Time course of lung ischemia-reperfusion-induced ICAM-1 expression and its role in ischemia-reperfusion lung injury

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1Division of Chest Medicine and Department of Medical Research, Mackay Memorial Hospital, Taipei Medical University, Taipei, Taiwan; and 2Division of Pulmonary and Critical Care Medicine, Long Island Jewish Medical Center, The Long Island Campus for the Albert Einstein College of Medicine, New Hyde Park, New York 11040

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Lu, Yen-Ta, Pai-Gene Chen, and Shu Fang Liu. Time course of lung ischemia-reperfusion-induced ICAM-1 expression and its role in ischemia-reperfusion lung injury. J Appl Physiol 93: 620–628, 2002; 10.1152/japplphysiol.01200.2001.—Upregulation of intercellular adhesion molecule-1 (ICAM-1) expression is an important mechanism underlying ischemia-reperfusion (I/R) induced neutrophil activation and tissue injury in other organs. However, I/R of the lungs has not been shown to upregulate ICAM-1 expression. We determined the time course profile of lung I/R-induced ICAM-1 expression and assessed the role of ICAM-1 in mediating neutrophil sequestration, transmigration, and I/R injury in the isolated blood-perfused rat lungs. I/R had a biphasic effect on ICAM-1 expression, an early downregulation and a late-phase upregulation. Superoxide dismutase and neutrophil depletion prevented the early ICAM-1 downregulation. The late-phase ICAM-1 upregulation coincided with the I/R-induced increase in pulmonary microvascular leakage index. ICAM-1 monoclonal antibody (MAb) reversed the I/R-induced increase in pulmonary microvascular leakage index, with control antibody being ineffective. Neither I/R nor ICAM-1 MAb affected lung MPO activity and circulating neutrophil count. Lung I/R significantly increased bronchoalveolar lavage fluid neutrophil count and the GSSG-to-(GSSG+GSH) ratio. ICAM-1 MAb blocked the I/R-induced increase in GSSG-to-(GSSG+GSH) ratio but had no effect on bronchoalveolar lavage fluid neutrophil count. Our results demonstrated that lung I/R up- and downregulates ICAM-1 expression depending on the duration of reperfusion. ICAM-1 upregulation is an important mechanism of I/R-induced pulmonary endothelial injury.

intercellular adhesion molecule-1; redox status; neutrophil; rat; microvascular permeability

The lungs suffer from ischemia-reperfusion (I/R) injury under a variety of clinical conditions, including cardiopulmonary bypass, lung transplantation, circulatory shock, thromboembolism, and thrombolysis. One common feature in the pathogenesis of various types of I/R lung injuries is neutrophil accumulation and emigration into the lungs. Thus neutrophil is generally believed to be the primary cellular mediator mediating the I/R lung injury (17, 33–35, 39). Two approaches have been used to define the role of neutrophil in I/R lung injury: first, neutrophil depletion using antineutrophil antibody, and second, blocking of neutrophil adhesion by using antibody against adhesion molecules.

Adhesion molecules on both endothelial cells and neutrophils are key factors that mediate the sequential events of neutrophil rolling, adherence, activation, and emigration in the tissue (3, 17, 39). The contribution of adhesion molecules to the pathogenesis of I/R lung injury has been examined by using monoclonal antibodies (MAbs) against specific adhesion molecules: CD11a, CD11b, CD18, intercellular adhesion molecule-1 (ICAM-1), E-selectin, P-selectin, or combination of multiple MAbs (6, 20, 31). These studies revealed that adhesion molecules, particularly CD11/CD18 and ICAM-1, play important roles in the I/R lung injury (6, 20, 31). However, neutrophil depletion studies have provided conflicting results (5, 9, 27, 28, 33–35). Some investigators reported that neutrophil depletion is protective against I/R lung injury (33–35), whereas others reported that neutrophils are not necessary for the induction of I/R lung injury (5, 27). It has also been reported that the neutrophil dependency of I/R lung injury depends on the duration of reperfusion (9, 28). Neutropenia had no effect on the I/R-induced increase in pulmonary microvascular permeability at 30 min of reperfusion but significantly reduced the I/R-induced increase in microvascular permeability at 3 or 4 h of reperfusion (9, 28). The reason for this discrepancy is unclear. However, because interaction between ICAM-1 and its counterreceptor, integrins, is believed to be the crucial step in neutrophil adhesion and activation (3, 17, 39), determining the kinetics of ICAM-1 expression in the lungs may shed some light at least on the reperfusion duration-dependent variation in the neutrophil dependency of I/R lung injury.

