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Full paper

# Soluble form of the receptor for advanced glycation end-products attenuates inflammatory pathogenesis in a rat model of lipopolysaccharide-induced lung injury

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ABSTRACT

Acute respiratory distress syndrome (ARDS) is a severe respiratory failure caused by acute lung inflammation. Recently, the receptor for advanced glycation end-products (RAGE) has attracted attention in the lung inflammatory response. However, the function of soluble form of RAGE (sRAGE), which is composed of an extracellular domain of RAGE, in ARDS remains elusive. Therefore, we investigated the dynamics of pulmonary sRAGE and the effects of exogenous recombinant human sRAGE (rsRAGE) under intratracheal lipopolysaccharide (LPS)-induced lung inflammation. Our result revealed that RAGE was highly expressed on the alveolar type I epithelial cells in the healthy rat lung including sRAGE isoform sized 45 kDa. Under LPS-induced injured lung, the release of sRAGE into the alveolar space was increased, whereas the expression of RAGE was decreased with alveolar disruption. Treatment of the injured lung with rsRAGE significantly suppressed the lung edema, the neutrophils infiltration, the release of high mobility group box-1 (HMGB1), and the expressions of TNF-α, IL-1β and iNOS. These results suggest that the alveolar release of sRAGE may play a protective role against HMGB1 as well as exogenous pathogen-associated molecular patterns. Supplementary therapy with sRAGE may be an effective therapeutic strategy for ARDS.



## 1. Introduction

Acute respiratory distress syndrome (ARDS) is characterized by the rapid onset of life-threatening respiratory failure as a consequence of severe acute inflammatory diseases (1). ARDS are induced by a diverse range of risk factors, including direct lung injury (e.g., bacterial or viral pneumonia, lung contusion, or toxic inhalation) and indirect lung injury induced by systemic insults (e.g., sepsis, burn, or pancreatitis) (2). The infiltrated neutrophils and macrophages exacerbate the lung inflammation by releasing pro-inflammatory mediators and reactive oxygen species, activating proteolytic enzymes, and so on (3). The pathophysiology of severe pulmonary inflammation is characterized by diffuse alveolar damage, alveolar capillary leakage, and protein rich pulmonary edema with alveolar epithelial and endothelial injury (3,4). Despite our current knowledge of the pathophysiology of ARDS, as described above, and the various therapeutic strategies that have been examined, there remains no established therapy for clinical use (4).

In 1992, an advanced glycation end-products (AGEs)-binding protein was initially purified and identified from the bovine lung and designated a receptor for AGEs (RAGE) (5). RAGE is a single

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transmembrane receptor that is composed of an extracellular ligand-binding domain, a transmembrane domain, and a short cytoplasmic domain which is essential for signal transduction (6,7). Moreover, RAGE has a soluble form protein (soluble RAGE (sRAGE)) composed of an extracellular ligand-binding domain without the transmembrane and cytoplasmic domains (7).

RAGE can bind to not only AGEs but also endogenous damage-associated molecular patterns (DAMPs) such as high mobility group box-1 (HMGB1), several members of the S100 protein family, amyloid β peptide, β2-integrin and pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) (8,9). In addition, RAGE is considered to be a typical pattern-recognition receptors (PRRs), with potential similarity to members of the toll-like receptors (TLRs) family in the innate immune system as a first line of host defense (10,11).

It is particularly worth noting that RAGE is constitutively and predominantly expressed in the lung at high levels, whereas it is expressed at low levels in almost all cells under normal conditions (12). Therefore, it is considered that RAGE may have an important function in lung homeostasis (13).

Recently, it has been reported that sRAGE levels are increased in the plasma and bronchoalveolar lavage fluid (BALF) of rodent models and human patients with ARDS (14). In particular, sRAGE release into BALF may be a good biomarker to estimate the severity of lung injury (15). However, the physiological function of sRAGE remains elusive. *In vivo* experiments using a lung injury model have been inconsistent and controversial in terms of the protective effects of sRAGE (16,17).

In the present study, we investigated the *in vivo* effects of purified recombinant human sRAGE (rsRAGE) administered intratracheally to a rat model of LPS-induced lung injury, and the underlying mechanism of its anti-inflammatory actions in the environment of the injured lung.

## 2. Material and methods

### 2.1. Animals and LPS-induced lung injury model

All animal protocols were approved and conducted according to the recommendations of the Okayama University Animal Care and Use Committee. Male Wistar rats at 8–11 weeks old and weighing 300 ± 50 g were purchased from Japan SLC (Shizuoka, Japan) or Japan Charles River (Yokohama, Japan). The LPS-induced lung injury model was established according to the method described previously (14). Under anesthesia, LPS (*Escherichia coli* O111:B4) (Sigma–Aldrich, St. Louis, MO, USA) at 5 mg/kg as a solution of 5 mg/mL or an equivalent volume of saline as a vehicle control was administered intratracheally through a catheter. Rats were sacrificed to obtain tissue samples at 0.5, 6, 24, and 48 h after LPS-induced lung injury. To evaluate the *in vivo* effects of rsRAGE for LPS-induced lung injury, rats were also intratracheally treated with rsRAGE at 1 mg/kg as a solution of 1 mg/mL or an equivalent dose of human serum albumin (HSA) (Sigma–Aldrich) as a protein control after 1 h of LPS-induced lung injury.

### 2.2. Purification of recombinant human soluble RAGE

rsRAGE was produced by the method as previously described (18).

### 2.3. BALF analysis

Twenty-four hours after LPS administration, BALF was collected by irrigating the lung 3 times with 5 mL of cold saline for analysis.

### 2.4. Immunoblot analysis

Immunoblot analysis was performed to detect the RAGE by using the antibody against rsRAGE, which was house-made and raised in a rabbit, and detected both the soluble isoform and transmembrane isoform.

