Sepsis impairs alveolar epithelial function by downregulating Na-K-ATPase pump

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Sepsis impairs alveolar epithelial function by downregulating Na-K-ATPase pump. Am J Physiol Lung Cell Mol Physiol 301: L23–L30, 2011. First published April 8, 2011; doi:10.1152/ajplung.00010.2010.—Widespread vascular endothelial injury is thought to be the major mechanism for multiorgan dysfunction and ARDS in sepsis, thereby augmenting the permeability of alveolar capillaries with subsequent influx of protein-rich edema fluid into the air spaces (17).

The resolution of both cardiogenic pulmonary edema and ARDS depends on the clearance of fluid from the alveolar space, a process that requires an intact, functional alveolar epithelium (22, 24). The primary driving force for alveolar fluid clearance (AFC) is the active transport of sodium from the alveolar space to the interstitium by alveolar epithelial type II cells (ATII cells) (2, 21, 23, 34, 36). Little is known about ATII function in sepsis. Therefore, we aimed to examine the effects of remote sepsis on AFC and active sodium transport.

MATERIALS AND METHODS

Animals

Specific pathogen-free male Sprague-Dawley rats (250–350 g; Harlan Laboratories, Jerusalem, Israel) were used in this study. All animals received humane care, in compliance with the guidelines of the Ruth and Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology. All animal experiments were conducted in accordance with the “Principles of Laboratory Animal Care” and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health. The animal studies were approved by the Technion Animal Care Committee.

Animal Preparation

Housing included day and night light cycles, with temperature control. Standard chow and water were administered ad libitum.

CLP Model for Sepsis

As previously described (41), following anesthesia a 2-cm midline laparotomy was performed and the cecum was ligated with a 3-0 silk ligature below the ileocecal valve and punctured twice with a 21-gauge needle [cecal ligation and puncture (CLP)]. The procedure was performed without obstructing the intestinal continuity. The cecum was then returned to the abdominal cavity and the incision was closed in two layers with 3-0 silk running suture. NaCl solution 0.9% 30 ml/kg was injected subcutaneously to replace fluid losses during laparotomy, and the animals were returned to their cages for follow-up. In sham control rats, the cecum was exposed and the bowel was massaged as described above, but the cecum was not ligated or punctured.
Alveolar Fluid Clearance and Alveolar Epithelial Permeability

To study AFC and alveolar epithelial permeability, the isolated-perfused fluid-filled lung model was used as previously described (30). Briefly, rats were anesthetized with 50 mg/kg body wt of pentobarbital sodium, tracheotomized, and mechanically ventilated with a tidal volume of 2.5 ml, a peak airway pressure of 8–10 cmH2O, and 100% oxygen for 5 min. The chest was opened via a median sternotomy. The heart and lungs were removed en bloc. The pulmonary artery and left atrium were catheterized, and the pulmonary circulation was perfused with a buffered physiological salt solution containing bovine serum albumin (BSA). The lungs were then instilled with 5 ml of BSA containing Evans blue-tagged albumin (EBD; Sigma). The lungs were then instilled with 5 ml of BSA containing Evans blue-tagged albumin (EBD; Sigma).

Perfusion of the lungs was performed with 90 ml of the same BSA containing 0.16 mg/ml of fluorescein-tagged albumin (FITC-albumin; Sigma). The perfusate was pumped from a lower reservoir to an upper reservoir by a peristaltic pump and from there flowed through the pulmonary artery and exited via the left atrium. AFC was determined from EBD-tagged albumin concentration changes.

Alveolar epithelial permeability was determined from the fraction of FITC-albumin that appeared in the alveolar space during the experimental protocol.

WD Lung Weight Ratio

To determine wet to dry (WD) weight ratio the whole lung was weighed on excision from the animal and then dried in an oven (70°C) overnight to constant weight as previously described (19).