Although the role of neutrophil adhesion and activation in I/R lung injury has been well studied (6, 20, 31), little is known how lung I/R promotes neutrophil adhesion and activation. ICAM-1 is constitutively ex-
pressed at relatively high levels on the vascular endothelial cell of the lungs, but lung injury does not occur under physiological conditions. It is not known whether I/R promotes neutrophil adhesion and lung injury through mobilizing the constitutive ICAM-1, or through an upregulation of ICAM-1 expression, or through both. ICAM-1 is known to be significantly upregulated in the lungs under various inflammatory conditions (4, 38, 40). ICAM-1 is also upregulated during I/R injury in other organs (22, 23, 25). The inducible expression of ICAM-1 appears to be a principal determinant of neutrophil recruitment and tissue injury during inflammation (4, 22, 23, 25, 38, 40). However, it remains unknown whether I/R of the lungs upregulates ICAM-1 expression.

In the present study, we determined the time course profile of ICAM-1 mRNA and protein expressions in lungs subjected to various periods of I/R and assessed the role of ICAM-1 in mediating neutrophil sequestration, emigration, and I/R injury in the lungs. Because reactive oxidant species (ROS) play an important role in ICAM-1 expression and lung injury, the effects of the superoxide scavenger superoxide dismutase (SOD) and neutrophil depletion on the I/R-induced ICAM-1 expression were also studied. We showed that I/R had biphasic effects in ICAM-1 expression: an early downregulation and a late-phase upregulation. Pretreatment with SOD and anti-rat neutrophil antibody (to deplete circulatory neutrophil) prevented the early ICAM-1 downregulation. The late-phase ICAM-1 upregulation temporally coincided with the I/R-induced increase in pulmonary vascular leakage index.

METHODS

Isolated, blood-perfused rat lung preparation. The isolated, blood-perfused, and ventilated lung preparation was used throughout (27). Male Wistar rats (275–299 g) were anesthetized and ventilated with a Harvard small-animal ventilator using room air plus 5% CO2 at a rate of 50 breaths/min, tidal volume of 2.5 ml, and positive end-expiratory pressure of 2.0 cmH2O. Animals were exsanguinated, and the lung preparations were set up as previously described (27). A 30-min equilibrium was allowed before any protocol was carried out. Blood samples were taken anaerobically from the left atrial cannula for monitoring gas tensions and pH. Base excess was maintained between −2 and 2 mmol/l by adding small volumes of NaHCO3. Ischemia was created by switching off the perfusion pump, and reperfusion was established by switching it on. The lungs were ventilated throughout the experiment.

Experimental groups. We studied 18 groups of lungs: fresh lungs, Group I30R180, C0, C30, C90, C150, and C210 groups, isolated perfused lungs subjected to 30 min before they were subjected to I30R60 or I30R120. To determine the role of neutrophil, lungs from neutrophil-depleted animals were subjected to I30R60 or I30R120. Lungs were frozen in liquid nitrogen and used for Northern or Western blot or other biochemical assays. To evaluate the role of ICAM-1 in mediating I/R lung injury, lungs were pretreated with control antibody (MOPC21, mouse IgG1, 10 μg/ml, Sigma Chemical, St. Louis, MO) or mouse anti-ICAM-1 antibody (1A29, 10 μg/ml, R & D Systems, Minneapolis, MN) for 15 min before they were subjected to I30R120. Lungs in the C210 group were used as control. Pulmonary microvascular leakage index was calculated and compared among these groups. The effects of these antibodies on I/R-induced neutrophil sequestration and emigration were assessed in these groups of lungs by counting circulating neutrophil, by measuring lung MPO activity, and by counting bronchoalveolar lavage (BAL) fluid neutrophil.

Evaluation of lung injury. We used extravascular accumulation of 125I-labeled human serum albumin (125I-HSA) as an index of pulmonary microvascular leakage. This dual-tracer method has been demonstrated to be accurate in estimating tracer albumin leakage rate and permeability in the rat lungs (15) and has been widely used to assess microvascular permeability in experimental animals (27, 21, 24). 125I-HSA (500 nCi/lung) was added to the reservoir at 15 min of the initial 30-min equilibrium period. The intravascular blood and 125I-HSA retentions were corrected by adding 131I-HSA (250 nCi/lung) to the reservoir at the end of each experiment. 131I-HSA was prepared as previously described (21). Five minutes after 131I-HSA addition, lungs and plasma were collected. Whole lungs from each animal (divided into 8 pieces) and plasma samples (100 μl each, in triplicate) were counted by using a gamma counter. Extravascular albumin accumulation, expressed as microliters of plasma equivalent, was calculated by using the following formula

