Receptor for Advanced Glycation End-Products Is a Marker of Type I Cell Injury in Acute Lung Injury

Tokujiro Uchida*, Madoka Shirasawa*, Lorraine B. Ware, Katsuo Kojima, Yutaka Hata, Koshi Makita, Gabe Mednick, Zachary A. Matthay, and Michael A. Matthay

Departments of Anesthesiology, Cardiothoracic Surgery, and Medical Biochemistry, Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan; Division of Allergy, Pulmonary, and Critical Care Medicine, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee; and Departments of Medicine and Anesthesia, Cardiovascular Research Institute, University of California–San Francisco, San Francisco, California

Rationale: Receptor for advanced glycation end-products (RAGE) is one of the alveolar type I cell-associated proteins in the lung.

Objectives: To test the hypothesis that RAGE is a marker of alveolar epithelial type I cell injury.

Methods: Rats were instilled intratracheally with 10 mg/kg lipopolysaccharide or hydrochloric acid. RAGE levels were measured in the bronchoalveolar lavage (BAL) and serum in the rats and in the pulmonary edema fluid and plasma from patients with acute lung injury (ALI; $n = 22$) and hydrostatic pulmonary edema ($n = 11$).

Main Results: In the rat lung injury studies, RAGE was released into the BAL and serum as a single soluble isoform sized ~ 48 kD. The elevated levels of RAGE in the BAL correlated well with the severity of experimentally induced lung injury. In the human studies, the RAGE level in the pulmonary edema fluid was significantly higher than the plasma level ($p < 0.0001$). The median edema fluid/plasma ratio of RAGE levels was 105 (interquartile range, 55–243). The RAGE levels in the pulmonary edema fluid from patients with ALI were higher than the levels from patients with hydrostatic pulmonary edema ($p < 0.05$), and the plasma RAGE level in patients with ALI were significantly higher than the healthy volunteers ($p < 0.001$) or patients with hydrostatic pulmonary edema ($p < 0.05$).

Conclusion: RAGE is a marker of type I alveolar epithelial cell injury based on experimental studies in rats and in patients with ALI.

Keywords: acute respiratory distress syndrome; alveolar epithelium; biological markers; pulmonary edema

Receptor for advanced glycation end-products (RAGE) is one of the alveolar type I cell-associated proteins in the lung (1, 2). Although it is also expressed in endothelial cells in large vessels (3, 4) and nervous tissues (5, 6), the transcript of RAGE is most prominent in the lung (3) and apparently not expressed in lung microvascular endothelia (7, 8). Immunoelectron microscopy of RAGE demonstrated that its expression is localized to the basal membrane of alveolar type I epithelial cells (7, 8). This protein belongs to the immunoglobulin superfamily of cell-surface molecules, consisting of an extracellular region (one V-type ligand

binding site and two C-type immunoglobulin-like regions), a transmembrane domain, and a cytosolic tail, that are essential for post-RAGE signaling (9). In general, RAGE is a multiligand-binding receptor that can bind advanced glycation end products, amyloid β -peptide, S100 proteins, and high-mobility group box-1 (10–12). RAGE–ligand interaction results in intracellular signaling, which leads to activation of the proinflammatory transcription factor nuclear factor- κ B (NF- κ B). This cellular activation is related to inflammatory processes or tissue injury, such as diabetic microvascular injury, amyloidosis, and immune-inflammatory process (10–12). RAGE knockout mice were recently reported to be resistant to septic shock induced by cecal ligation and puncture (13), suggesting that RAGE potentially plays a role in systemic acute inflammation. However, the biochemical characteristics of RAGE in the lung in response to acute lung injury (ALI) have not been determined.

Alveolar type I epithelial cells cover more than 95% of the internal surface area of the lung, and damage to alveolar type I epithelial cells is an important feature of ALI and acute respiratory distress syndrome (ARDS). In this context, some alveolar type I epithelial cell proteins have been demonstrated to be markers of the severity of lung injury in animal and human studies (14, 15). Because of the new evidence regarding high concentrations of RAGE in the lung apparently localized primarily to alveolar epithelial type I cells (1, 2), we hypothesized that RAGE may be a useful biochemical marker of alveolar type I epithelial cell injury in ALI and ARDS.