ATII cell isolation. Alveolar epithelial type II (ATII) cells were isolated as described (10). The lungs were perfused, lavaged, and digested by elastase (4.4 U/mg, Worthington) for 20 min at 37°C. The tissue was minced and cells were filtrated through cotton gauze and 80-μm nylon mesh. The crude cell suspension was purified by differential adhesion to IgG-coated petri dishes. After 60-min incubation at 37°C, ATII cells were removed from the dishes by gentle panning. The purity of ATII cells was assessed by modified Papanicolaou stain based on the presence of dark blue inclusions. Cell viability was assessed by Trypan blue exclusion (>95%).

Western blot analysis. Ten micrograms of peripheral lung BLMs in homogenization or 50 μg of total ATII cell lysate buffer were resolved in a polyacrylamide gel. Proteins were transferred onto nitrocellulose membranes by a semidry transfer apparatus (Bio-Rad) and resolved in a polyacrylamide gel. Proteins were transferred onto nitrocellulose membranes by a semidry transfer apparatus (Bio-Rad) and resolved in a polyacrylamide gel.

Preparation of basolateral plasma membranes. After separation of early and late endosomes, another fraction (500 μl) was collected at the 16 and 42% sucrose interface corresponding to cell ghosts, mitochondria, and plasma membranes. BLMs were further purified according to Hammond and Verroust (15) by using a Percoll gradient. Briefly, the collected material was diluted by adding 500 μl of imidazole (3 mM, pH 7.4) buffer containing protease inhibitors (final sucrose concentration 25%/26%, wt/wt), and spun at 20,000 g for 20 min. The yellow layer was resuspended again in the supernatant (carefully removed from the brown pellet containing mitochondria and cell ghosts) and centrifuged at 48,000 g for 30 min. The supernatant was discarded, and the pellet was resuspended in 1 ml of buffer (300 mM mannitol and 12 mM HEPES, pH 7.6, adjusted with Tris) by gentle pipetting. To form a Percoll gradient, 0.19 g of undiluted Percoll (Amersham Biosciences) was added to a 1-ml suspension (0.2–1 mg of protein). The suspension was gently mixed and centrifuged at 48,000 g for 30 min, and the ring of BLMs was collected.

Immunocytochemistry. ATII cells were isolated from septic and control rats and seeded on coverslips. Cells were fixed in 95% ethanol 18 h after isolation and then washed in PBS. Endogenous peroxidase reactive samples were quenched in 3% hydrogen peroxide in wash buffer solution; immunoperoxidase staining was performed by using a streptavidin-biotin system kit (Zymed Laboratories, San Francisco, CA) for specific antibodies: anti-Na+–K+–ATPase α1- or β1-subunit and anti-actin (Santa Cruz Biotechnology).

Computerized morphometry. Immunohistochemistry images were captured and digitized by using a computerized image analysis system consisting of a high-resolution digital camera (Retiga 200, QIMAGING, Burnaby, BC, Canada) attached to a microscope (Olympus BX51, Japan) and to a computer. Images were displayed at a resolution of 1,024 × 768 pixels (spatial resolution = 0.11 μ per pixel). The Image Pro Plus version 6.3 software (MediaCybernetics, Bethesda, MD) was used for image segmentation, threshold establishment, and signal intensity measurements. Manual corrections were performed as needed. For each sample, the entire section was digitally scanned at a magnification ×400. All areas on the sections were sampled for morphometrical analysis and final data expressed as the value of average pixel density (optical density).

Na-K-ATPase Pump Activity

Na-K-ATPase activity was determined by measuring the hydrolysis of ATP and detecting P release with the malachite-based PColorLock Gold Kit (Innova Biosciences).

To permeabilize the membranes, 1 mg/ml BLM were preincubated with the detergent C12E8 0.01 mg/ml, for 1 h on ice. Next the activity of Na-K-ATPase was measured in a reaction media containing 130 mM NaCl, 20 mM KCl, 3 mM MgCl2, 25 mM histidine, 1 mM EGTA, 0.5 mM ATP. The permeabilized membranes were incubated in the reaction media for 10 min at 37°C, comparing activities in the presence and absence of 5 mM ouabain, in media plus and minus Na+, and in the presence or absence of phosphates inhibitors (18).