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\text{Extravascular albumin volume (μl)} = \frac{[125\text{I-HSA}_{\text{Lung}} \text{ (cpm)}]/[125\text{I-HSA}_{\text{Plasma}} \text{ (cpm/μl)}]}{[131\text{I-HSA}_{\text{Lung}} \text{ (cpm)}]/[131\text{I-HSA}_{\text{Plasma}} \text{ (cpm/μl)}]}
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Northern blot analysis. Rat ICAM-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probes were generated by using RT-PCR as previously described (26). Authenticities of PCR product were confirmed by dideoxy chain termination sequencing. RNA isolation and Northern hybridization were carried out as previously described (26). The ICAM-1 and GAPDH bands were quantified by using laser densitometry linked to a computer analysis system (PDI, Huntington Station, NY) and expressed as a ratio of ICAM-1 to GAPDH.

Western blot analysis. Lungs were homogenized in protein extracting buffer containing 25 mM Tris-HCl, 0.5 mM EDTA, 0.5 mM EGTA, 0.1 mg/ml phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 1 μM pepstatin. The homogenate was centrifuged at 17,500 g at 4°C for 15 min, and the resulting supernatant was collected as cytosolic fraction. Protein concentration was determined by using bicinchoninic acid assay kit with BSA as standard (Pierce, Rockford, IL).

Membrane fraction proteins (20 μg/lane) were loaded, separated on 7.5% SDS-PAGE under denaturing conditions, and transferred onto nitrocellulose membrane. After incubation in 5% dry milk in Tris-buffered saline, 0.05% Tween 20 at room temperature for 2 h, the membrane was incubated with antirat ICAM-1 antibody at room temperature for 1 h, fol-
lowed by washing and incubation in alkaline phosphatase-conjugated secondary antibody. The blot was developed with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt. The ICAM-1 bands were quantified by using a Master VDS scanner linked to a Master 1D Elite software program (Amersham Pharmacia, Piscataway, NJ) and expressed as arbitrary optical density units.

Myeloperoxidase assay. We used myeloperoxidase (MPO) activity as an indicator of lung neutrophil sequestration. At the end of each protocol, the lungs were removed en bloc, blotted dry, frozen in liquid nitrogen, and stored at −80°C until assayed. Lung was weighed, homogenized in 0.2% NaCl buffer (pH 4.7), and centrifuged at 260 g for 10 min. Supernatant was collected and centrifuged at 100,000 g for 1 h. The pellet was resuspended in hexadecyltrimethyl-ammonium bromide (0.5%), pH 5.4) and incubated with equal volumes of 3,3,5,5-tetramethylenimin (16 mM) and sodium acetate (0.02 M, pH 5.4) for 5 min. The reaction was initiated by adding H2O2, incubated for 5 min, and stopped by addition of catalase. MPO activity in the final mixture was assayed by measuring the change in spectrophotometric absorbance at 690 nm and expressed as optical density unit per gram of tissue.

Neutrophil depletion. Rats were injected intraperitoneally with 0.5 ml rabbit anti-rat neutrophil polyclonal antibody (Accurate Chemical and Scientific, Westbury, NY), or 0.5 ml rabbit serum as a control, 40–48 h before experimentation. Neutrophil depletion was confirmed by blood leukocyte counting.

BAL. BAL was performed at the end of the experiment by irrigation with 1.5–2.0 ml saline (total 15 ml) through a trachea intubation tube. BAL fluid was centrifuged at 1,300 rpm at 4°C for 20 min, and the supernatant was collected for measuring GSSG and GSH. The pellet was resuspended in 1 ml sulfobromophthalein (in 1.5% bovine albumin without Ca2+ and Mg2+), and total neutrophil number was counted.

Measurement of GSSG-to-(GSSG + GSH) ratio. GSH and GSSG were measured by using HPLC (Variant 9002, Varian Associated) with an electrochemical detector based on the method of Smith et al. (36). BAL fluid was deproteinized and concentrated in a vacuum dryer. The concentrated fluid was resuspended in 200 μl running buffer, and 20 μl of sample were injected onto a Hypersil ODS column (Jones chromatography, Mid Glamorgan, UK) with an applied electrode potential of 1.3 V. Peaks were quantified by standard curves for GSH and GSSG, and GSSG/GSSG + GSH) was calculated.

Statistics. All results are presented as means ± SE. Data were analyzed by unpaired t-test and one-way ANOVA followed by Newman-Keuls posttest. P values <0.05 were regarded as statistically significant.