The initial objectives of this study were to investigate whether RAGE can be detected in the bronchoalveolar lavage (BAL) and serum and whether the level of RAGE in the BAL or serum reflects the severity of experimentally induced ALI. For this purpose, we used a rat model of hydrochloric acid (HCl)-induced lung injury and a rat model of lipopolysaccharide (LPS)-induced lung injury. The second objective was to measure RAGE in the pulmonary edema fluid from patients with ALI/ARDS and compare these edema fluid levels with a group of patients with hydrostatic pulmonary edema. Plasma RAGE levels in patients with ALI/ARDS were also compared with those in patients with hydrostatic pulmonary edema and normal healthy volunteers.

METHODS

Additional details are provided in the online supplement.

Animal Studies

Protocols were approved by the institutional animal care and use committee of Tokyo Medical and Dental University and by the University of California at San Francisco.

Acid Aspiration Lung Injury Model. Male Sprague-Dawley rats (250–350 g) were anesthetized with pentobarbital (50 mg/kg intraperitoneally). After tracheostomy, animals were ventilated with a tidal volume of 7 ml/kg, 100% oxygen, and positive end-expiratory pressure of 5 cm H₂O. Several concentrations and volumes of HCl or saline were instilled

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*These investigators contributed equally to this article.

Correspondence and requests for reprints should be addressed to Tokujiro Uchida, M.D., Department of Anesthesiology, Graduate School of Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. E-mail: uchida.mane@tmd.ac.jp

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into the trachea. The rats were killed 2 h after instillation of HCl (pentobarbital 150 mg/kg intravenously).

LPS-induced Lung Injury Model. Male Sprague-Dawley rats (250–350 g) were anesthetized with ether and a single dose of LPS (10 mg/kg) in 250 μ l of saline was administered by intratracheal instillation with the same volume of intratracheal saline in control animals. The animals were killed 6 h after instillation under deep anesthesia (pentobarbital 150 mg/kg intravenously).

Lung Tissue Homogenate and Its Fractionation. The lung tissues were homogenized in the lysis buffer (20 mM Tris/HCl, 100 mM NaCl). After low-speed centrifugation, lysates were centrifuged at $100,000 \times g$ for 15 min to separate into the soluble and the membrane fractions.

Western Blot Analysis. RAGE expression in the rat BAL and serum was characterized by Western blot analysis using a rabbit polyclonal anti-RAGE antibody, which was well characterized in a recent study (8) (*see online supplement*).

Quantification of RAGE Level in the BAL by Dot Blotting. RAGE level in rat BAL samples was quantified by a dot blot method using the same rabbit polyclonal anti-RAGE antibody as the one used in the Western blots. Serial dilution of recombinant RAGE-Fc chimera protein (R&D Systems, Inc., Minneapolis, MN) and rat BAL samples were assayed on the same piece of nitrocellulose, and the RAGE level was quantified in arbitrary units of RAGE antigen concentration expressed as μ g/ml of RAGE-Fc chimera protein equal to that in the sample (*see online supplement*).

Immunohistochemistry. Rat lungs were fixed by 4% paraformaldehyde and embedded. Sections (5 μ m) were blocked and incubated with the primary antibody at 4°C overnight and visualized with appropriate secondary antibodies (*see online supplement*).

Human Studies

Protocols were approved by the Ethical Committee for Human Research of Tokyo Medical and Dental University and the Committee for Human Research of the University of California, San Francisco.

Sampling of normal lung tissue. A portion of normal lung tissue, obtained from a patient undergoing lobectomy, was homogenized using the same method as for the rat lung tissue described previously. RAGE expression was characterized by Western blot analysis using mouse monoclonal anti-human RAGE antibody (Chemicon International, Inc., Temecula, CA; *see online supplement*).