Catecholamine Assay

Blood samples for epinephrine and norepinephrine were collected into EDTA tubes. All samples were centrifuged immediately for 10 min to separate the plasma and stored at −70°C. Plasma catecholamines were determined by reverse-phase HPLC with electrochemical detection, using as a mobile phase a mixture of monobasic sodium phosphate 0.1 M, EDTA (80 mg/ml), methanol (1%), and heptane sulfonic acid (200 mg/l).

Additionally, the lungs were lavaged with 5 × 1 ml of normal saline. Bronchoalveolar lavage (BAL) fluid (BALF) was pooled for measurement of cell count and protein content. Blood pressure was measured following anesthesia and blood sample was drawn for measurement of arterial blood gases (ABG), blood count, and catecholamine level.

Study Groups

The experimental groups were as follows, with the number of animals in each group is given in parentheses.

AFC. AFC and alveolar epithelial permeability were studied in control group (n = 8) and rats with sepsis for 24 h (n = 6) and for 48 h (n = 6).

The effects of the adrenergic system on AFC were investigated by perfusing 10−6 M isoproterenol in control rats (n = 4) and in rats with sepsis for 24 h (n = 4). In addition, the lungs of control (n = 4) and sepsis (n = 4) rats were instilled with 10−5 M propranolol. Both isoproterenol and propranolol were purchased from Sigma-Aldrich, St. Louis, MO.

To examine the role of the apical Na+ channels, we instilled the lungs with Na+ channel blocker, 10−6 Mamiloride (Sigma-Aldrich),
in both control rats and rats with sepsis for 24 h ($n = 6$ and $n = 4$, respectively).

**WD weight ratio.** The W/D lung weight ratio was studied in control rats ($n = 8$) and 24 h after the induction of sepsis ($n = 8$).

**Western Blot, Immunohistochemistry Analysis, and Na-K-ATPase Pump Activity**

Western blot, immunohistochemistry analysis, and Na-K-ATPase pump activity were examined in control rats ($n = 4$) and in rats with sepsis for 24 h ($n = 4$) for each set of experiments.

**Hemodynamic and Laboratory Measurements**

Blood pressure, blood cell count, ABG, and catecholamine levels were measured in control group and in rats with 24-h sepsis; $n = 4$ each group.

The protein content and white blood cell count in BALF were also measured in both control rats ($n = 4$) and rats with sepsis for 24 h ($n = 4$).

**Statistical Analysis**

Data are presented as means ± SE; $n$ is the number of animals in each study group. One-way analysis of variance was used, and when multiple comparisons were made it was followed by a multiple-comparison test (Tukey’s test) when the $F$ statistic indicated significance. Differences between groups were determined by paired Student’s $t$-test, by analysis of variance (Newman-Keuls test), as well as by the nonparametric rank test. Significance was accepted at a $P$ value of less than 0.05.

**RESULTS**

**Hemodynamic and Laboratory Measurements**

As shown in Table 1, the mortality rate was 20% in CLP rats, the mean arterial pressure was lower in sepsis rats compared with control group ($122 ± 10.7$ vs. $97.2 ± 5.7$ mmHg, respectively); it is noteworthy that septic rats were not in shock. Hemoglobin levels decreased acutely in septic rats; concordantly, serum lactate and BAL leukocytes increased significantly, indicating the validity of the CLP model for sepsis.

**Alterations in Plasma Catecholamines**

There was a 30-fold and 5-fold increase in plasma epinephrine and norepinephrine levels, respectively, at 24 h in CLP rats compared with shams (Table 1).