### 2.5. Immunohistochemistry

Immunohistochemical staining was performed as previously described (19) using antibody against rsRAGE, Podoplanin (Acris, San Diego, CA, USA), P180 LBP (Abcam, Cambridge, UK), CD68 (Abcam), Myeloperoxidase (MPO) (Abcam) and HMGB1 (Abcam and R&D, Minneapolis, MN, USA).

### 2.6. Measurement of HMGB1 by ELISA

A sensitive and specific anti-HMGB1 monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (ELISA) was established. The capture and detection antibodies were produced by our group as described previously (19).

### 2.7. Naphthol AS-D chloroacetate esterase stain

Naphthol AS-D chloroacetate esterase stain was performed to count the infiltrating active neutrophils as previously described (20).

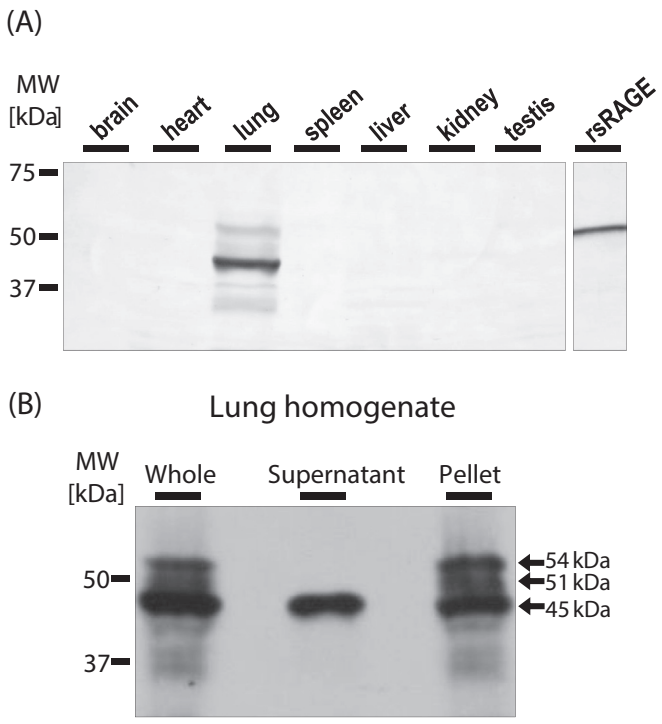


**Fig. 1. Tissue distribution of RAGE isoforms in normal rat tissues**. (A) Tissue homogenates were prepared from normal rat organs including lung tissue and subjected to immunoblot analysis with anti-rsRAGE antibody. RAGE isoforms in the indicated organs were detected by using a DAB reaction (A) or ECL system (B). Purified rsRAGE with 6 tandem histidine-tags was used as a positive control in (A). The left lane in (B) shows the result when loading a whole fraction of the lung homogenate, including the insoluble components of the plasma membrane, and the middle lane the result for a supernatant fraction including only soluble components. The right lane in (B) shows the result when loading a pellet fraction including the insoluble fraction.

