, IL-8 bound to α2-M retained its biological activity, and this fraction of IL-8 was protected from proteolytic degradation. Thus, complex formation may modulate the acute inflammatory process in the lung.

α2-M in pulmonary edema fluid; neutrophils; IL-8 receptors; specific binding

Acute lung injury and acute respiratory distress syndrome (ALI/ARDS) are characterized by diffuse lung injury resulting in deterioration of lung function. Due to an increase in capillary-alveolar membrane permeability, α2-macroglobulin (α2-M) translocates into the air spaces in patients with ALI/ARDS (30, 32). α2-M is a proteinase inhibitor that exists both in a “native” (or “slow”) and modified (or “fast”) form. This latter form of α2-M migrates faster than native α2-M during electrophoresis (9). Slow (native) α2-M displays anti-proteinase activity. The fast or inactive form of α2-M is generated on the reaction with neutrophil elastase, a product of the neutrophils that accumulates in the alveolar space in patients with ALI/ARDS (29, 31). α2-M binds several cytokines, including interleukin-8 (IL-8) (12, 14, 17). IL-8 is a potent neutrophil attractant and activator (23). Several studies have demonstrated that high concentrations of IL-8 are present in bronchoalveolar lavage (BAL) and pulmonary edema fluids from patients with ALI/ARDS (5, 7, 18–20). Furthermore, a significant fraction of IL-8 in BAL fluid is associated with α2-M (14). IL-8 binds only to fast (methylamine-treated) α2-M. [Methylamine is routinely used to convert slow (native) α2-M to fast form (9).] In addition, in vitro both IL-8 and α2-M/IL-8 complexes bind to specific receptors for IL-8 on human neutrophils with similar affinity (13).

Most of α2-M in BAL fluid of patients with ALI/ARDS is associated with neutrophil elastase (fast form) (32). Therefore, we hypothesized that lower levels of active α2-M were present in pulmonary edema fluid of patients with ALI/ARDS than in patients with hydrostatic pulmonary edema. Because IL-8 binds to the inactive, fast form of α2-M, we also hypothesized that the levels of α2-M/IL-8 complexes would be increased in ALI/ARDS patients compared with control patients with hydrostatic pulmonary edema. Furthermore, we wanted to test the hypothesis that the IL-8 bound to α2-M in pulmonary edema fluid of ALI/ARDS patients is biologically active. In contrast to our previous studies using BAL fluid from patients with ALI/ARDS and from healthy volunteers, we used undiluted pulmonary edema fluid in this study because pulmonary edema fluid reflects the actual lung environment more accurately than BAL fluid. Because BAL fluids from individual patients are diluted, interpretation of the data is also somewhat difficult. In addition, we compared the interaction between IL-8 and α2-M in pulmonary edema fluids from patients with ALI/ARDS and from patients with hydrostatic edema, an ideal group of control patients who are ventilated and critically ill but have pulmonary edema primarily due to elevated pul-
monary vascular pressure (28). Furthermore, because of a sustained inflammatory response in the latter group, no significant increase in concentrations of IL-8 and α2-M is expected (25, 28).

MATERIALS AND METHODS

Human subjects. All studies involving human blood and pulmonary edema fluid were approved by the Human Subjects Investigation Committees of the University of California, San Francisco, and the University of Texas Health Center at Tyler. Informed written consent was obtained from all the subjects or their representatives. ALI was diagnosed according to the following criteria: 1) PaO2-to-FIO2 ratio <300 mmHg, 2) bilateral infiltrates on the chest radiograph, and 3) a pulmonary artery wedge pressure of ≤18 mmHg and/or no clinical evidence of elevated left atrial pressure (1). Hydrostatic pulmonary edema was defined as published before (28).