**Alveolar Fluid Clearance Was Decreased In Sepsis**

Twenty-four hours after the induction of sepsis AFC decreased in isolated perfused rat lungs by ~40% compared with control group ($0.30 ± 0.02$ vs. $0.51 ± 0.02$ ml/h, respectively, $P < 0.0001$). This inhibitory effect was consistent 48 h after the induction of sepsis ($0.33 ± 0.03$ ml/h, $P < 0.0001$ compared with sham-operated controls). Notably, the difference between AFC at 48 h compared with 24 h was not statistically significant (Fig. 1A).

**Effects of the Adrenergic System on Alveolar Fluid Clearance**

Isoproterenol perfused through the pulmonary circulation of septic lungs at concentrations of $10^{-6}$ M significantly increased AFC to control levels.

The instillation of the β-adrenergic-receptor antagonist, propranolol did not affect AFC in control rats ($0.47 ± 0.03$ ml/h); however, AFC was significantly decreased in sepsis rats treated with propranolol to $0.11 ± 0.01$ ml/h (Fig. 1B).

**Effect of Amiloride on Sepsis Rat Lungs**

As shown in Fig. 1C, instilling $10^{-6}$ M amiloride to the air spaces significantly decreased AFC in both control ($0.51$ ml/h ± 0.02 vs. $0.30 ± 0.02$) and sepsis ($0.31 ± 0.02$ vs. $0.19 ± 0.03$) rat lungs; $P < 0.05$. Amiloride decreased AFC by ~41% in both control and sepsis rat lungs.

**Effects of Sepsis on Extravascular Lung Water and Permeability**

As depicted in Fig. 2A, the W/D lung weight ratio was increased in the 24-h sepsis group compared with control rats, $3.65 ± 0.38$ and $2.27 ± 0.15$, respectively ($P = 0.017$), indicating that the extravascular lung water was increased. At the same time, the permeability to large solutes as measured by the fraction of FITC-albumin that appeared in the alveolar space during the experimental protocol was not different in the control and 24-h sepsis groups, indicating that the alveolar-capillary barrier was intact and its permeability did not increase (Fig. 2B).

**Expression of Na-K-ATPase and ENaC Proteins**

**Western blot analysis.** Qualitative and quantitative Western blot analysis of whole ATII cells from control and septic rats ($n = 4$ each group) did not reveal significant difference in Na-K-ATPase α1-subunit protein expression at whole ATII cells (Fig. 3A). However, Western blot analysis of Na-K-ATPase α1-subunit protein expression in BLMs from sepsis rat lungs demonstrated a significant decrease (~40%) compared with control BLMs, $P < 0.05$ (Fig. 3B). These findings suggest that sepsis decreased AFC by endocytosing the Na-K-ATPase.

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**Table 1. Catecholamines, hemodynamic and BAL characteristics of sham-operated rats and rats with 24-h sepsis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>Sepsis</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>122 ± 10.7</td>
<td>97.2 ± 5.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>0</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>300 ± 50</td>
<td>550 ± 64</td>
<td>0.04</td>
</tr>
<tr>
<td>Protein</td>
<td>33.1 ± 6.6</td>
<td>28.0 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>115.1 ± 11.4</td>
<td>725.6 ± 66.8</td>
<td>0.0005</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>71 ± 45</td>
<td>2,193.6 ± 515.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>16.1 ± 0.12</td>
<td>10.6 ± 0.53</td>
<td>0.0004</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.2 ± 0.35</td>
<td>10.7 ± 2.8</td>
<td>0.05</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.01</td>
<td>7.26 ± 0.55</td>
<td>0.079</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>27.79 ± 1.11</td>
<td>24.45 ± 1.76</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP: mean arterial pressure; WBC: white blood cells; NS: nonsignificant. The hemodynamic and laboratory characteristics of the study groups. The mortality rate was 20% in rats with sepsis, whereas the mean arterial pressure was lower in sepsis rats as compared to control group. Hemoglobin levels decreased acutely in septic rats; concordantly, serum lactate and leukocytes measured in the bronchoalveolar lavage, increased significantly. There was a 30-fold and 5-fold increase in plasma epinephrine and norepinephrine levels, respectively, in sepsis rats as compared to control.