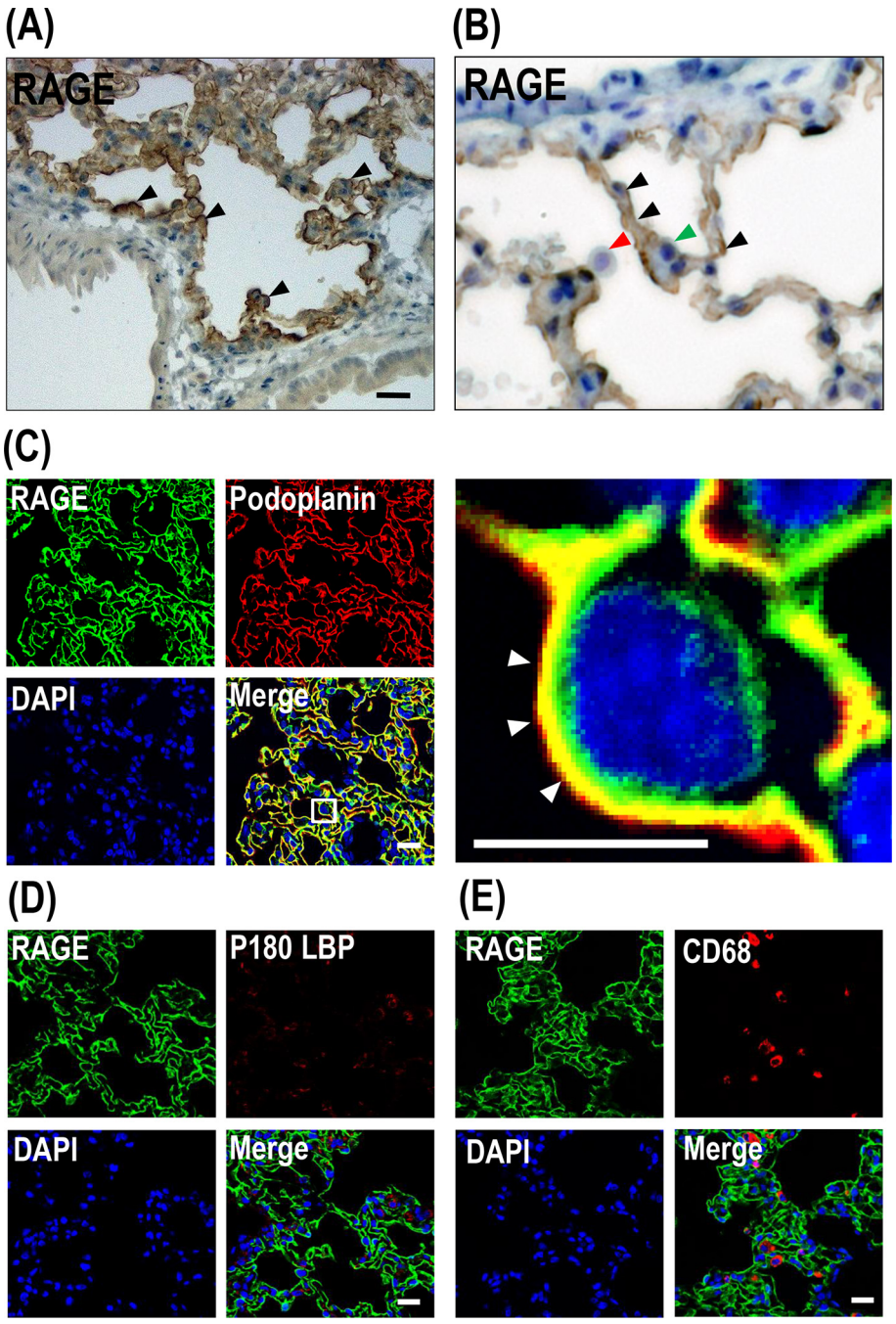


**Fig. 2. Immunohistochemical localization of RAGE in the normal rat lung.** (A–B) Lung sections were immunostained with anti-rsRAGE antibody followed by initiation of a DAB reaction. The black, green and red arrowheads indicate a RAGE-positive AECI, a RAGE-negative AECII and a RAGE-negative alveolar macrophage, respectively. (C) Double-immunofluorescent staining of lung tissue with anti-rsRAGE (Alexa Fluor 488) and with anti-podoplanin (Alexa Fluor 555) antibodies. The right panel shows a high magnification of the region in the white square in the left panel. (D) Double-immunofluorescent staining of lung tissue with anti-rsRAGE (Alexa Fluor 488) and anti-P180 LBP (Alexa Fluor 555) antibodies. (E) Double-immunofluorescent staining of lung tissue with anti-rsRAGE (Alexa Fluor 488) and with anti-CD68 (Alexa Fluor 555) antibodies. The sections were counterstained with hematoxylin (A–B) or DAPI (C–E). The scale bars indicate 20 μm (A, C-E) or 10 μm (magnification panel in (C)).

### 2.8. Measurement of lung wet/dry weight ratio

Lung edema was assessed by measuring the tissue wet/dry weight ratio as previously described (21).

### 2.9. Real-time quantitative PCR analysis

Total RNA was extracted from the lung tissue by using an RNeasy kit (QIAGEN, Hilden, Germany), and then total RNA was reverse-transcribed by using a Takara PrimeScript RT reagent kit (Takara, Siga, Japan). Real-time quantitative PCR analysis was performed described previously (19). The amplification of each PCR product was confirmed by analyzing a melting curve of the PCR products (22).

### 2.10. Statistical analysis

Statistical comparisons of experiments were performed using a one-way ANOVA followed by the *post hoc* Dunnett's test. The statistical comparisons of BALF analysis were performed using a

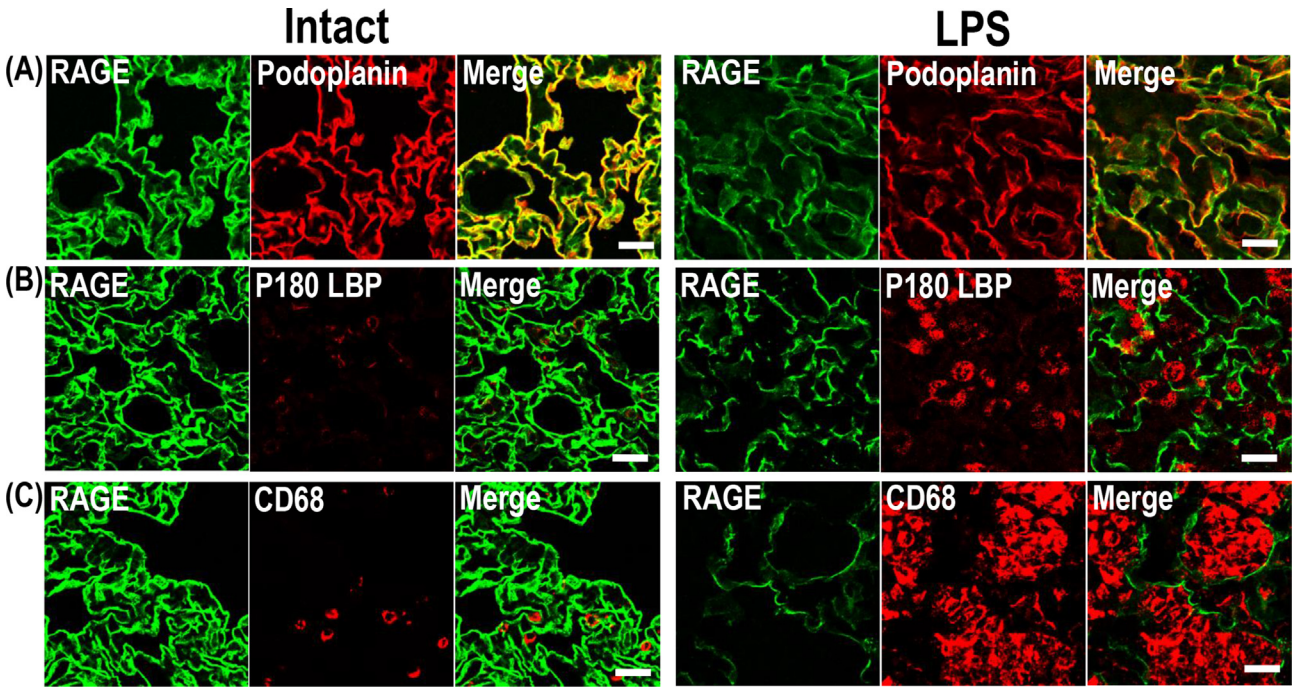


**Fig. 3. LPS-induced changes in RAGE immunoreactivities in the injured lung**. Lung sections were prepared from the intact rats in the 9 panels at left or from rats 48 h after intratracheal LPS administration in the 9 panels at right. Double-immunofluorescent staining for RAGE (Alexa Fluor 488) and podoplanin (Alexa Fluor 555), P180 LBP (Alexa Fluor 555), or CD68 (Alexa Fluor 555) were performed as in Fig. 2. The scale bars indicate 20 μm.

