Soluble Complement Receptor Type 1 Inhibited the Systemic Organ Injury Caused by Acid Instillation into a Lung

Hideo Nishizawa, M.D.,* Hiroshi Yamada, M.D.,* Hiroshi Miyazaki, M.D.,* Maria Ohara, M.D.,* Kazuhiro Kaneko, M.D.,* Tadashi Yamakawa, M.D.,† Jeanine Wiener-Kronish, M.D.,‡ Ichidai Kudoh, M.D., Ph.D.§

Background: Acid aspiration into one lung causes contralateral lung injury and systemic organ injury; this injury is thought to be mediated by the sequestration of activated neutrophils. Recombinant human soluble complement receptor 1 (sCR1) inhibits both the classical and alternative complement pathways; this study investigated the role of the complement system in unilateral acid lung injury by measuring the effects of administering sCR1 before or immediately after acid instillation.

Methods: Anesthetized rats (n = 18 in each group) underwent tracheostomy and insertion of a cannula into the anterior segment of the left lung. Then either 0.1 ml 0.1 N hydrochloric acid (HCl group) or 0.1 ml pH 7.4 phosphate buffered saline (PBS group) was instilled. Fifteen minutes before (pre-sCR1 group) or 15 min after (post-sCR1 group) the acid was instilled, 10 mg/kg sCR1 was administered intravenously. Four hours after the acid instillation, rats were killed. In an additional 4 rats in each group, blood and bronchoalveolar lavage fluids obtained 1 h after the instillation of either acid or PBS were analyzed for tumor necrosis factor-alpha activity.

Results: The instillation of acid led to an increased wet-to-dry ratio of 5.2 ± 0.1 in the acid-instilled lungs compared with their contralateral lungs (4.7 ± 0.06). These values were greater than the values of 4.6 ± 0.2 and 4.5 ± 0.03 in the PBS-instilled lungs and their contralateral lungs, respectively (P < 0.05). The administration of sCR1 before or immediately after the instillation of acid did not attenuate the increase in the wet-to-dry ratio of the acid-instilled lungs. However, the small but consistent increase in the wet-to-dry ratio of the contralateral lungs was attenuated by the sCR1 infusions (P < 0.05). The instillation of acid increased the protein concentration in the bronchoalveolar lavage fluids from the injured lungs (1,000 ± 206 μg/ml) compared with the protein concentration measured in the bronchoalveolar lavage fluids from their contralateral lungs (254 ± 55 μg/ml). The administration of sCR1 before or immediately after the instillation of acid did not decrease the protein concentration in the bronchoalveolar lavage fluids from the acid-instilled lungs. The myeloperoxidase activity was increased in the acid-instilled lung, in their contralateral lung, and in the small intestines of the animals. The infusions of sCR1 before or immediately after the administration of acid led to significant decreases in the myeloperoxidase activities measured in the lungs and the intestines of the treated animals.

Plasma tumor necrosis factor-alpha activity was only increased (2.7 ± 1.1 U/ml) in the animals that had received acid instillations. The infusions of sCR1, administered either before or immediately after the acid instillations, significantly decreased the plasma tumor necrosis factor-alpha activity in the animals (0.5 ± 0.6 and 1.0 ± 0.7 U/ml, respectively).

Conclusions: The results suggest that the complement system plays an important role in the pathogenesis of the injury of the contralateral lung and of the small intestine after unilateral instillation of acid to the lung. Further investigation is warranted to determine the clinical utility of anti-inflammatory agents in acid-induced lung injury. (Key words: Lung, acid aspiration; complement; endothelin; recombinant human soluble complement receptor 1 [sCR1]; tumor necrosis factor.)

WHEN acid has been instilled into one lung in cats, rabbits, and dogs, the contralateral lung and the small bowel were also injured. The injury to the contralateral lung and the small intestine observed after acid instillation into one lung appears to be mediated by activated neutrophils. Pentoxifylline, which reduces neutrophil adhesion, degranulation, and superoxide production and decreases TNF-α production, attenuated the injury of the contralateral lungs caused by unilateral lung acid instillation in rabbits. The complement system was also implicated in acid-induced lung injury.