As in our prior studies (28), the definition of hydrostatic edema was based on clinical evidence of cardiac dysfunction from an acute myocardial infarction, exacerbation of chronic heart failure, or volume overload with either a pulmonary arterial wedge pressure >18 mmHg or a two-dimensional echocardiogram demonstrating a reduction in the left ventricular ejection fraction plus the presence of a transudative pulmonary edema fluid-to-plasma total protein ratio <0.65. Pulmonary edema fluid samples from patients with ALI/ARDS or hydrostatic pulmonary edema were obtained as previously described (30). Briefly, pulmonary edema fluid samples were obtained within 15 min of intubation and mechanical ventilation. A 14-French suction catheter (Becton-Dickinson, Lincoln Park, NJ) was passed through the endotracheal tube and wedged into the distal airways. Then, edema fluid samples were suctioned gently through the inserted catheter. The samples were centrifuged at 3,000 g for 10 min, and the supernatants were stored at −70°C until use.

Measurement of total protein. The total protein concentration in the edema fluid samples was measured by using Coomassie Plus protein assay reagent (Pierce, Rockford, IL) according to the manufacturer’s instructions. For the measurement of edema fluid-to-plasma protein concentration ratios, protein concentration was measured by the biuret method as previously described (28).

Measurement of concentration of α2-M. The concentration of α2-M in the edema fluid samples was measured in an ELISA assay as previously described (2, 14).

Visualization of different forms of α2-M in the edema fluid samples (electrophoresis I). To establish the state of α2-M (slow vs. fast), pulmonary edema fluid samples were subjected to nondenaturing polyacrylamide gel electrophoresis (5% Tris-borate) and autoradiography (22). In this type of electrophoresis only α2-M bands can be detected using Coomassie blue stain (see Fig. 1). When samples are incubated with 125I-rhIL-8, radioactive bands are visualized by autoradiography. These bands represent 125I-rhIL-8 bound to α2-M and migrate as does α2-M alone.

Purification of α2-M/IL-8 complexes (gel filtration chromatography). To purify α2-M/IL-8 complexes, we separated the pulmonary edema fluid samples on an HPLC gel filtration column, TSK-250. PBS was used as elution buffer at a flow rate of 0.25 ml/min, and the fractions were analyzed using IL-8 ELISA.

Measurement of activity of purified α2-M/IL-8 complexes (neutrophil chemotaxis). Chemotactic activity of neutrophils (0.4 × 106/chemotactic chamber) was assessed by the leading edge of the monolayer of neutrophils (25) at the back of the test tube (25). The activity was expressed as the number of neutrophils migrated into the wells (25). The results were expressed as means ± SE.

Measurement of concentration of IL-8. IL-8 concentration in the edema fluid samples was measured in an ELISA assay developed in our laboratory by using a matched antibody pair according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN).

Measurement of concentration of α2-M/IL-8 complexes. The concentration of α2-M/IL-8 complexes in the edema fluid samples was measured by using a specific ELISA assay developed in our laboratory (14).

Visualization of binding of 125I-recombinant human IL-8 to α2-M in the edema fluid samples (electrophoresis II). Recombinant human IL-8 (rhIL-8; R&D Systems) was labeled with 125I (11). Pulmonary edema fluid samples were incubated with labeled IL-8, and complexes of 125I-rhIL-8 with α2-M were detected by nondenaturing polyacrylamide gel electrophoresis (5% Tris-borate) and autoradiography (22). In this type of electrophoresis only α2-M bands can be detected using Coomassie blue stain (see Fig. 1). When samples are incubated with 125I-rhIL-8, radioactive bands are visualized by autoradiography. These bands represent 125I-rhIL-8 bound to α2-M and migrate as does α2-M alone.

Purification of α2-M/IL-8 complexes (gel filtration chromatography). To purify α2-M/IL-8 complexes, we separated the pulmonary edema fluid samples on an HPLC gel filtration column, TSK-250. PBS was used as elution buffer at a flow rate of 0.25 ml/min, and the fractions were analyzed using IL-8 ELISA.

Preparation of neutrophils. Human neutrophils from healthy volunteers were separated by dextran sedimentation and erythrocyte lysis by the method of Boyum (3). The purity of neutrophils was usually 80–90%.

Measurement of slow/native α2-M (trypsin binding assay). To calculate the amount of slow or native form, α2-M was used for trypsin binding activity by the method of Ganrot (8). The activity of trypsin (Sigma, St. Louis, MO) was determined by active site titration with the substrate, p-nitrophenyl-p'-guanidinobenzoate hydrochloride (4).