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proteins. Notably, β1-subunit of Na-K-ATPase was not changed in sepsis rats compared with control group (Fig. 3C).

**Qualitative and quantitative Western blot analysis of whole ATII cells from control and septic rats (n = 3 each group) that was performed to test whether sepsis affects ENaC protein abundance at whole ATII cells did not reveal significant difference in ENaC protein expression (Fig. 4).**

**Immunohistochemistry analysis.** As depicted in Fig. 5, immunostaining of ATII cells with specific antibodies to Na-K-ATPase proteins at a magnification of ×400 and optical density of Na-K-ATPase did not show a significant difference between control and sepsis rats.

**Na-K-ATPase Pump Activity**

Twenty-four hours after the induction of sepsis, Na-K-ATPase pump activity was examined and was decreased compared with control group. However, the activity was low in both groups compared with the high background of other enzymes; therefore, we cannot determine with certainty the statistical significance.

**DISCUSSION**

Severe systemic and pulmonary endothelial injury that occur in sepsis may be associated with a marked increase in epithelial permeability to proteins with an inability to transport fluid from the air spaces of the lung (6, 7).

Our study was designed to determine whether AFC is changed in sepsis before the development of severe epithelial injury and progression to ARDS.

Rats with sepsis were hypotensive (but not in septic shock) with marked accumulation of lactate and acute decrease in hemoglobin levels. Sepsis signs were also demonstrated in the BALF as white blood cell count was significantly higher in septic rats. Notably, the mortality rate after 24 h of sepsis was 20% (Table 1).

In the present study, we provide, to our knowledge for the first time, evidence that AFC is decreased in rats with sepsis (but without ARDS) secondary to CLP. This effect was noted...
at 24 and 48 h after the induction of sepsis. Remarkably, the inhibition of active sodium transport and decreased AFC occurred despite increased endogenous release of catecholamines. Conceivably, the well-recognized stimulatory effect of endogenous catecholamines on transepithelial sodium transport and AFC could compensate only partially (26). Indeed, neutralization of this compensatory mechanism by treatment with β-antagonist resulted in further decrease of AFC without significant water influx.

Our results are consistent with findings of Ware and Matthay (43), who measured AFC in patients with ALI and found that in a subset of patients with sepsis AFC was impaired, probably because sepsis is associated with more severe and lasting injury to the alveolar capillary barrier than other causes of lung injury, such as aspiration of gastric content or primary pneumonia. In contrast to our findings, several experimental models of sepsis were not associated with impaired AFC. Pittet et al. (27) reported that, in a rat model of bacteremia and septic shock, AFC was increased. Later on, it was demonstrated that sheep with septic shock exhibited an intact functional alveolar barrier, even though there was an increase in lung endothelial permeability to protein and an increase in extravascular lung water (28). However, in these two studies, AFC was examined under different conditions of sepsis in regard to model, duration of illness, and severity. The preservation of AFC is attributed to several known mechanisms, such as the activation of the adrenergic system with subsequent increase of endogenous catecholamine’s levels (8, 32), direct stretch-sensitive

Fig. 3. A: Western blot analysis of Na-K ATPase α-subunit protein in whole type II alveolar epithelial (ATII) cells did not reveal significant difference in Na-K-ATPase α1-subunit expression in sepsis rats compared with control. Equal amounts of protein were loaded in each lane. B: Western blot analysis of Na-K-ATPase α1-subunit abundance at the basolateral membranes (BLMs) of distal lung tissue from sepsis rat lungs revealed a significant protein expression decrease compared with control rats. Top: quantitative Western blot analysis of Na-K-ATPase α1-subunit protein. Bottom: representative Western blot of Na-K-ATPase α1-subunit expression. Graph represents the fold of control means ± SE. β-Actin

Fig. 4. Western blot analysis of epithelial sodium channel (ENaC) protein in the ATII cells did not reveal significant difference in sepsis rats compared with control. Top: quantitative Western blot analysis of ENaC protein. Bottom: representative Western blot of ENaC expression. Graph represents the fold of control means ± SE. Cell, whole ATII cells.
mechanism in the alveolar wall for detecting volume overload, and proliferation of ATII cells (44).