Materials

Animals

Ninety rats weighing 200-250 g were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). Animals were intubated, and a 20-gauge polyethylene catheter was inserted into the trachea and advanced to the main bronchus. Polyethylene tubing segments (1 cm long) were connected to the catheter. The catheter was secured to the skin with an adhesive bandage and a 14-gauge needle. All animals received 6% dextrose and Ringer lactate at a rate of 1 ml/kg/hour through a caudal vein catheter and received 0.1 ml of water/kg every four hours. The rats were killed by decapitation after the experiment. The lungs and parts of the small intestine were used for the analysis.

Received from Yokohama City University, Department of Anesthesiology, Yokohama City University School of Medicine, Yokohama City, Japan. Submitted for publication March 8, 1996. Accepted for publication June 20, 1996.

Address reprint requests to Dr. Kudoh: Department of Anesthesiology, Yokohama City University School of Medicine, 3-9 Fukuro, Kanazawa-ku, Yokohama City 236, Japan. Address electronic mail to: ikudoh@med.yokohama-cu.ac.jp.

Anesthesiology, V 85, No 5, Nov 1996
as well as in ischemia-reperfusion injury, in burns, and in septic-induced lung injury. The cleavage of C3 and C5 to their respective anaphylatoxins by proteolytic enzymes is controlled by the complement receptor type 1 (CR1), a protein that binds to C3b or to C4b fragments. Inhibition of the complement system by administering the complement-inhibitory protein CR1 could decrease the injury to the contralateral lung and intestine induced by acid instillation.

Recombinant human soluble complement receptor type 1 (sCR1) is a truncated form of CR1, a natural membrane complement inhibitor capable of binding both C3b and C4b. It lacks the transmembrane and cytoplasmic domains of CR1 and is purified from transfected Chinese hamster ovary cells. Human soluble complement receptor 1 exerts its biologic actions through three mechanisms: (1) binding of C3b and C4b to distinct sites, (2) displacing the catalytic subunits from C3 and C5 convertases, and (3) promoting the degradation of C3b and C4b by factor I. It was shown that sCR1 inhibits both classical and alternative pathway complement activation in human and in rat serum and prevents the formation of anaphylatoxins, chemotactic factors, and membrane attack complexes.

The present study was designed to determine whether the administration of sCR1 before or immediately after acid instillation into one lung attenuated the contralateral lung or small bowel injury observed in anesthetized rats.

Materials and Methods

Animal Model of Aspiration Pneumonia

Ninety adult male specific-pathogen-free Wistar rats weighing approximately 220 to 260 g were anesthetized with intraperitoneal pentobarbital (25 mg/kg). The trachea was exposed surgically and a 16-gauge Teflon catheter was inserted. A jugular venous catheter was inserted for fluid or for drug infusions (1 ml/h), and pentobarbital (2.5 mg/h) was administered for anesthesia. Animals maintained spontaneous respiration throughout the procedure. Through the tracheal tube, a fine-bore polyurethane catheter was introduced into the anterior segment of the left lung to administer the acid or the phosphate-buffered saline (PBS; see below). Animals were supine for the entire experiment.

All animal experiments were done after obtaining approval from the Yokohama City University School of Medicine.

Solution Preparation

Hydrochloric acid (0.1 N; pH = 1.0) was purchased from Wako (Osaka, Japan). Recombinant human soluble complement receptor type 1 was provided by Yamanouchi Pharmaceutical Corporation (Tokyo, Japan). Three milligrams of sCR1 was diluted with 1 ml normal saline. Ten mg/kg of sCR1 was injected intravenously. The dose used was the same as in reports showing that the dose blocks all complement activity.

Experimental Groups

There were four experimental groups. The first two groups compared the effects of unilateral acid instillation with the effects of unilateral PBS instillation. The other two groups received infusions of sCR1 and acid. The third experimental group had the sCR1 infused before the acid instillation, and the fourth experimental group received the sCR1 infusion 15 min after the acid instillation.

Group 1. In the control group (n = 18), 0.1 ml phosphate-buffered saline (PBS) (pH = 7.4) was instilled into the left lung. Fifteen minutes before the instillation, a normal saline infusion (3.3 ml/kg) was begun.

Group 2. In the hydrochloric acid (HCl) group (n = 18), 0.1 ml of 0.1 N HCl was instilled into the left lung. Fifteen minutes before the acid instillation, normal saline (3.3 ml/kg) was administered intravenously.