Measurement of RAGE level in the edema fluid and the plasma. Samples were selected randomly from a stored sample bank of pulmonary edema fluid and plasma from patients with ALI/ARDS and hydrostatic pulmonary edema. Eligibility for inclusion in the study was based solely on availability of an adequate stored volume of plasma and edema fluid for measurement of RAGE levels. Patients with ALI or ARDS were identified based on the American European Consensus Conference definitions (16). Patients with hydrostatic pulmonary edema were identified as previously described (17). Undiluted pulmonary edema fluid and plasma were collected simultaneously as previously described (17, 18). Plasma was also sampled from healthy volunteers. The concentrations of RAGE were measured in duplicate by ELISA (*see online supplement*).

Data analysis. Continuous variables were compared by Student's *t* test or by analysis of variance, with Scheffé test for multiple comparisons. Nonparametric data were analyzed by Mann-Whitney U test or Kruskal-Wallis test, with Dunn's test for multiple comparisons where appropriate. All data are reported as means $\pm$ SD unless otherwise noted, and statistical significance was defined as $p < 0.05$.

RESULTS

RAGE Is Released into the Alveolar Space and Serum of Rats with Acid Aspiration Lung Injury

Rats were divided into three groups: (1) an HCl group, in which rats were intratracheally instilled with 4 ml/kg of 0.1 N HCl and mechanically ventilated for 2 h; (2) a saline group, in which rats were intratracheally instilled with 4 ml/kg of saline and mechanically ventilated for 2 h; and (3) a control group that was mechanically ventilated without fluid instillation. The degree of lung injury was evaluated by measuring total protein recovered

in the BAL and wet-to-dry lung weight ratio (W/D ratio). Total protein recovered in the BAL and W/D ratio were significantly increased in HCl-instilled rats (Figures 1A and 1B). RAGE was detected by Western blot as a single band of approximately 48 kD in the BAL and serum in the HCl group (Figure 1C). The saline challenge caused a weak expression of RAGE in the BAL, and the RAGE levels in the BAL were significantly higher in the HCl group than the saline group (Figures 1C and 1D). RAGE was also detected in the serum of HCl-instilled rats but not in the control or saline-instilled rats (Figure 1C).

RAGE levels in the BAL and serum varied with the severity of lung injury. When the volume of HCl was increased from 1 to 4 ml/kg, the total protein recovered in the BAL and the W/D ratio were increased in a dose-dependent manner (Figures 2A and 2B). The levels of RAGE in the BAL and the serum increased (Figures 2C and 2D), and 4 ml/kg of HCl increased RAGE level to ninefold higher than the control value (Figure 2D). When the concentration of HCl was increased from 0.025 to 0.1 N, total protein recovered in the BAL and W/D ratio were increased in a dose-dependent manner (Figures 3A and 3B). RAGE levels in the BAL also increased in a dose-dependent manner (Figures 3C and 3D). RAGE levels in the BAL were sixfold higher in the rats instilled with 0.1 N HCl than in the rat instilled with normal saline (Figure 3D). RAGE was not detected in serum unless the concentration of HCl exceeded 0.075 N (data not shown).

LPS Induced the Release of RAGE into the Alveolar Space in Rats

To study acute inflammatory lung injury, we assessed the effect of LPS on the expression of RAGE. Rats were instilled intratracheally with LPS 10 mg/kg or saline, and the expression of RAGE was evaluated 6 h after the instillation. Total protein in the BAL and the lung W/D ratio increased moderately in LPS-instilled rats (Figures 4A and 4B). The levels of RAGE in the BAL increased after LPS administration (Figure 4C), whereas RAGE was not detected in serum from LPS-instilled rats (data not shown).

RAGE Is Expressed by Alveolar Type I Epithelial Cells during LPS-induced Inflammation in Rats

In normal rats, we previously reported that RAGE was expressed in alveolar type I epithelial cells but not in alveolar type II cells or endothelial cells (8). Because LPS induces activation of macrophages, rat lung tissue sections were double stained with anti-RAGE antibody and anti-CD68 antibody (a macrophage marker). We also double stained rat lung tissue with anti-RAGE and anti-p180 lamellar body protein antibody (a marker of alveolar type II epithelial cells) to study the possibility of LPS-induced expression of RAGE in alveolar type II epithelial cells. In normal rat lung, RAGE was detected in alveolar type I epithelial cells but not in alveolar type II epithelial cells or macrophages (Figures 5 and 6, Control). LPS instillation caused thickening of the interalveolar septa due to interstitial edema. However, there was no change in the distribution of RAGE-expressing cells in the alveoli, and neither alveolar type II epithelial cells nor alveolar macrophages were stained positively with anti-RAGE antibody (Figures 5 and 6, LPS).