Measurement of the quantity of α2-M bound to neutrophil elastase (α2-M/elastase complexes) in the edema fluid samples. The concentration of neutrophil elastase bound to α2-M in pulmonary edema fluid was determined by measuring the rate of hydrolysis of the elastase substrate MeOSuc-Ala-Ala-Pro-Val-NH2p in the presence and absence of an α1-proteinase inhibitor (a generous gift from Dr. Hall James, the University of Texas Health Center, Tyler, TX) (21).

Fig. 1. Gel electrophoresis of α2-macroglobulin (α2-M) in pulmonary edema fluids from patients with acute lung injury/acute respiratory distress syndrome (ALI/ARDS) and hydrostatic pulmonary edema (Coomassie blue staining). Lane 1: “slow” form of purified human α2-M; lanes 2 (ALI/ARDS), 4 (ALI/ARDS), and 6 (hydrostatic pulmonary edema): edema fluid samples from 3 different patients; lanes 3, 5, and 7: same edema fluid samples as in lanes 2, 4, and 6 but treated with methylene blue; lane 8: “fast” form of purified human α2-M.
of sepsis and renal failure in the ALI/ARDS patients (Table 1).

Edema fluid/plasma protein concentration ratio. The edema fluid-to-plasma protein concentration ratio was significantly higher in patients with ALI/ARDS compared with patients with hydrostatic edema (0.98 ± 0.21 and 0.63 ± 0.28) (P < 0.01). The total protein concentration in the edema fluid was also increased in ALI/ARDS patients compared with patients with hydrostatic edema (4.7 ± 1.6 and 3.0 ± 0.6 g/100 ml, respectively) (P < 0.05).

**α2-M in pulmonary edema fluid.** The different forms of α2-M present in pulmonary edema fluid of patients with ALI/ARDS and patients with hydrostatic edema are depicted in Fig. 1. Samples were chosen according to their ability to inhibit trypsin. Edema fluid samples from three different patient untreated [lanes 2 (ALI/ARDS), 4 (ALI/ARDS), and 6 (hydrostatic pulmonary edema)] and the same edema fluid samples treated with methylamine [lanes 3 (ALI/ARDS), 5 (ALI/ARDS), and 7 (hydrostatic pulmonary edema)] were run on a native gel. Samples containing slow or native α2-M (capable of interacting with trypsin or methylamine) are shown in lanes 2 and 4, and a sample containing both slow and fast α2-M is shown in lane 6. Patients with ALI/ARDS had a higher total α2-M concentration in edema fluid compared with patients with hydrostatic edema (P < 0.01) (Fig. 2A) due to the increase in capillary-alveolar permeability. As hypothesized, due to differences in the nature of pulmonary inflammation, the relative amount of slow to native α2-M (expressed as a percentage of total α2-M) was decreased in patients with ALI/ARDS. Pulmonary edema fluid from patients with ALI/ARDS contained significantly less slow or native α2-M (expressed as a percentage of total α2-M) than did pulmonary edema fluid from hydrostatic edema patients (P < 0.001) (Fig. 2B). In addition, the quantity of α2-M bound to neutrophil elastase or fast α2-M (expressed as a percentage of total α2-M) was higher in patients with ALI/ARDS compared with patients with hydrostatic edema (34 ± 33% vs. 8 ± 19%), though that difference did not quite reach statistical significance (P = 0.06). Because the total α2-M concentration was significantly higher in patients with ALI/ARDS, the actual concentration of the slow form did not differ between patient groups (0.33 ± 0.19 and 0.31 ± 0.20 mg/ml, for ALI/ARDS and hydrostatic edema groups, respectively) (P = 0.83). However, the concentration of α2-M bound to neutrophil elastase was significantly increased in patients with ALI/ARDS compared with patients with hydrostatic edema (0.28 ± 0.29 and 0.03 ± 0.06 mg/ml, respectively) (P < 0.05).