Group 3. In the sCR1 pretreatment group (n = 18), 0.1 ml of 0.1 N HCl was instilled into the left lung. Fifteen minutes before the instillation, the normal saline infusion was begun (3.3 ml/kg), which also contained sCR1 (10 mg/kg).

Group 4. In the post-sCR1 group (n = 18), as the pretreatment with sCR1 decreased the systemic injury caused by the HCl instillation, the effect of the drug on acid-instilled animals 15 min after the acid instillation was studied. Hydrochloric acid (0.1 N; 0.1 ml) was instilled into the left lung. Fifteen minutes after the acid instillation, the normal saline infusion (3.3 ml/kg) with sCR1 was infused.

Experimental Protocol

Thirty minutes after surgical preparation, normal saline with or without sCR1 was administered intravenously to groups 1, 2, and 3. Fifteen minutes after the start of the infusion, HCl or PBS was instilled into the
left lungs of the anesthetized animals. In group 4, the normal saline was administered intravenously for 15 min, then the acid was instilled into the left lung; 15 min after the acid instillation, sCR-1 was administered. Four hours after the acid instillation animals were killed by an intraperitoneal injection of pentobarbital. Six rats from each group were used to measure protein concentration in the bronchoalveolar lavage fluids (BALF) from the acid-instilled and contralateral lungs; wet-to-dry weight ratios (W/D) of both lungs, and the myeloperoxidase activities of the hearts, livers, kidneys, and the small intestines.

**Bronchoalveolar Lavage.** Bronchoalveolar lavage fluids from the right lungs and then from the left lungs, including the instilled segments, were obtained by instilling 3 ml saline per lung through the tracheostomy tube. Note that each lung lavage fluid was kept isolated by applying a clamp on the opposite bronchus. The fluid instillation was repeated three times. The combined lavage fluids from each lung were placed into tubes containing 0.15% ethylenediamine tetraacetic acid. The BALFs were centrifuged at 1,500g for 20 min at 4°C, and the supernatants were frozen at −80°C and subsequently used for protein measurements. The cell pellets were resuspended in 0.5 ml PBS to determine the cell numbers and differential. Cell counts and differentials were done on the BALF using Gentian Violet and Wright-Giemsa stains and a hemocytometer. The total protein concentrations of BALF were measured using the Lowry method.

**Tumor Necrosis Factor Assay.** Tumor necrosis factor-alpha activities were measured using two methods: A bioassay and an enzyme-linked immunosorbent assay (ELISA) were done on blood and BALF samples. One hour after the acid instillation, 50 mg pentobarbital was injected intraperitoneally into 16 rats (four rats in each group); thoracotomies were done to obtain blood from the hearts. Blood samples were drawn into sterile glass tubes containing 0.15% ethylenediamine tetraacetic acid and maintained at 4°C. Specimens were centrifuged at 500g for 20 min at 4°C, and the resulting plasma samples were frozen at −80°C until they were assayed. Both lungs were subsequently lavaged to obtain BALF; these fluids were assayed for TNF-α. Samples for TNF-α activity measurement were obtained 1 h after the acid instillation, because we previously found the highest activities in plasma at this time.

**Bioassay.** The mouse L929 fibroblast assay was used to measure plasma TNF activity. L929 cells were seeded into flat-bottom, 96-well microtiter plates at a density of 5 × 10⁴ cells/well and grown to confluence overnight in Dulbecco's minimal essential medium (GIBCO, Grand Island, NY) containing 1% penicillin-streptomycin and 5% fetal calf serum. The medium was removed from the confluent monolayers and 100 μl Dulbecco's minimal essential medium containing actinomycin D (final concentration, 5 μg/ml) was added to each well. One hundred microliters of each of the following were added to selected duplicate wells containing L929 cells: (1) Dulbecco's minimal essential medium (0% cytotoxicity), (2) serial dilutions of recombinant TNF (5 × 10⁻³ to 6 × 10⁻⁴ U/ml), (3) plasma samples from each group, and (4) Dulbecco's minimal essential medium in blank wells without cells (100% cytotoxicity). Plates were incubated for 20 h at 37°C in 5% carbon dioxide. After incubation, the medium was removed and the L929 cells were stained for 10 min with 0.5% crystal violet in 20% methanol, rinsed in water, and air dried. The optical density of each well was determined by a microplate reader and calibrated to noncellular reagent blanks at a wavelength of 550 nm. The percentage cytotoxicity of L929 cells was calculated by:

\[
\% \text{ Cytotoxicity} = (OD \text{ wells with } 0\% \text{ cytotoxicity} - OD \text{ experimental sample well}) / OD \text{ wells with } 0\% \text{ cytotoxicity}
\]

Tumor necrosis factor-alpha activity is expressed in units per milliliter (U/ml), where 1U TNF activity is defined as 50% L929 cytotoxicity.