To exclude the possibility that the lung microvascular endothelium is another source of RAGE during lung inflammation, we stimulated human lung microvascular endothelial cells (HMVEC-L; Cambrex Bio Science, Inc., Walkersville, MD) with 10 ng/ml, 10 μ g/ml, or 10 mg/ml of LPS for 6 h. The RAGE level was below the detection limit of ELISA in the medium and whole-cell extract of these cells, whereas RAGE was detected by

Figure 1. RAGE protein levels in the bronchoalveolar lavage (BAL) and serum in the rat hydrochloric acid (HCl) instillation lung injury model. Rats were divided into three groups: (1) an HCl group, in which rats were intratracheally instilled with 4 ml/kg of 0.1 N HCl and mechanically ventilated for 2 h ($n = 7$); (2) a saline group, in which rats were instilled with 4 ml/kg of saline intratracheally and mechanically ventilated for 2 h ($n = 3$); and (3) a control group that was mechanically ventilated without fluid instillation ($n = 3$). (A) Protein recovery in the BAL. (B) Wet-to-dry ratio of the lung. (C) Immunoblot assay using anti-RAGE polyclonal rabbit antibody. After 0.1 N HCl, RAGE was detected in the BAL (10 μ l) and in serum (10 μ g protein) as an approximately 48 kD band. (D) Quantification of RAGE level in the BAL by dot blot analysis. Results are expressed in arbitrary units of RAGE antigen concentration expressed as μ g/ml of recombinant RAGE-Fc chimera protein equal to that in the sample. Instillation of 0.1 N HCl caused significantly higher RAGE level in the BAL as well as protein recovery in the BAL (A) and lung wet-to-dry ratio (B). * $p < 0.05$.

this ELISA using a positive control of human lung homogenate prepared in the buffer used for whole-cell extract (data not shown).

The RAGE Isoform in Serum and the Alveolar Space Is the Same Isoform That Is Expressed in the Soluble Fraction in Rats

We compared the expression of RAGE in lung tissue fractions and BAL. Anti-RAGE antibodies recognized three isoforms of RAGE in homogenate and membrane fractions, but only the smallest isoform of RAGE (~ 48 kD) was detected in the soluble fraction of the homogenate. RAGE in the BAL was also expressed as one band with the same size as RAGE in the soluble fraction (Figures 1–4 and 7A). These results indicated that RAGE in the BAL and the serum was a soluble isoform; the

larger isoforms observed in the membrane fraction were not present in these samples.

RAGE Is Expressed in Normal Human Lung, and RAGE Levels Are High in the Pulmonary Edema Fluid and Plasma from Patients with ALI

We examined the homogenate of normal human lung and pulmonary edema fluid from patients with ALI/ARDS. Although the membrane fraction of normal human lung homogenate demonstrated multiple bands of RAGE, the soluble fraction of the homogenate and the edema fluid demonstrated a single band sized approximately 48 kD (Figure 7B).

We compared RAGE levels in pulmonary edema fluid and plasma from patients with ALI/ARDS with a group of patients with hydrostatic pulmonary edema. Plasma RAGE levels in

Figure 2. The relationship between the severity of lung injury and abundance of RAGE in the BAL and serum in the rat HCl lung injury model. The volume of instilled HCl (0.1 N) was increased from 1 to 4 ml/kg ($n = 3$ in each setting except 4 ml/kg [$n = 7$]), and the severity of lung injury and the RAGE levels were evaluated. (A) BAL protein recovery. (B) Wet-dry ratio of the lung. (C) RAGE abundance in the BAL (10 μ l) and in serum (10 μ g protein) demonstrated by representative Western blot. (D) Quantification of RAGE level in the BAL by the dot blot analysis. Results are expressed in arbitrary units of RAGE antigen concentration expressed as μ g/ml of recombinant RAGE-Fc chimera protein equal to that in the sample. Instillation of 4 ml/kg HCl resulted in significantly higher level of RAGE than the control level. * $p < 0.05$ versus control.