Student's *t*-test. The mean values of data are shown along with the SE. *P*-values less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Distribution and localization of RAGE expression in normal rats

Several major organs were examined by immunoblot analysis, and the RAGE protein levels were highest in the lungs (Fig. 1A). While three major bands corresponding to molecular weights of approximately 45, 51 and 54 kDa were detected in the whole and insoluble pellet fraction of the lung homogenate, the supernatant fraction of the lung homogenate showed only a single band with a molecular weight of 45 kDa (Fig. 1B).

To confirm that RAGE expression was localized in the lung, immunohistochemical staining was performed. Anti-rsRAGE immunoreactivity was prominent along with the alveolar epithelium cell layer in the histological structure (Fig. 2A), but was not observed in AECII and alveolar macrophages on the basis of the morphological features (Fig. 2B). We also confirmed that the expression of RAGE was colocalized quite clearly to the podoplanin-positive AECI (Fig. 2C), but not observed in the P180 LBP-positive AECII (Fig. 2D) or CD68-positive alveolar macrophages (Fig. 2E).

### 3.2. Alteration of RAGE expression under LPS-induced lung injury

LPS has a toxic component, endotoxin, that is derived from a component of the Gram-negative bacterial cell wall and is the leading cause of ARDS. We used the intratracheal LPS-induced lung injury rat model to examine the role of RAGE under a pathological condition. Forty-eight hours after LPS administration, the alveolar structure was dramatically altered and showed inflammatory features, leading to a marked decrease in the podoplanin-immunoreactivities (Fig. 3A), an increase in the P180 LBP-positive AECII (Fig. 3B), and a severe infiltration of the CD68-positive alveolar macrophages into the pulmonary alveolus (Fig. 3C). In addition, the fluorescence intensity for RAGE was also remarkably decreased in the injured lung along with the decreased podoplanin-immunoreactivities (Fig. 3A).

Following the immunohistological analysis, we performed quantitative determination of the alteration of RAGE expression in the injured lung. Immunoblot analysis showed that administration of LPS to the lung resulted in a significant decrease of RAGE isoforms with molecular weights of 45, 51 and 54 kDa in a time-dependent manner (Fig. 4A–D). The 54-kDa RAGE isoform was almost undetectable at 0.5 h after LPS administration. RAGE levels in the saline-treated group were not significantly different from those in the intact rat group. Essentially identical results were also observed following intravenous LPS administration to the rat tail vein (date not shown). Real-time quantitative PCR analysis revealed that the pulmonary expression level of RAGE mRNA was significantly decreased by LPS administration in a time-dependent manner (Fig. 4E), which was consistent with the results of the immunoblot analysis.

### 3.3. sRAGE release into the alveolar space under LPS-induced lung injury

To assess the inflammation grade of the LPS-induced lung injury model, BALF samples were prepared from the lungs at 24 h after LPS administration. Immunoblot analysis did not detect any isoforms of RAGE from the control BALF. However, the BALF derived from the injured lung showed the clear existence of the 45-kDa RAGE isoform, which was considered to be a soluble isoform (Fig. 5A). The release into BALF of HMGB1, which is an initial inflammatory mediator, was significantly increased by LPS stimulus (Fig. 5B).