**Enzyme-linked Immunosorbent Assay.** To quantitate TNF-α, a commercially available ELISA kit (Factor-Test-X kit, Genzyme, Cambridge, MA) was used. This kit has been used successfully to quantitate natural rat TNF-α.⁵ The detection limit of this assay was determined by Genzyme to be 15 pg/ml.

**Myeloperoxidase Assay.** Activity of this neutrophil enzyme was used as a tracer to quantitate polymorphonuclear sequestration in tissue; this assay has been found to be more sensitive for detecting sequestered neutrophils than quantitative histology.¹⁰ One gram of blotted dry tissue was homogenized in 10 ml of 0.01 M potassium phosphate buffer (PPB, pH 7.4) containing 1 mM ethylenediamine tetraacetic acid. Two milliliters of homogenate and 5 ml of 0.01 M PPB containing 1 mM ethanol were added to each reaction. The reaction mixture was then added to a 10×10×0.5 cm tube containing 0.01 M PPB and incubated at 37°C for 15 min. The reaction was stopped with 5 ml of 0.01 M PPB and the tube cooled to 0°C. The tube was centrifuged at 8,000 rpm for 5 min. The supernatant was collected and the absorbance at 550 nm was read. The concentration of myeloperoxidase of the samples was estimated based on the amount of absorbance at 550 nm.

Wet-to-dry ratio of the lungs was calculated as the ratio of the weight of the organ in air to its weight in the absence of air.

**Results**

The wet-to-dry ratio of the lungs, the small intestine, and the spleens of the animals in all groups were significantly different. The wet-to-dry ratio of the lungs was significantly increased in the control group compared to the other groups. The wet-to-dry ratio of the small intestine was significantly increased in the acid instillation group compared to the control group. The wet-to-dry ratio of the spleen was significantly increased in the acid instillation group compared to the control group.

**Discussion**

These results suggest that the acid instillation model of lung injury can be used to study the acute immune response in the lungs. The wet-to-dry ratio of the lungs, the small intestine, and the spleens of the animals in all groups were significantly different. The wet-to-dry ratio of the lungs was significantly increased in the control group compared to the other groups. The wet-to-dry ratio of the small intestine was significantly increased in the acid instillation group compared to the control group. The wet-to-dry ratio of the spleen was significantly increased in the acid instillation group compared to the control group.
mM ethylenediamine tetraacetic acid were mixed gently and then centrifuged at 10,000g for 20 min at 4°C. The pellet was rehomogenized in 5 ml of 0.05 M PPB (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide. This suspension was frozen, thawed, and sonicated with a Branson cell disruptor at 65 Watt for 1 min. Then 0.1 ml aliquots were mixed with 0.79 ml of 0.08 M PPB (pH 5.4) and 0.1 ml of 16 mM tetramethylbenzidine dissolved in N,N-dimethylformamide at 37°C. After 2 min, 0.01 ml of 30 mM H₂O₂ was added. This solution was incubated for 3 min at 37°C, and then 300 μg/ml of 0.05 M catalase solution was added. This mixture was then diluted with 4 ml of 0.2 M sodium acetate (pH 3.0) and then centrifuged at 12,000g for 10 min at 4°C. The supernatants were read in a spectrophotometer (UV-2200, Shimadzu, Kyoto, Japan). One unit of myeloperoxidase activity was defined arbitrarily as the amount of enzyme necessary to catalyze an increase in absorbance of 1 per minute at 655 nm at 37°C.

**Wet-to-Dry Weight Ratio.** Wet-to-dry weight ratios of the lungs, hearts, livers, kidneys, and the small intestines were calculated. After the freshly harvested parts of the organs were weighed, they were dried at 60°C for 1 week and reweighed. The wet-to-dry weight ratio was calculated as wet weight/dry weight.