**Interaction of IL-8 with α2-M.** The pulmonary edema fluid concentrations of IL-8 were not significantly different between the two groups of patients (P = 0.15), although in patients with ALI/ARDS, levels of IL-8 showed a tendency to increase (39 ± 38 and 17 ± 22 ng/ml, respectively). However, the concentration of α2-M/IL-8 complexes was significantly higher in pulmonary edema fluid from patients with ALI/ARDS (P < 0.05) (Fig. 3). Similarly, comparison of the ratios of

---

**Table 1. Characteristics of patients with ALI/ARDS and hydrostatic pulmonary edema**

<table>
<thead>
<tr>
<th>Hydrostatic Edema (n = 7)</th>
<th>ALI/ARDS (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>52 ± 15</td>
</tr>
<tr>
<td>Smoker</td>
<td>38%</td>
</tr>
<tr>
<td>SAPS II score</td>
<td>51 ± 22</td>
</tr>
<tr>
<td>LIS</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>Sepsis</td>
<td>0%</td>
</tr>
<tr>
<td>Renal failure</td>
<td>0%</td>
</tr>
<tr>
<td>Liver failure</td>
<td>12%</td>
</tr>
</tbody>
</table>

Values are mean ± SD. ALI/ARDS, Acute lung injury; SAPS, simplified acute physiology score; LIS, lung injury score. *P < 0.05.
α₂-M/IL-8 complex concentrations to total protein concentrations using Fisher’s exact test (α₂-M/IL-8 complexes:total protein > 1.10 ng/mg) showed that patients with ALI/ARDS (18:1) significantly differed from patients with hydrostatic pulmonary edema (4:3) (P < 0.05). The mean ratio was 0.81 ± 0.26 and 2.06 ± 0.60 ng/mg for patients with ALI/ARDS and for patients with hydrostatic pulmonary edema, respectively.

In addition, edema fluid samples containing fast α₂-M were capable of binding 125I-labeled rhIL-8. Samples containing slow α₂-M bound IL-8 after they were treated with methylamine (conversion of slow form to fast form). [Slow α₂-M does not bind IL-8 (14).] For example, the first two samples that were run on the gel presented in Fig. 1 did not bind 125I-labeled rhIL-8 without prior methylamine treatment, whereas the third sample did. We observed a similar phenomenon in our previous studies, except that plasma or commercial α₂-M were examined before (13, 14).

Moreover, IL-8 that was present in α₂-M/IL-8 complexes, purified from edema fluids, retained its biological activity as shown by binding to IL-8 receptors on neutrophils. α₂-M/IL-8 complexes competed with 125I-labeled rhIL-8 to bind to IL-8 receptors (Fig. 4). The typical inhibition curve was obtained when different concentrations of purified complexes were incubated with 125I-labeled rhIL-8 and the cells (Fig. 4) (14). This suggests that IL-8 in purified complexes bound to IL-8 receptors on neutrophils. [α₂-M does not bind to neutrophils (13).] In addition, the neutralizing antibody against α₂-M substantially decreased the ability of α₂-M/IL-8 complexes to compete with 125I-labeled rhIL-8 to bind to receptors on neutrophils (P < 0.01) (Fig. 4). The two inhibition curves were compared with

Fig. 2. α₂-M in pulmonary edema fluids from patients with ALI/ARDS and hydrostatic pulmonary edema. A: total α₂-M concentration in pulmonary edema fluids from patients with ALI/ARDS and hydrostatic pulmonary edema (P < 0.01). B: slow α₂-M in pulmonary edema fluids from patients with ALI/ARDS and hydrostatic pulmonary edema (P < 0.001).

Fig. 3. α₂-M/interleukin (IL)-8 complex concentration in pulmonary edema fluids from patients with ALI/ARDS and hydrostatic pulmonary edema (*P < 0.05).

Fig. 4. Competition for binding of 125I-labeled recombinant human (rh) IL-8 to human neutrophils by α₂-M/IL-8 complexes purified from pulmonary edema fluid in the absence (solid line) and presence (dotted line) of the antibody against α₂-M. The results represent the average of 4 experiments.
each other. The antibody or α2-M alone did not affect the binding (i.e., did not inhibit the binding of 125I-labeled rhIL-8; data not shown). This antibody binds specifically to α2-M/IL-8 complexes (14) and suppresses their binding to IL-8 receptors on neutrophils. Complexed IL-8 was also protected from proteolytic degradation (α2-M/IL-8 complexes treated with elastase displayed similar activity to untreated complexes in the competition assay) (P > 0.05). Furthermore, the neutrophil chemotactic activity of the complexes (80 ± 7%) was similar to that of IL-8 alone suspended in buffer (100%) (P > 0.05).