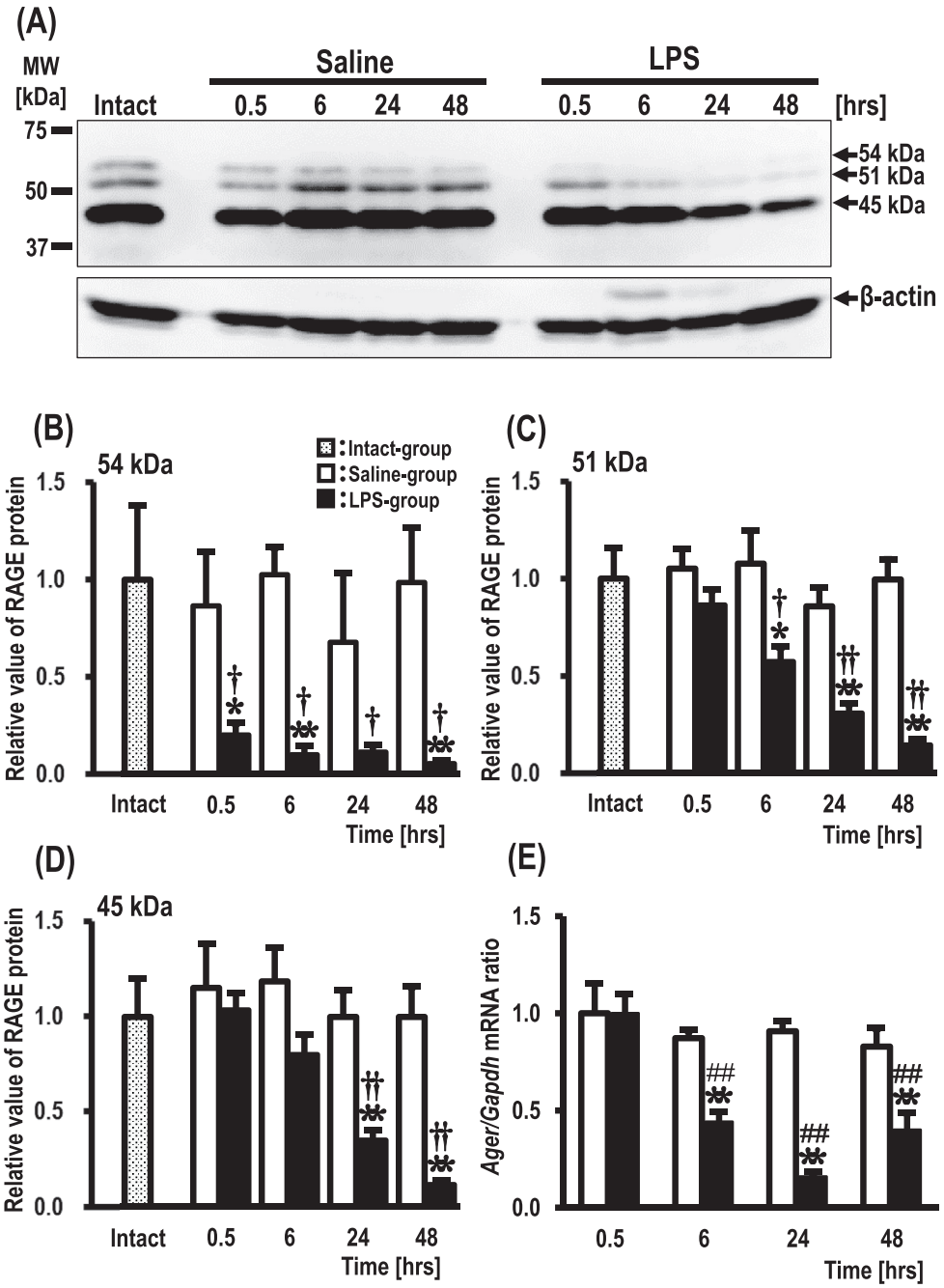


**Fig. 4. Change in RAGE isoforms in lung tissue after intratracheal LPS administration**. Whole lung homogenate was prepared from the intratracheal LPS- or saline-administered rats at the indicated times and subjected to immunoblot analysis with anti-rsRAGE antibody. (A) The left-most lane represents the intact rat sample as a control. β-actin was used as a loading control. (B–D) The 45-, 51-, and 54-kDa isoforms of RAGE were quantified, respectively. (E) Total RNA was isolated from lung tissue under the same conditions as in (A) and subjected to real-time quantitative PCR for analysis of the expression of RAGE mRNA. GAPDH was used as a housekeeping gene. Values represent the means ± SE (n = 4–5 rats for (A–D), n = 7–8 rats for (E)). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the saline-group at each of the indicated time points. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with the intact rat group in (B–D). $^{\#\#}P < 0.01$ compared with the control saline-group at 0.5 h in (E).

Similarly, both the total cell number and protein contents in BALF were significantly increased by LPS administration (Fig. 5C–D).

### 3.4. Anti-inflammatory effects of rsRAGE treatment on the LPS-induced lung injury

Finally, we evaluated whether the inflammatory condition of LPS-induced lung injury was ameliorated by therapeutic treatment with intratracheally-injected purified rsRAGE.

Consistent with the LPS-induced inflammatory responses shown in Fig. 3 and Fig. 5, the number of naphthol AS-D chloroacetate esterase-positive neutrophils in lung tissue and the lung wet/dry weight ratio were significantly increased at 24 h after LPS administration. The rsRAGE treatment of the injured lung significantly decreased these pathological parameters (Fig. 6A–B and Fig. 7A). In immunohistochemical staining, the anti-inflammatory effects of rsRAGE on the injured lung were also apparent based on the reduction in excessive infiltrating inflammatory cells, and

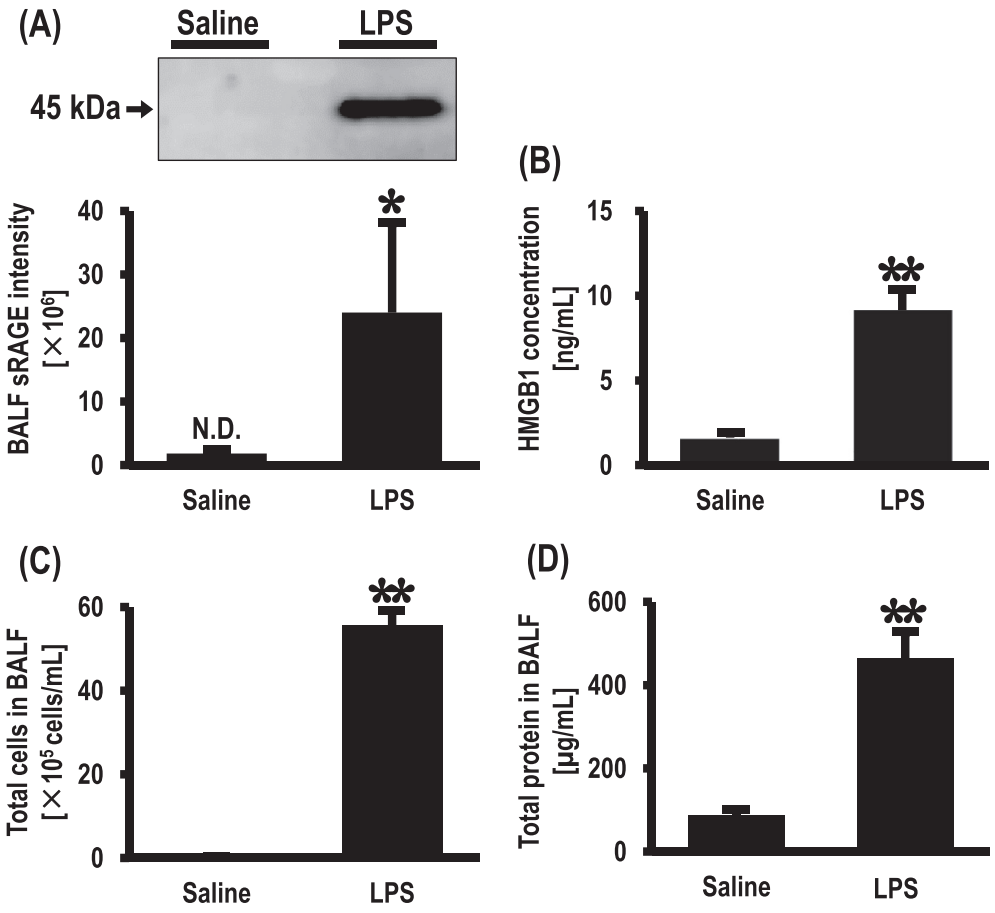


**Fig. 5. Release of the sRAGE isoform and HMGB1 into the BALF after intratracheal administration of LPS**. The BALF was collected from the intratracheal LPS- or saline-administered rats at 24 h. (A) Immunoblot analysis with anti-rsRAGE antibody was performed and the results were quantified. The background area in each lane was used to adjust the quantification. (B) The HMGB1 concentration in the BALF was measured by ELISA. (C) Total cells in BALF were counted. (D) The protein concentration was measured. Values represent the means ± SE (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control saline-group. N.D.: not detectable.