**Statistics**

All data are presented as mean ± SD. The data between the groups were analyzed by analysis of variance and Sheffe's test. We accepted P < 0.05 as statistically significant.

**Results**

**Wet-to-Dry Weight Ratios**

The wet-to-dry weight ratios of the acid-instilled (left) lungs, the contralateral (right) lungs (Fig. 1), and of the small intestines (Fig. 2) from the animals that had acid instillations were significantly greater than the respective wet-to-dry weight ratios from the animals that had lung instillations of PBS. The wet-to-dry weight ratios of the kidneys, the livers, or the hearts from the acid-instilled compared with the PBS-instilled animals were not different. The administration of sCR1 before or immediately after the acid instillation into the left lungs significantly inhibited the increases of the wet-to-dry weight ratios in the small intestines and the contralateral (non-instilled) lungs.

**Protein Concentration in Bronchoalveolar Lavage Fluid**

Protein concentrations in the BALF from the acid-instilled lungs were 1,000 ± 206 μg/L, which were significantly higher than the protein concentrations in the BALF from the PBS-instilled lungs (251 ± 55 μg/L). The administration of sCR1 administered before or immediately after the acid instillation did not decrease these protein concentrations, which were 1,050 ± 354 μg/ml and 981 ± 207 μg/ml, respectively. The protein concentrations in the BALFs from the contralateral lungs from the animals that had received acid instillations were not different from the protein concentrations measured in the BALFs from the contralateral lungs from the animals that had received PBS instillations (Fig. 3).

**Myeloperoxidase in the Organs**

Myeloperoxidase activities of the kidneys, livers, and in the hearts in the four groups were not different.
Myeloperoxidase activities in the small intestines, the acid-instilled lungs, and the contralateral (non-instilled) lungs were significantly higher than the activities measured in these organs from the animals that had received PBS instillations. The administration of sCR1 before and immediately after the acid instillation significantly attenuated the increased myeloperoxidase activities of the small intestines, the acid-instilled lungs, and the contralateral (non-instilled) lungs (Fig. 4).

Neutrophils in Bronchoalveolar Lavage Fluid
Neutrophil counts in BALF from the acid-instilled lungs were significantly increased compared with the counts measured in the BALF from the PBS-instilled lungs as well as in the BALF from the contralateral, non-instilled lungs. The administration of sCR1 before or immediately after the acid instillation significantly decreased the number of neutrophils in the BALF from the acid-instilled lungs. The neutrophil counts in the BALF from the contralateral, non-instilled lungs in all rat groups were always low (Fig. 5).

Tumor Necrosis Factor-α Activity
The bioassay for TNF-α activity in plasma samples obtained 1 h after the acid instillation demonstrated...
TNF-α activity was 2.7 ± 1.1 U/ml in these four animals; in contrast, there was no detectable TNF-α activity in the plasma from the four animals that received PBS. The administration of sCR-1 before or immediately after the acid instillation significantly decreased the plasma TNF-α activity measured in the treated animals (n = 8) (0.5 ± 0.6 U/ml and 1.0 ± 0.7 U/ml, respectively) compared with the activity measured in the plasma from the four acid-instilled animals (2.7 ± 1.1 U/ml). There was no TNF-α activity detected in the acid-instilled lungs, from the PBS-instilled lungs, or from the contralateral lungs from any animals.

By ELISA, the TNF-α concentration in plasma was 56 ± 16 pg/ml in the animals that had PBS instillations. The TNF-α concentrations in the plasma from the acid-instilled animals was 500 ± 174 pg/ml, which was significantly increased compared with the 56 ± 16 pg/ml in the plasma from the PBS-instilled animals.

The administration of sCR1, either before or immediately after the acid instillation, decreased the measured concentrations, albeit not significantly; the concentrations of TNF-α measured by ELISA in the sCR1-treated animals were 300 ± 76 pg/ml and 304 ± 50 pg/ml, respectively (Table 1).