RESULTS

Effect of lung I/R on ICAM-1 expression. The effects of lung I/R on ICAM-1 mRNA and protein expressions were evaluated by Northern and Western blot analyses using mRNAs and proteins from fresh lungs; lungs subjected to 0, 30, 90, 150, and 210 min of continuous perfusion; and lungs that underwent 30 min of ischemia followed by various periods of reperfusion. Northern blot using rat ICAM-1 probe and Western blot using ICAM-1 MAbs showed that there was a relatively high level of constitutive ICAM-1 expression in the control lungs as expected (Figs. 1 and 2). Membrane ICAM-1 protein level was reduced with time of perfusion in lungs that underwent continuous perfusion (Fig. 1, C90, C150, and C210 compared with C0 and C30) but was significantly increased in lungs subjected to 30 min of ischemia without reperfusion (Fig. 1, I30R0 compared with C0 and C30). Thirty minutes of ischemia followed by 60 min of reperfusion significantly downregulated ICAM-1 protein level (Fig. 1, I30R60 compared with C90). However, ICAM-1 protein expression significantly increased in lungs subjected to 30 min of ischemia followed by 120 and 180 min of reperfusion (Fig. 1, I30R120 and I30R180 compared with C150 and C210). Thus, although ischemia alone upregulated ICAM-1 protein expression, ischemia followed by reperfusion had a biphasic effect on the ICAM-1 protein level, an initial downregulation followed by a late-phase upregulation. Northern blot showed that neither ischemia alone nor ischemia followed by a short period of reperfusion (<60 min) had an effect on ICAM-1 mRNA expression (Fig. 2; C0, I30R0, I30R60, and I30R90). Ischemia followed by 90, 120, and 180 min of reperfusion showed a trend of upregulation of ICAM-1 mRNA (Fig. 2, I30R90, I30R120, and I30R180).

Roles of ROS and neutrophil in I/R-induced ICAM-1 expression. To determine the contribution of a humoral component (superoxide) and a cellular component (neutrophil) in the I/R-induced down- and upregulation of ICAM-1 protein, isolated lung preparations were prepared from rats being depleted of neutrophil or were pretreated with SOD before they were subjected to I30R60 or I30R180. Lungs underwent C90 and C210 as controls. ICAM-1 protein level was determined by
Western blot analysis. Compared with controls (C90 group), I/R (I30R60) significantly reduced the ICAM-1 protein level in the lung membrane extract (Fig. 3, IR group I30R60). Pretreatment with SOD and neutrophil depletion prevented the I/R-induced reduction in ICAM-1 protein level (Fig. 3, I30R60 in IR+SOD and neutrophil depletion groups). Compared with the C210 group, I30R180 significantly upregulated ICAM-1 protein level (Fig. 3, IR group I30R180), which was not prevented by SOD or neutrophil depletion (Fig. 3, I30R180 in IR+SOD and neutrophil depletion groups).

Role of ICAM-1 in I/R lung injury. To ascertain whether the I/R-induced ICAM-1 upregulation contributes to the I/R lung injury, we evaluated the effect of ICAM-1 MAb on I/R-induced increase in pulmonary microvascular leakage index as assessed by extravascular accumulation of 125I-HSA. Isolated lungs were pretreated with either ICAM-1 MAb (1A29 group) or a control antibody (MOPC21 group) before they were subjected to 30 min of ischemia followed by 180 min of reperfusion. A control group of lungs underwent 210 min of continuous perfusion. As illustrated in Fig. 4, I/R caused an approximately twofold increase in pulmonary microvascular leakage index in lungs treated with the control antibody (MOPC-21 group) over control group (C210). Pretreatment with ICAM-1 MAb reversed the I/R-induced increase of the leakage index (Fig. 4).

Role of ICAM-1 in I/R-induced neutrophil sequestration and transmigration in the lungs. We assessed neutrophil influx into the lungs by counting circulating neutrophils in the perfusate and by measuring lung MPO activity, which is widely used as an indicator of tissue neutrophil accumulation. There were initially 0.3–0.4 million neutrophils/ml in the perfusate. Circu-
Circulating neutrophils in the perfusate reduced by 95% after 1 h of perfusion and remained at a similar low level after 2, 3, and 4 h of perfusion, regardless of the treatment groups (Fig. 5). Circulating neutrophil counts were identical among C210, MOPC-21, and 1A29 groups at all time points. Consistent with the loss of circulating neutrophils, there was a significant increase in MPO activity in all three groups of lungs (Fig. 6). Compared with fresh lungs, there was an approximately 1.5-fold increase in MPO activity in all groups of perfused lungs (Fig. 6). The MPO activity was similar in C210, MOPC-21, and 1A29 groups of lungs (Fig. 6), indicating that I/R alone, I/R plus ICAM-1 MAb, or I/R plus control antibody had no effect on the low-flow perfusion-induced neutrophil sequestration in the lungs. We assessed neutrophil transmigration into bronchoalveolar air space by counting the retrievable neutrophils in BAL fluids. Total neutrophil count in BAL fluids was compared among lungs subjected to continuous perfusion (C210 group), lungs subjected to I30R180 and pretreated with control antibody (MOPC-21 group), and lungs subjected to I30R180 and were pretreated with ICAM-1 MAb (1A29 group). I/R caused a ninefold increase in BAL fluid neutrophil count (Fig. 7, MOPC-21 group), which was not significantly