the suppression of HMGB1 release from nuclear to cytosol (white arrows in Fig. 6E) in AECI and macrophages (Fig. 6C–E). However, the infiltrated neutrophils did not translocate the nuclear HMGB1 24 h after LPS stimulation. Furthermore, under the same conditions as in Fig. 6, real-time quantitative PCR analysis showed that the gene expressions for inflammatory cytokines such as IL-1β, TNF-α and iNOS were significantly increased in the injured lung (Fig. 7B–D). Treatment of the injured lung with rsRAGE significantly suppressed the increased expression of these genes (Fig. 7B–D).

## 4. Discussion

We have shown that intratracheal treatment with purified rsRAGE ameliorated the inflammatory conditions in an LPS-induced model of lung injury. The pathological alteration of sRAGE expression revealed in the present study is potentially important for understanding the overall inflammatory responses in the pulmonary alveolus, which in turn is crucial for understanding the pathogenesis of ARDS. The contribution of rsRAGE to the anti-inflammatory effects in the injured lung was revealed by the attenuation of pulmonary edema, the decreased infiltration of inflammatory cells and the suppressed expression of inflammatory cytokines. The treatment of rsRAGE appeared to play an important role in protecting the lung against LPS-induced damage.

In the normal rat lung tissue, RAGE was predominantly expressed in three main isoforms detected as approximately 45, 51 and 54 kDa bands (Fig. 1B). The 45-kDa isoform of RAGE was present in the supernatant fraction of lung homogenate, which is considered a soluble isoform lacking a transmembrane domain. These results are consistent with the findings previously described (14).

RAGE protein was predominantly localized to the plasma membrane of AECI in the pulmonary alveolus under normal conditions (Fig. 2). The AECI linings constitute more than 98% of the internal surface area in the rodent pulmonary alveolus, which is essential to pulmonary homeostasis through the integrity of the alveolar epithelial barrier, gas exchange and alveolar fluid clearance (23).

On the other hand, AECI is constantly exposed to invasions of a wide range of infectious pathogens and/or foreign antigens from the external environment and host-derived danger signals. Under AECI injury, podoplanin is suggested to be a sensitive marker of ARDS based on the findings that podoplanin and its related homologs were released into BALF in bleomycin-induced or *Pseudomonas aeruginosa*-induced lung injury (24,25). Thus, the apparent decrease in podoplanin immunoreactivities in LPS-induced lung injury strongly suggests damage to the AECI in our rat model (Fig. 3). It is known that AECII proliferate, migrate and transform into AECI in ARDS (25,26). Therefore, the considerable increase in the number of AECII in LPS-induced lung injury observed in the