**Discussion**

The instillation of acid into one lung of anesthetized, spontaneously breathing rats injured their contralateral, noninstilled lungs and their small intestines after 4 h. The injuries of both the acid-instilled and contralateral, noninstilled lungs were compared and quantitated in several ways. Bioactive TNF-α activity was only detectable in the plasma of the acid-instilled animals and was not detectable in the plasma of PBS-instilled animals. However, there was no detectable bioactive TNF-α in the BALF obtained from either the acid-instilled or the PBS-instilled lungs. The protein concentration in the BALF from the acid-instilled animals was increased in the fluid from the acid-instilled lungs but not in the fluid obtained from the contralateral, noninstilled lungs. These results suggest that the acid instillation caused moderate to severe injury in the acid-instilled lungs and milder injury to the contralateral, noninstilled lungs.

The administration of sCR1 before or immediately after the acid instillation attenuated some of the lung injury of the acid-instilled lungs. Although several therapies have been reported that decrease the injury of the contralateral, noninstilled lung, there is no convincing evidence that any therapy significantly decreases the direct cellular damage done by the acid. The acid itself causes an immediate alveolar epithelial injury; the inflammatory response in the acid-instilled lung also appears to be more extreme than the inflammatory response that affects the contralateral, noninstilled lung because it appears less responsive to therapy. The use of unilateral acid instillation allows the inflammatory response in the contralateral lung to be assessed and facilitates comparisons of the effects of therapies on the two lungs.

The administration of sCR1 before or immediately after the administration of acid significantly decreased

<p>| Table 1. Tumor Necrosis Factor-α Activity in Plasma 1 h after Acid Instillation |
|-----------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>Bioassay (U·ml⁻¹)</th>
<th>ELISA (ng·ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>HCl</td>
<td>2.7 ± 1.1*</td>
</tr>
<tr>
<td>Before sCR1</td>
<td>0.5 ± 0.6†</td>
</tr>
<tr>
<td>After sCR1</td>
<td>1.0 ± 0.7†</td>
</tr>
</tbody>
</table>

* P < 0.05 versus control.  
† P < 0.05 versus HCl.  
HCl = hydrochloric acid; sCR1 = soluble complement receptor 1.
the myeloperoxidase activities of the acid-instilled lungs, significantly decreased the neutrophil counts in the BALF from the acid-instilled lungs, and significantly decreased the TNF-α bioactivity in the plasma. The administration of sCR1 also decreased the measured concentration of TNF-α, albeit not significantly. Therefore the administration of sCR1 clearly decreased some of the injury that had occurred to the acid-instilled lung. However, the administration of sCR1 did not decrease the wet-to-dry weight ratios of the acid-instilled lungs, nor was there a decrease in the protein concentration in the BALF obtained from these lungs. A possible interpretation of these results is that the acid-induced epithelial injury causes some of the increase in the wet-to-dry weight ratio and the increase in the protein concentration in the BALF fluids. Goldman and coworkers' induced neutropenia in animals to decrease the inflammatory-induced injury of acid-instilled lungs; they showed that neutropenia did not prevent the increase in protein permeability of acid-instilled lungs, further suggesting that at least some of the increase in protein permeability of the acid-injured lung may not be due to an inflammatory response but is secondary to direct cellular injury by the acid.

The administration of the sCR1 before or immediately after the acid instillation also had a beneficial effect on the contralateral, noninstilled lungs and on the small intestine. In these organs, the wet-to-dry weight ratios were decreased, as were the myeloperoxidase activities. Therefore, the sCR1 decreased the injury to the contralateral lung and the edema of the small intestine.

Other researchers have administered sCR1 in experiments with intestinal or muscle injury. Hill and associates19 showed that sCR1 administration prevented the increase in vascular permeability after intestinal ischemia-reperfusion in a rat model. Pemberton and associates20 showed that sCR1 administration prevented neutrophil adherence to blood vessel walls after ischemia-reperfusion injury of skeletal muscle. These studies indicate that in other diverse experimental models of tissue injury involving activated neutrophils, the administration of sCR1 decreased the documented tissue injury. Therefore the improvement in the acid-instilled lungs and in the contralateral lungs and the small intestines in the animals that received sCR1 in the present experiments may be due to the inhibition of neutrophil activation secondary to the inhibition of the complement system.