Figure 3. The relationship between the severity of lung injury and abundance of RAGE in the BAL and serum in the rat HCl lung injury model. The concentration of instilled HCl (4 ml/kg) was increased from 0 N (saline without HCl) to 0.1 N ($n = 3$ in each setting except 0.1 N [$n = 7$]), and the severity of lung injury and the RAGE levels were evaluated. (A) BAL protein recovery. (B) Wet-to-dry ratio of the lung. (C) RAGE abundance in the BAL (10 µl) demonstrated by representative Western blot. (D) Quantification of RAGE level in the BAL by the dot blot analysis. Results are expressed in arbitrary units of RAGE antigen concentration expressed as µg/ml of recombinant RAGE-Fc chimera protein equal to that in the sample. Instillation of 0.1 N HCl resulted in a significantly higher level of RAGE than the control level. * $p < 0.05$ versus saline.

healthy volunteers were also measured. The clinical characteristics of the patients and healthy volunteers are summarized in Table 1. In patients with pulmonary edema, RAGE levels in the alveolar edema fluid were significantly higher than plasma ($p < 0.0001$). The median edema fluid/plasma ratio of RAGE levels was 105 (interquartile range [IQR], 55–243). This finding suggests that the source of RAGE in these samples was predominantly from the lungs rather than from the circulation. In the pulmonary edema fluid, the RAGE levels in ALI/ARDS were significantly higher than those from patients with hydrostatic pulmonary edema (median, 1.3 [IQR, 0.9–3.2] vs. 0.7 µg/ml [IQR, 0.4–1.4]; $p < 0.05$; Figure 8A). Plasma RAGE levels in patients with ALI/ARDS were significantly higher than those in healthy volunteers (median, 15.5 [IQR, 6.0–21.3] vs. 1.4 ng/ml [IQR, 1.1–1.7]; $p < 0.001$; Figure 8B). Plasma RAGE levels were also significantly higher in patients with ALI/ARDS than in patients with hydrostatic pulmonary edema (median, 15.5 [IQR, 6.0–21.3] vs. 6.4 ng/ml [IQR, 3.8–10.7]; $p < 0.05$; Figure 8B).

DISCUSSION

The results of the present study indicate that (1) RAGE was released into the alveolar space by injurious and inflammatory stimuli in the rat lung, and the source was predominantly alveolar type I cells; (2) ALI in rats from hydrochloric acid resulted in

a dose-dependent increase in the BAL levels of RAGE; (3) in the injured rat and human lung, RAGE in the alveolar space and in serum was a soluble isoform sized approximately 48 kD, whereas the membrane fraction of lung tissue demonstrated three isoforms; and (4) RAGE levels in the pulmonary edema fluid from patients with ALI/ARDS were significantly higher than levels in the patients with severe hydrostatic pulmonary edema. Plasma RAGE levels in patients with ALI/ARDS were also significantly higher than the level in the healthy volunteers and in the patients with severe hydrostatic pulmonary edema. These findings indicate that RAGE may be useful as a marker of alveolar epithelial type I cell injury in the acutely injured lung.