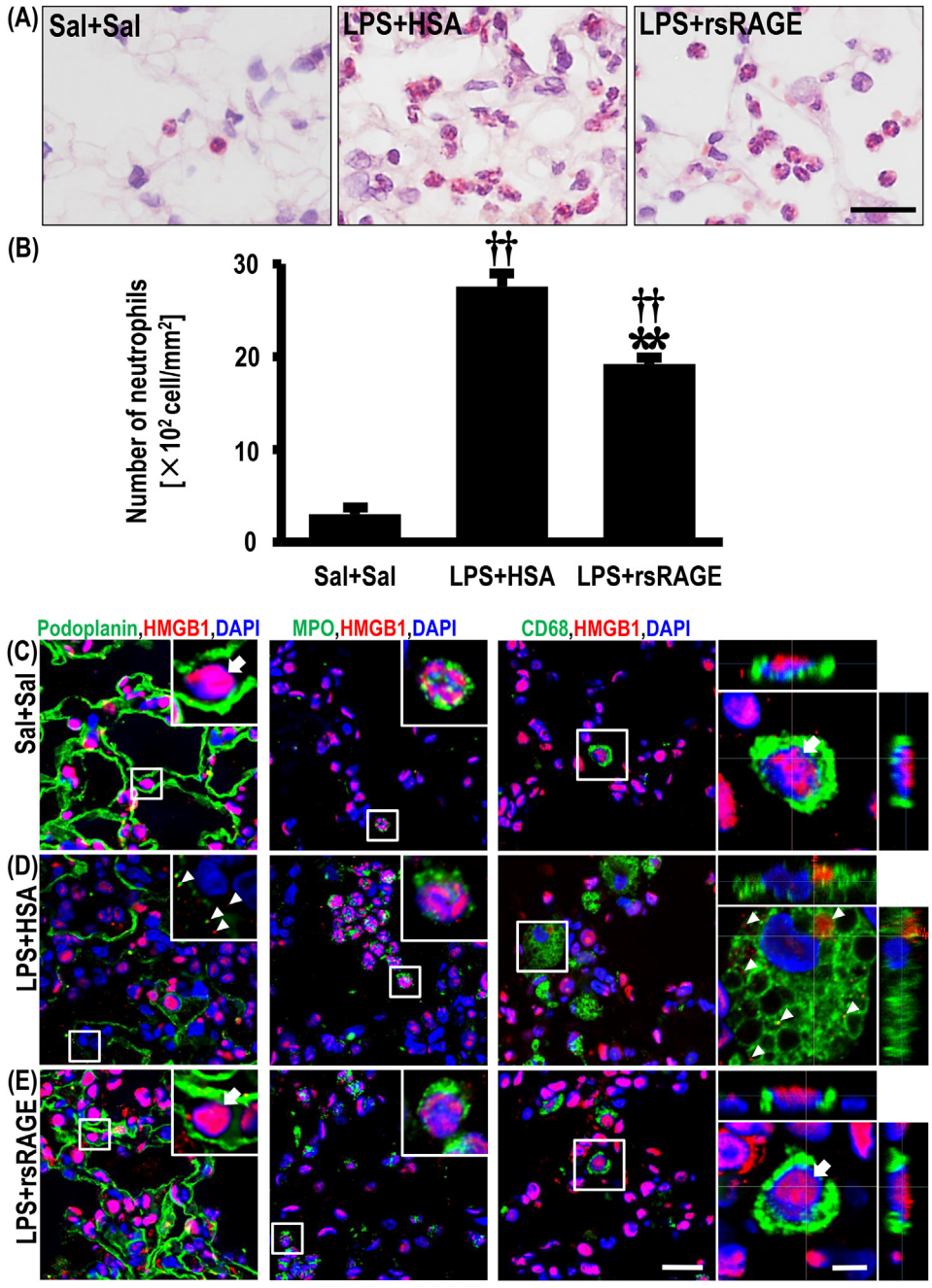


**Fig. 6. Effects of rsRAGE treatment on the number of neutrophils and the HMGB1 localization in LPS-induced injured lung.** Lung tissues were prepared after intratracheal LPS administration with rsRAGE (LPS + rsRAGE) or HSA (LPS + HSA). The Sham group (Sal + Sal) was administered saline alone. (A) Naphthol AS-D chloroacetate esterase staining was performed for the identification of neutrophils. The infiltrated neutrophils in the lung are represented by Naphthol AS-D-positive cells as the deep dye staining. The sections were counterstained with hematoxylin. (B) Cell density of the activated neutrophils was quantified by counting Naphthol AS-D-positive cells. (C–E) Double-immunofluorescent staining of lung tissue with anti-HMGB1 (Alexa Fluor 555) and anti-Podoplanin (Alexa Fluor 488), anti-MPO (Alexa Fluor 488), or anti-CD68 (Alexa Fluor 488) antibodies were performed on three groups. The white squares and their magnified pictures show the typical patterns of HMGB1 distribution in each group. The most right panel in each group represent the spot-checked distribution of HMGB1 by Z-stack analysis in CD68-positive alveolar macrophage in the adjacent picture (white square). White arrows in (C) and (E) indicate the intra-nuclear localization of HMGB1. White arrowheads in (D) indicate the extranuclear localization of HMGB1. The sections were counterstained with DAPI. The scale bars indicate 20 μm (A, C-E) or 5 μm (3D confocal analysis in (C–E)). Values represent the means ± SE (n = 4–6 rats). $^{**}P < 0.01$ compared with the LPS + HSA group. $^{\dagger\dagger}P < 0.01$ compared with the Sal + Sal group.

present study supports the notion that the AECI were severely injured. Taken together, our observations suggest that the AECI suffered pathological alteration or lethal damage leading to alveolar disruption. Such a feature of AECI injury could result in the respiratory failure with high mortality seen in ARDS (1).

Interestingly, it has been reported that AECI may be a more important and active player than AECII in host defense through the innate immune response in the lungs (27). RAGE has the potential to induce inflammatory responses by directly binding with LPS (9). Therefore, it might be possible that RAGE on AECI constitute a sensory alarm system in the pulmonary innate immune response against PAMPs/DAMPs.

In the presence of LPS-induced lung injury, both the expression and production of RAGE were decreased with striking damage to