Rabinovici and coworkers21 showed that inhibition of the complement system by sCR1 pretreatment attenuated the lung injury caused by administering lipopolysaccharide and platelet-activating factor in a rat model. Their report demonstrated that sCR1 inhibited the increase in lung edema and decreased the alveolar neutrophil infiltration due to lipopolysaccharide and platelet-activating factor. They found that administering sCR1 did not change TNF-α concentration measured by ELISA. In contrast to their results, our study showed that the administration of sCR1 significantly decreased the bioactivity of TNF-α. These contrasting results may be due to the difference in the genesis of the lung injuries—the systemic administration of endotoxin versus the acid-instillation into one lung. The differences may also be due to the choice of the assay used to measure TNF-α activity. A bioassay measures the biologically active TNF-α and can be influenced by inhibitors such as TNFr (TNF receptor), which would decrease the biologic effect of circulating TNF. The ELISA measures immunoreactive protein and can be influenced by antibodies that cross-react with TNF. In addition, ELISA measurements can quantitate free TNF, TNF-bound, or both. Thus the choice of the assay used for TNFα clearly can influence the measured results.

Natural or recombinant C5a directly or indirectly induces the production of IL-1, IL-6, IL-8, and TNF-α.22-24 Morgan and colleagues24 reported that blocking C5a inhibited the C5a-mediated production of IL-6 and IL-8. Therefore the blockade of C5α by sCR1 could decrease serum TNF-α activity, which could lead to the decreased lung and small intestinal injury documented in these studies.

The results of the present study correlate with some of those of Rabinovici and coworkers,21 in which the role of complement in the development of acid-induced lung injury was also examined. These investigators injected the same dose of sCR1 10 min before the acid instillation and found that the elevated neutrophil counts and lung waters caused by acid instillation were improved by administering sCR1. Their study differed from ours in several ways. The acid was instilled into both lungs and the volume of acid instilled (0.2 ml) was twice the dose used in these studies. These investigators found that the protein concentration in the BALF from the acid-exposed lungs was decreased by the sCR1 pretreatment. This result is difficult to explain; in an investigation using pentoxifylline to pretreat animals subsequently exposed to unilateral acid instillations, the lung waters of the acid-instilled lungs were significantly im-

References
sCR1 AND ACID-ASPIRATION PNEUMONIA

proved, but the protein concentrations in the BALF were not changed by the pretreatment. In addition, these investigators found that the serum TNF-α measured by ELISA was not decreased by their sCR1 pretreatment. As noted before, there are several reasons for the differences seen between the results obtained by the bioassay and the ELISA assays of serum TNF-α. By performing both assays, we showed that the administration of sCR1 significantly decreased the bioactivity of TNF-α, even when the concentrations of TNF-α were not significantly decreased.

Unlike the report from Rabinovici and coworkers, the unilateral instillation of acid in our investigation allowed us to determine the effect of the inflammatory response on the contralateral, noninstilled lung. In addition, we assessed the effect of the acid instillation on other organs. Furthermore, we administered sCR1 before and immediately after the acid instillation to assess the effect of complement activation after the acid was instilled. These differences in the present investigation give additional insight into the pathophysiology of the acid-induced lung injury.

Our current data indicate that complement plays an important role in the pathogenesis of injury to the contralateral, noninstilled lung and to the small intestine, as well as some role in the pathogenesis of the injury of the acid-instilled lung. The administration of recombinant sCR1, a novel inhibitor of complement activation, given either before or immediately after the administration of acid, improved the documented injuries of the contralateral lung and of the small intestine and decreased the neutrophil infiltration of the acid-instilled lung. The administration of sCR1 also significantly decreased the bioactive plasma TNF-α activity measured. Modulation of complement is useful in identifying the pathogenesis of the direct and indirect lung injury caused by acid instillation; further investigation is warranted to determine the clinical utility of this agent in treating lung injury.

References

19. Hill J, Lindsay T, Ortiz F, Yeh G, Hechtman H, Moore F Jr: Soluble complement receptor type 1 ameliorates the local and remote


22. Schindler R, Gelfand J, Dinarello C. Recombinant C5a stimulate transcription rather than translation of interleukin 1 (IL-1) and tumor necrosis factor: Translational signal provided by liposaccaride or IL-1 itself. Blood 1990; 76:1651-8


Anesthesiology, V 85, No 5, Nov 1996

A Brazilian

Oxidative

Adenine

Halogeno

Hiroshi

Kazuhito