The Source of RAGE in the Alveolar Space and in Blood

Since its first purification from a bovine lung cDNA library (19), RAGE has been found to be most abundant in the lung (3). As several recent studies demonstrated, RAGE seems to be expressed in the lung exclusively in alveolar type I epithelial cells (1, 7, 8), and its expression is localized to the basolateral membrane in the normal lung (7, 8). In the present study, the immunohistochemistry of rat lung demonstrated that neither alveolar type II epithelial cells nor alveolar macrophages expressed RAGE under LPS-stimulated conditions or in the normal lung. Another possible source of RAGE is the lung endothelium. However, human lung microvascular endothelial cells in culture demonstrated no expression of RAGE regardless of LPS stimulation. Although there are extrapulmonary sources of RAGE, the vast majority of RAGE seems to be from the lung because the RAGE levels in the BAL in the rats and pulmonary edema fluid in the patients were much higher than levels in the circulation. In the setting of less severe lung inflammation, RAGE was not detectable in the serum despite its presence in the BAL in the rat studies. These results strongly suggest that the source of RAGE in the alveolar space and in the serum was primarily alveolar type I epithelial cells. Recently, Hanford and colleagues (20) reported that bleomycin-induced lung injury caused significant loss of the expression of RAGE in the lung tissue after 7 d of bleomycin administration. These experimental data support the hypothesis that the major source of RAGE in the lung is the alveolar type I epithelial cell because the number of intact alveolar type I epithelial cells is significantly decreased after bleomycin administration and because expression of other

Figure 4. RAGE expression in a rat LPS-induced lung injury model. Rats were divided into an LPS group ($n = 3$) and a control group ($n = 3$). In the LPS group, LPS (10 mg/kg) in 250 µl of saline was instilled intratracheally. The same volume of saline was instilled in control animals. Instillation of LPS into the distal airspaces increased protein recovery in the BAL (10 µl) (A), the lung wet-to-dry weight ratio (B), and the level of RAGE in the BAL (C). * $p < 0.05$ versus saline.

Figure 5. RAGE expression in the normal rat lung and in the LPS-induced lung injury model. RAGE-positive cells (red) were observed on the alveolar septa, but they did not colocalize with p180 lamellar body protein-positive cells (alveolar type II epithelial cells; green). Intratracheal instillation of LPS (10 mg/kg) resulted in edematous alveolar septa, but the pattern of RAGE positivity did not change compared with control conditions. White scale bar indicates 10 μ m.

alveolar type I epithelial cell specific proteins, such as aquaporin 5 (21) and RT140 (22), were also decreased.

The Isoform of RAGE Detected in the Fluid of Alveolar Space

In general, RAGE consists of an extracellular immunoglobulin-like region, a single transmembrane-spanning domain, and a cytosolic tail. Although a full-length form is essential for RAGE-mediated cell activation, there is another isoform, designated as soluble RAGE, that consists of only the extracellular region (9–12). Yonekura and colleagues (23) transfected full-length and soluble-isoform mRNA to COS-7 cells and identified that the

size of these isoforms were 55 kD (full-length) and 46 to $\sim$ 50 kD (soluble), respectively. Furthermore, the soluble isoform can be N-glycosylated, and they reported that the 48- to approximately 50-kD soluble isoform was detected in human sera (23). Our results demonstrate that the membrane fraction of lung homogenate contained multiple isoforms consisting of approximately 55, 50, and 48 kD protein, whereas the BAL and the edema fluid contained a single isoform of approximately 48 kD protein that was identical to the isoform observed in the soluble fraction of the homogenate. These observations indicate that the isoform detected in the fluid of the alveolar space and the

Figure 6. RAGE expression in the normal rat lung and in the LPS-induced lung injury model. RAGE-positive cells (red) were observed on the alveolar septa, but they did not colocalize with CD68-positive cells (alveolar macrophages; green). Intratracheal instillation of LPS (10 mg/kg) resulted in edematous alveolar septa, but the pattern of RAGE positivity was not changed compared with control conditions. White scale bar indicates 10 μ m.

Figure 7. (A) RAGE expression in the BAL and lung homogenate of rat. The immunoblot assay using anti-RAGE polyclonal rabbit antibody demonstrates lung homogenate and its membrane fraction contained three bands sized approximately 48, 50, and 55 kD, whereas the soluble fraction of lung homogenate and BAL demonstrated a single band sized approximately 48 kD. (B) RAGE expression in the pulmonary edema fluid and lung homogenate of human lung. The immunoblot assay using anti-RAGE monoclonal antibody demonstrates that lung homogenate and its membrane fraction contained three bands sized approximately 48, 50, and 55 kD, whereas the soluble fraction of lung homogenate and the pulmonary edema fluid demonstrated a single band sized approximately 48 kD. Homo, homogenate; Mem, membrane fraction; Sol, soluble fraction.

circulating plasma is the soluble isoform consisting of the extracellular domain, based on its solubility and molecular size, whereas tissue homogenate contained the full-length isoform sized approximately 55 kD.