Interaction of IL-8 with elastase-modified α2-M in vitro. Because purified α2-M/IL-8 complexes contained elastase (bound to α2-M), we studied the interaction of IL-8 with α2-M, which was treated with elastase in vitro. IL-8 bound to elastase-modified α2-M in a saturable manner, and the calculated \( K_d \) was \( 6 \times 10^{-8} \) M. We also examined the effect of IL-8 in complex with elastase-modified human α2-M on the release of myeloperoxidase, a marker of neutrophil activation (Fig. 5). The complexed IL-8 did not differ from free IL-8 in its ability to release myeloperoxidase from human neutrophils (P > 0.05). In addition, α2-M alone did not exhibit any activity (Fig. 5).

DISCUSSION

The major findings of this study can be summarized as follows. In patients with ALI/ARDS, the quantity of α2-M complexed with elastase in pulmonary edema fluid was increased, and consequently the quantity of slow (native) α2-M was decreased. There was a corresponding increase in the quantity of fast α2-M in pulmonary edema fluid from patients with ALI/ARDS. The increase in the fast form of α2-M may result in increased binding to proinflammatory cytokines. Accordingly, as we hypothesized, there was a significant increase in α2-M/IL-8 complexes in pulmonary edema fluid from patients with ALI/ARDS compared with control patients with hydrostatic edema. In addition, the results indicate that IL-8 complexed to α2-M remains biologically active. This is the first study to describe the function of IL-8 complexed to α2-M in pulmonary edema fluid. In addition, this is the first study to define the activity of IL-8 in α2-M/IL-8 complexes purified from pulmonary edema fluid. Thus our observations are more closely related to in vivo conditions than our prior studies of the in vitro activity of IL-8 (13–15).

An important feature of ALI/ARDS is that large proteins, such as α2-M, translocate across the injured endothelial and epithelial barriers of the lung and accumulate in the air spaces (10, 29). Wewers et al. (32) showed that the majority (70%) of α2-M present in BAL fluid from patients with ALI/ARDS is complexed to neutrophil elastase. However, only 34% of the total α2-M was complexed with neutrophil elastase in our study. The difference between the studies may be due to the fact that the former used BAL fluid whereas our study used pulmonary edema fluid. Furthermore, elastase complexed to α2-M retains its enzymatic activity. This form of elastase was present in samples from five of seven patients with ALI/ARDS but only in one of 13 normal volunteers (32). In our study, neutrophil elastase complexed with α2-M was detected in 13 of the 19 patients with ALI/ARDS. Thus a similar number of patients with ALI/ARDS had the complexed elastase (70 vs. 68%, respectively) in both studies.

The intensity and character of inflammation are quite different in patients with ALI/ARDS and patients with hydrostatic pulmonary edema (24). Accordingly, more patients with ALI/ARDS than with hydrostatic edema had elastase bound to α2-M (13 in 19 patients and 2 in 7 patients, respectively). In addition, 34% of the total α2-M was complexed with neutrophil elastase in patients with ALI/ARDS whereas only 8% was complexed in patients with hydrostatic edema. The concentration of α2-M associated with elastase was significantly higher in these patients (P < 0.05). Furthermore, the activity of α2-M (the ability to inhibit trypsin) ranged from 47% (%total) for ALI/ARDS to 85% (%total) for the hydrostatic edema group. However, because of the increased permeability to protein in patients with ALI/ARDS, the total amount of active α2-M was similar in both groups of patients. The remaining α2-M (not active or complexed with elastase) could be bound to other proteinases or modified by oxidation (25).