Wiener-Kronish et al. (45) studied the effect of early sepsis on lung epithelium using Escherichia coli endotoxin, administered intravenously or in high doses into the air spaces of the lung and found that alveolar epithelial permeability to protein and AFC were normal. The resistance of the alveolar epithelial barrier to E. coli endotoxin injury is one important mechanism that prevents the development of alveolar edema in the presence of endotoxin-induced lung endothelial injury (45). Nevertheless, these studies used different models of lung injury and included administration of intravenous live bacteria, intravenous LPS, and intrapulmonary bacteria. Bacteremia alone (in the absence of sepsis syndrome) is associated with an extremely low risk of ARDS. Thus the relevance of bacteremia or endotoxemia models for human lung injury is unclear, whereas local administration of live bacteria into the lungs results in ARDS by causing pneumonia. In contrast, the CLP model in which peritonitis is followed by sepsis and lung injury is probably the single best animal model simulating lung injury due to sepsis (25).

As depicted in Fig. 2, the W/D weight ratio was significantly increased in rats with 24 h of sepsis, indicating that the extravascular lung water was increased; however, the movement of albumin across the alveolar-capillary barrier was not significantly different in rats with sepsis indicating that the alveolar-capillary barrier was intact. Consistent with this finding, the total protein content in BALF was not increased in septic rats. The preserved integrity of the alveolar-capillary barrier together with normal protein content in alveolar fluid confirms a septic state without ARDS and suggesting accumulation of a protein-poor extravascular lung water in early sepsis. The most reasonable explanation for impaired AFC is the association of sepsis with severe and lasting injury of the alveolar capillary barrier. Bachofen and Weibel’s (3) findings showing that sepsis-associated lung injury was characterized by necrosis and sloughing of the alveolar epithelial barrier support this explanation. However, our observation of normal epithelial permeability indicating that the alveolar-capillary barrier was not grossly damaged differs from the previously reported and supports the importance of alveolar epithelial active sodium transport in sepsis.

Keeping with this observation of decreased alveolar epithelial active sodium transport in sepsis in the presence normal epithelial permeability, we advanced into epithelial type II cell to study the process of active Na\(^+\)/H\(^+\) transport in sepsis and found two complementary findings. First, we found a significant decrease in Na-K-ATPase \(\alpha_1\)-subunit proteins in the basolateral membranes of sepsis rats compared with control. Second, the abundance of ENaC and Na-K-ATPase proteins at whole ATII cells was not different in the CLP group compared with control rats. Therefore, we suggest that sepsis remote from the lungs caused endocytosis of the Na-K pump proteins from the ATII cell plasma membrane into intracellular pools, with resultant inhibition of active sodium transport and AFC. Concordant with this observation, it has been reported that endocytosis of Na-K-ATPase proteins plays an important role in the modulation of AFC in ALI (39). In this regard, Dada and Sznajder (9) have shown that in acute hypoxia, reactive oxygen species (ROS), which are also important in the pathogenesis of sepsis, inhibits Na-K-ATPase function by promoting its endocytosis. Furthermore, Vadasz et al. (38) examined the role of thrombin on pulmonary fluid balance and found a dual role for thrombin. It provokes concomitant edema formation by increasing endothelial permeability, and inhibits alveolar edema resolution by promoting endocytosis of Na-K-ATPase proteins. Thus thrombin signaling via ROS and protein kinase C-\(\varepsilon\) promotes Na\(^+\)-K\(^+\)-ATPase endocytosis, resulting in loss of function. Lee et al. (20) reported that alveolar edema fluid from patients with ALI induced a significant reduction in sodium
and chloride transport genes and proteins in human ATII cells such as α-ENaC, α1-Na-K-ATPase, and cystic fibrosis transmembrane conductance regulator, effects that were associated with a decrease in net vectorial fluid transport across human alveolar type II cell monolayers.