The Mechanism of Augmentation of RAGE Levels in the Alveolar Compartment

In humans, there is evidence that the soluble isoform of RAGE may be a product of splicing variant mRNA (23, 24). However, the transcriptional regulation of RAGE has not been well studied. One prior study has reported that some inflammatory stimuli, such as LPS, can increase transcription of RAGE via NF- κ B binding to the RAGE promoter region, resulting in increased expression of full-length RAGE (25). However, this mechanism cannot sufficiently explain the elevation of the levels of the soluble isoform in the alveolar space. Recently, Hanford and colleagues (26) demonstrated that the soluble isoform of RAGE was produced by carboxyl terminal truncation, not by alternative splicing, in mouse lung. Considering that Devaux and colleagues (27) showed that matrix metalloproteinase-9 caused murine pulmonary epithelial cells to shed the soluble isoform into the culture medium, proteolysis of full-length RAGE may be a mechanism for the increase in the level of the soluble isoform in the alveolar space in human and rat. Another possible explanation is that all isoforms of RAGE are released into the alveolar space due to tissue injury (e.g., necrosis or apoptosis) and then undergo

proteolysis in the alveolar space. The acutely injured human lung contains several proteases (28, 29). More studies are needed to elucidate the mechanisms that account for the release of the soluble isoform of RAGE in the alveolar space.

RAGE as a Marker of Alveolar Type I Epithelial Cell Injury in ALI/ARDS

In the present human study, RAGE levels in the pulmonary edema fluid from patients with ALI/ARDS were significantly higher than the levels in patients with hydrostatic pulmonary edema. Although many investigators have used BAL of normal subjects for controls in studies of ALI (29, 30), mechanically ventilated patients with severe hydrostatic pulmonary edema are a valuable comparison population with pulmonary edema primarily from a hydrostatic stress. Because the source of RAGE is the alveolar type I epithelial cell, it seems likely that the significant increase in the RAGE levels in the edema fluid in ALI/ARDS compared with hydrostatic pulmonary edema reflected enhanced release from the alveolar type I epithelium. Furthermore, RAGE levels in the plasma from patients with ALI/ARDS were significantly higher than in the plasma of healthy volunteers and patients with hydrostatic pulmonary edema. Although the plasma RAGE levels were 100 times lower in the plasma than in the edema fluid, plasma RAGE levels might also reflect the injury to alveolar epithelial type I cells.

TABLE 1. CLINICAL CHARACTERISTICS OF HYDROSTATIC PATIENTS AND PATIENTS WITH ACUTE LUNG INJURY WITH PULMONARY EDEMA AND HEALTHY VOLUNTEERS

	HYDRO	ALI/ARDS	Healthy Volunteers	p Value
No. patients	11	22	11	
Male sex	55%	59%	55%	NS
Smoker	13%	35%	0%	NS
Sepsis	0%	50%		0.005
Age, yr	45 $\pm$ 21	42 $\pm$ 14	35 $\pm$ 8	NS
Lung injury score	2.5 $\pm$ 0.7	3.1 $\pm$ 0.5		0.039
PaO ₂ /FIO ₂ ratio	143 $\pm$ 90	74 $\pm$ 27		0.002
Arterial-alveolar gradient, mm Hg	434 $\pm$ 174	525 $\pm$ 130		NS
Vt, ml/kg	9.7 $\pm$ 2.1	8.2 $\pm$ 2.6		NS
SAPS II score	37 $\pm$ 20	49 $\pm$ 17		NS
Ventilator-free days	19 $\pm$ 9	7 $\pm$ 10		0.002
Hospital mortality	18%	68%		0.01

Definition of abbreviations: ALI/ARDS = acute lung injury/acute respiratory distress syndrome; HYDRO = hydrostatic pulmonary edema; NS = statistically not significant; SAPS II = Simplified Acute Physiology Score (44).