In agreement with our previous study (14), only a small fraction of the total α2-M was associated with IL-8 (<1%). However, fast α2-M present in pulmonary edema fluid was still able to bind IL-8. Accordingly, we found that the extent of 125I-labeled rhIL-8 binding to pulmonary edema fluid α2-M depended on the α2-M state (slow vs. fast). Edema fluid samples that contained mostly native α2-M bound 125I-rhIL-8 only after treatment with methylamine (IL-8 does not bind to the slow form) (14). Furthermore, the concentration of the α2-M/IL-8 complexes was higher in patients with ALI/ARDS.
ARDS than in patients with hydrostatic edema \((P < 0.05)\). To determine whether the differences in \(\alpha_2\)-M/IL-8 complex concentrations were due to augmented intra-alveolar production of \(\alpha_2\)-M versus translocation from the intravascular to the intra-alveolar compartment, we also examined the \(\alpha_2\)-M/IL-8 complex concentration-to-total protein concentration ratios. The difference between ALI/ARDS and hydrostatic groups was still significant \((P < 0.05)\).

Because \(\alpha_2\)-M complexed to IL-8 was associated with elastase, the interaction between IL-8 and elastase-treated \(\alpha_2\)-M was also studied in vitro. We found for the first time that IL-8 bound to elastase-modified \(\alpha_2\)-M in a saturable manner in vitro, and the binding was of high affinity compared with other cytokines \((6)\). The calculated \(K_d\) was \(\sim 6 \times 10^{-8}\)M. In addition, IL-8 in complex with elastase-modified \(\alpha_2\)-M retained its biological activity.

IL-8 is an important neutrophil chemoattractant in lung fluids from patients with ALI/ARDS \((19, 20)\). Several studies have indicated the potential use of IL-8 as a marker of the development or outcome of ALI/ARDS \((5, 7, 18–20)\). However, some ELISA assays do not recognize IL-8 that is bound to \(\alpha_2\)-M \((14)\). The results of this study demonstrate that the IL-8 concentration in pulmonary edema fluid is at least two to three times higher that that measured by ELISA assay. Furthermore, purified \(\alpha_2\)-M/IL-8 complexes displayed unchanged biological activity (hemotactic activity). Also, IL-8 complexed to \(\alpha_2\)-M was protected from proteolytic degradation. Therefore, when the appearance of \(\alpha_2\)-M/IL-8 complexes coincides with the influx of neutrophils, it may create a favorable environment for further neutrophil accumulation. This may explain why, in patients at risk for ALI/ARDS who subsequently developed ALI/ARDS, \(\alpha_2\)-M/IL-8 complexes and neutrophil concentrations were correlated \((P < 0.05; r^2 = 1.00)\) \((16)\).

On the other hand, our previous findings suggested that \(\alpha_2\)-M could be an important mediator of IL-8 clearance \((13, 15)\). Those studies indicated that alveolar macrophages have receptors for \(\alpha_2\)-M but not for IL-8. In addition, we found that complexes between rhIL-8 and fast human \(\alpha_2\)-M are cleared by human alveolar macrophages via \(\alpha_2\)-M receptors \((13)\). Furthermore, the instillation of IL-8 bound to the fast rabbit \(\alpha\)-macroglobulin to the rabbit lung abolished the influx of neutrophils induced by the instillation of the same concentration of IL-8 alone \((15)\). These findings suggested that formation of \(\alpha_2\)-M/IL-8 complexes facilitates clearance of IL-8. Accordingly, we found that the concentration of these complexes declined over time \((between days 1 and 7 of ALI/ARDS) but interestingly only in ALI/ARDS survivors. This finding was independent of the decline in total protein concentration \((14, 16)\). Thus the data in this study, coupled with our earlier work, indicate that \(\alpha_2\)-M/IL-8 complexes may modulate the acute inflammation in patients with ALI/ARDS since \(\alpha_2\)-M may affect degradation and clearance of proinflammatory IL-8. On the other hand, if availability of \(\alpha_2\)-M receptors on macrophages is decreased, then \(\alpha_2\)-M/IL-8 complexes could enhance the inflammatory process in the lung further by triggering the activation of neutrophils.

In summary, IL-8 in complex with \(\alpha_2\)-M retains its biological activity. The complexes thus bind to IL-8 receptors on neutrophils. The complexes are also cleared by alveolar macrophages \((via \alpha_2\)-M receptors). Therefore, the fate of these complexes depends on the immediate environment surrounding them, e.g., cell type and the availability of receptors.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-56768 (A. K. Kurdowska) and HL-51856 (M. A. Matthay).

REFERENCES