Sepsis results from a generalized inflammatory and procoagulant host response to infection. It has been recognized that endothelial cells play a key role in the pathogenesis of sepsis by expressing or releasing a number of substances, such as nitric oxide (NO) (40). Tsuibo et al. (37) found that AFC was significantly decreased at 6 h after endotoxin instillation, at a time when lung NO and cGMP levels were highest.

Considering the close endothelial-epithelial interaction, it is conceivable to assume that ATII cells function is under strong influence of some of those substances. Therefore, we can speculate that ROS, NO, thrombin, and other substances found in high levels in sepsis inhibit active sodium transport and AFC through its effect on the Na-K-ATPase pump already in the early phase of sepsis.

Moreover, proinflammatory mediators such as leukotriene D4 and tumor necrosis factor-α, found in substantial levels in sepsis, have recently been demonstrated to modulate alveolar epithelial sodium and fluid transport (11, 14, 35).

Notably, the inhibitory effect of sepsis on AFC was restored by exogenous β-adrenergic therapy. Considering our finding of Na+-K+-ATPase endocytosis as the mechanism leading to AFC decrease, it is reasonable to assume that AFC increased with isoproterenol as a result of increase in sodium pump α-subunit quantity delivered to the basolateral cell membrane, one of the well-recognized catecholamine-dependent mechanisms for stimulation of active sodium transport (26). This suggests that in this stage of sepsis the alveolar epithelium is still responsive to β2-agonist stimulation, probably because of absence of extensive injury and preservation of alveolar type II cells. This observation is concordant with the stimulatory effect of catecholamines on other models of lung injury such as acute cardiogenic heart failure (1, 13), hyperoxia (32), and high tidal volume injury (33).

The role of active sodium transport in keeping the alveolar spaces free of fluids is well established (4). To examine the contribution of active sodium transport in the rat alveolar epithelium with sepsis; we studied the effect of the sodium channel blocker and found that amiloride significantly inhibited the AFC in both control and sepsis. Notably, the extent of inhibition by amiloride was similar in both groups. Our data are consistent with the effects of amiloride-sensitive sodium channels on other models of ALI (16, 19, 20, 31).

We recognize several limitations to our study. First, widespread vascular endothelial injury is thought to be the major mechanism for the increase in pulmonary water content in sepsis. The alveolar-capillary barrier permeability in our study was determined only by the movement of albumin representing large molecule, whereas the passive movement of small molecule was not measured therefore we could not generate an accurate index of alveolar barrier function. Second, our study does not reveal the substance responsible for the inhibition of active sodium transport and decreased AFC in sepsis. This requires further studies.

Third, the Na-K-ATPase may be regulated at different levels, including protein degradation rate, recruitment from intracellular pools to the plasma membrane, and recycling from the plasma membrane. Fourth, the activity was low in both groups compared with the high background of other enzymes; therefore, we cannot determine with certainty the statistical significance.

Consequently, we cannot determine with certainty whether the decrease in BLM Na-K-ATPase results from decreased delivery or increased endocytosis. In conclusion, AFC is decreased already in early stages of sepsis. Conceivably, this effect is due to endocytosis of the Na-K-ATPase proteins with resultant inhibition of active sodium transport and AFC, reflecting early dysfunction of the epithelial type II cell. The inhibitory effect of sepsis was restored following the administration of isoproterenol.

This information provides new insights that are of clinical relevance to our understanding of the role of active sodium transport in preventing and managing ARDS in patients with sepsis.

DISCLOSURES
No conflicts of interest, financial or otherwise are declared by the author(s).

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