Figure 8. RAGE levels in pulmonary edema fluid and plasma. (A) In the pulmonary edema fluid, RAGE levels in the patients with acute lung injury/acute respiratory distress syndrome (ALI/ARDS) were significantly higher than the RAGE levels in the patients with hydrostatic pulmonary edema (HYDRO; $p < 0.05$). (B) In plasma, RAGE levels in the ALI/ARDS group were higher than in the healthy volunteers ($p < 0.001$). The median levels of plasma RAGE levels in ALI/ARDS were higher than hydrostatic pulmonary edema group ($p < 0.05$). Data expressed as median (horizontal line), with 25th to 75th confidence intervals in the boxes and the 10th to 90th percentiles in the whiskers.

Although the levels were lower than those in patients with ALI/ARDS, the detection of RAGE in the edema fluid and the moderate elevation of RAGE in the plasma of patients with severe hydrostatic pulmonary edema suggests that there may be some low-level injury to the alveolar epithelium in these patients that may be related to the stress-induced epithelial changes reported by West and colleagues (31, 32) or to the effects of positive pressure ventilation in patients with severe lung edema. Our prior studies of pulmonary edema fluid from hydrostatic patients demonstrate some low level of inflammation and detectable protease activity in most patients, although the magnitude of inflammation is less than in patients with ALI (28, 33–35).

Because of its important role in the barrier function between airspace and lung tissue, structural abnormalities of alveolar type I epithelial cells are an important factor in the pathogenesis of ALI/ARDS. For example, recent studies suggested a possible role for type I alveolar epithelial cells in alveolar fluid clearance (36–38), and structural properties of alveolar type I epithelial cells are also critical for maintaining the normal barrier properties of the alveolar capillary barrier. Therefore, biochemical markers that reflect abnormalities of alveolar type I epithelial cells have the potential to improve the detection and evaluation of severity of ALI/ARDS (2). In this context, some previous studies tried to establish alveolar type I epithelial cell markers in animal and human ALI. RTI40 is an apical membrane protein specific to lung alveolar type I epithelial cells and reflects the severity of several types of lung injury (22, 39–41). However, its use is limited to rat studies. Newman and colleagues (15, 42) reported that HTI56 is another alveolar type I cell specific protein that can be used as a marker in human ALI. However, in that study, the quantification of HTI56 was based on the standard curve generated by only partially purified HTI56, which was purified from normal lung tissue obtained from a resected lung specimen (15). In addition, HTI56 is poorly soluble in aqueous media and therefore difficult to assay.

There are some limitations to this study. More work is needed for us to understand the mechanisms by which RAGE is released into the air spaces of the acutely injured lung. The relationship of RAGE activation to inflammatory lung injury, particularly in relationship to its ligand high-mobility group box protein-1, deserves further study. Although this study included human samples from normal volunteers and patients with hydrostatic and lung injury edema, additional studies of larger groups of patients with ALI are needed at different time points, especially to determine if the cause of lung injury (e.g., aspiration, pneumonia, or sepsis) influences the levels of RAGE and to establish whether levels of RAGE correlate with the physiologic severity of lung injury and clinical outcomes. Although there is a need for better biochemical markers to distinguish cardiogenic from noncardiogenic pulmonary edema in patients (43), it is not clear that plasma levels of RAGE are useful for this purpose because plasma levels of RAGE in patients with severe hydrostatic and lung injury edema showed some overlap.

In summary, the results of this study indicate that RAGE may be useful as a biological marker of experimentally induced alveolar epithelial type I cell injury. In patients, RAGE was detected as a single soluble isoform in the samples of plasma, BAL, and pulmonary edema fluid. In the human samples, quantification of RAGE can be accomplished by an ELISA. Because a homolog of RAGE has been identified in several species, including humans, rats, and mice, RAGE may facilitate translational studies between animals and humans. The measurement of RAGE may stimulate more research into the relationship of injury to alveolar type I epithelial cells and the pathophysiology of ALI and other acute and chronic pulmonary diseases.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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