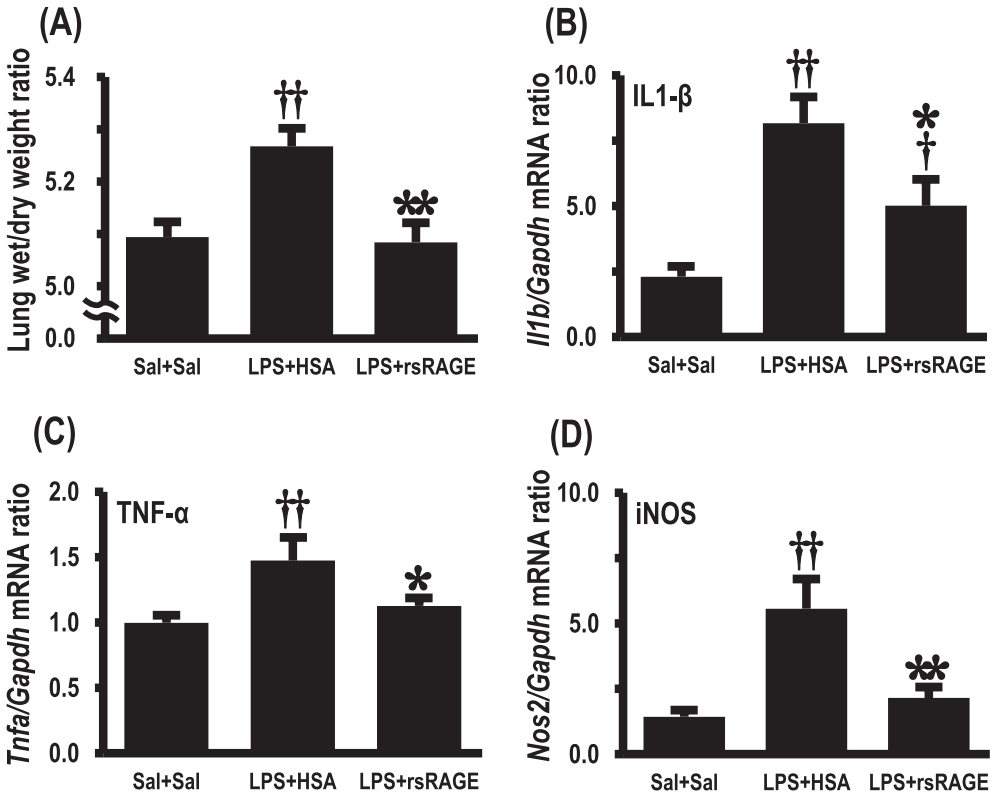


**Fig. 7. Effects of rsRAGE treatment on pulmonary edema and the expression of inflammatory cytokines in the LPS-induced injured lung**. Lung tissues were prepared from the intratracheal LPS-administered rats treated with rsRAGE (LPS + rsRAGE) or HSA (LPS + HSA). The Sham group (Sal + Sal) was administered saline alone. (A) The lung wet/dry weight ratio was determined in each group. (B–D) Total RNA was isolated from each group of lungs and subjected to real-time quantitative PCR analysis for determination of the expression of IL-1β (B), TNF-α (C) and iNOS (D). GAPDH was used as a housekeeping gene. Values represent the means ± SE (n = 4–6 rats for (A), n = 6–8 rats for (B–D)).*$P < 0.05$, **$P < 0.01$ compared with the LPS + HSA group. ††$P < 0.01$ compared with the Sal + Sal group.

the alveolar structure (Figs. 3 and 4). We also confirmed the remarkable decrease in RAGE isoforms in lung tissue under the intravenous LPS-induced lung injury in rats (date not shown). The decrease in RAGE protein in the injured lung has also been observed in other animal models of lung injury, such as *E. coli*-induced lung injury, asbestos-induced idiopathic pulmonary fibrosis, and bleomycin-induced lung fibrosis (17,28,29). These results suggest that RAGE expression in lung tissue is decreased in AECI under strong inflammatory conditions.

However, several have also reported that RAGE was increased in the lung tissue of experimental models of lung injury induced by cigarette smoke or hyperoxia (30,31). This discrepancy may be due to differences in the degree, frequency or persistence of inflammatory stimulation compared to our injury model. Thus, mild and low levels of inflammatory stimuli might induce an increase of RAGE expression in response to various PAMPs/DAMPs in a sensitive manner during mild lung injury. As a result, the increased RAGE expression may enhance an innate immune response in lung tissue as a host defense mechanism. However, when the stimulus levels by invading PAMPs/DAMPs exceed the homeostatic levels, lethal alternation of AECI may occur, leading to severe damage and cell death of AECI. Under such conditions, RAGE expression would decrease, attenuating the subsequent inflammatory response. The alteration of RAGE expression under severe lung injury may contribute to a decrease in excessive inflammatory responses through the reduced RAGE signaling in AECI. Thus, RAGE expression on AECI may be regulated by the severity of lung injury.

In the alveolar space, sRAGE was dramatically increased by LPS stimulation in BALF, in association with protein leakage and the infiltration of neutrophils and macrophages (Fig. 5), whereas both the mRNA expression and protein production of RAGE were significantly decreased in the injured lungs (Fig. 4). In experimental models of ARDS, we also consider that sRAGE is produced by proteolytic cleavage of membrane-bound RAGE isoforms by extracellular proteases (32).

Looking at the sRAGE findings more closely, it can be seen that, among the three major RAGE isoforms, sRAGE had the highest intensity in the whole homogenate and in the supernatant fraction of the intact rat lungs (Figs. 1B and 4A), whereas sRAGE could not be detected in BALF in the sham group (Fig. 5A). These results suggest that a large amount of 45 kDa sRAGE isoform might be constantly present in a storage pool in the normal rat lung that may be rapidly released into the alveolar space under the early stage of lung injury. Although further investigation is necessary, sRAGE would have a very important physiological function in the lung tissue.

To confirm the conjectured function of sRAGE in lung injury, we evaluated the effect of rsRAGE on the inflammatory responses under LPS-induced lung injury. Many pathological responses in the injured lung were significantly improved by rsRAGE treatment, including the excessive pulmonary infiltration of inflammatory cells, the increase of lung edema and the expression of inflammatory cytokines (Figs. 6 and 7). The anti-inflammatory effects of rsRAGE in the injured lung were also confirmed by the suppression of HMGB1 translocation in AECI and alveolar macrophages, as shown by a histological study (Fig. 6C–E). However, infiltrated neutrophils appeared not to be the source of released HMGB1 (Fig. 6C–E). Thus, HMGB1 is probably released from activated macrophages and AECI in the LPS-induced lung injury in the

present study. Not only RAGE but also TLR4 has been recognized as HMGB1 receptor (10). Since up-regulation of TLR4 was reported on the same LPS-induced lung injury model in rats (33), TLR4 also might be involved in the action of extracellular HMGB1. Therefore, we consider that one of the anti-inflammatory effects of rsRAGE in the injured lung is due to a neutralizing effect of sRAGE by binding to not only LPS but also the released HMGB1 from damaged alveolar epithelial cells and activated alveolar macrophages for inhibition of these ligands binding to RAGE, TLR4, or other cell surface receptors. Taken together, our present study suggests that sRAGE treatment has the potential to ameliorate lung injury in conditions such as ARDS.

Several reports have supported that sRAGE acts as a decoy receptor (9,16,34). However, another study reported that sRAGE did not function as a decoy (17). Although little is known about this discrepancy, our results have provided considerable evidence for the anti-inflammatory function of sRAGE.

In conclusion, our present study suggests that the expression of RAGE in AECI plays a role for lung inflammation. The release of soluble RAGE from AECI has the potential to attenuate the excessive inflammatory response in ARDS. Although further investigation is necessary, this study supports the notion that RAGE would be a promising candidate for a molecular target in the treatment of ARDS.

## Conflict of interest

The authors report no conflicts of interest.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jphs.2016.02.005.

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