**Fig. 4.** ICAM-1 monoclonal antibody (MAb) reversed I/R-induced increase in pulmonary microvascular leakage. Lungs were pretreated with control antibody (MOPC21 group, 10 μg/ml) or ICAM-1 MAb (1A29 group, 10 μg/ml) for 15 min before being subjected to I30R180. Lungs that underwent 210 min of continuous perfusion (C210 group) were used as control. Means ± SE are shown of 4–5 animals in each group. *P < 0.001 compared with C210 and 1A29 groups.

**Fig. 5.** Circulating neutrophil counts in lung perfusate. Circulating neutrophil in the lung perfusate was counted before (time 0) and during the experiments at 1-h intervals. Circulating neutrophils decreased immediately after initiation of perfusion and remained at very low levels throughout. Neither ICAM-1 MAb (1A29) nor control antibody (MOPC-21) affected the circulating neutrophil counts. Means ± SE are shown of 3 animals in each group.

**Fig. 6.** Lung myeloperoxidase (MPO) activity. MPO activity was measured at the end of 210 min of continuous perfusion (C210 group) or 30 min of ischemia followed by 180 min of reperfusion (MOPC-21 and 1A29 groups). Fresh lungs were collected immediately after animal was exsanguinated. Both continuous perfusion (C210 group) alone and I30R180 (MOPC-21 group) increased lung MPO activity significantly compared with fresh lungs. ICAM-1 MAb (1A29) had no effect on the increased lung MPO activity. Mean ± SE are shown of 3 lungs in each group. *P < 0.01 compared with Fresh group. OD, optical density; 690, 690 nm.

**Fig. 7.** ICAM-1 MAb had no effect on bronchoalveolar lavage (BAL) fluid neutrophil count. Total neutrophil count in BAL fluids was compared among lungs that underwent 210 min of continuous perfusion (C210 group), lungs pretreated with control antibody and subjected to I30R180 (MOPC-21 group), and lungs pretreated with ICAM-1 MAb and subjected to I30R180 (1A29 group). I30R180 (MOPC-21 group) markedly increased BAL fluid total neutrophil counts, which were not reduced by ICAM-1 MAb treatment (1A29 group). Mean ± SE are shown of 3 animals. *P < 0.05 compared with C210 group.
reduced by treatment with ICAM-1 MAb (Fig. 7, 1A29 group).

**Role of ICAM-1 in I/R-induced lung tissue oxidant stress.** To analyze the effect of ICAM-1 blockade on I/R-induced lung tissue oxidant stress, we measured the GSH and GSSG content in BAL fluids from three groups of lungs. Lungs in the C210 group were subjected to 210 min of continuous perfusion, lungs in the MOPC-21 group were pretreated with control antibody and subjected to I30R180, and lungs in the 1A29 group were pretreated with ICAM-1 MAb and underwent I30R180. We used the GSSG/GSSG+GSH as an index of tissue redox state. Compared with the C210 group (Fig. 8, C210), BAL fluids from I/R lungs showed a 4.7-fold increase in GSSG/GSSG+GSH (Fig. 8, MOPC-21). Treatment with ICAM-1 antibody significantly reduced BAL fluid GSSG/GSSG+GSH (Fig. 8, 1A29), indicating that blocking ICAM-1 function with ICAM-1 MAb reduced I/R-induced tissue oxidant stress.

**DISCUSSION**

Although studies using antibodies against adhesion molecules have established a role of ICAM-1 in mediating I/R-induced neutrophil adhesion and lung injury (6, 20, 31), how I/R promotes neutrophil adhesion and I/R lung injury is unclear. ICAM-1 expression is significantly upregulated in lungs under inflammatory conditions (4, 38, 40). ICAM-1 is also upregulated during I/R injury in most other organs studied (22, 23, 25). The inducible expression of ICAM-1 appears to be a major factor contributing to the increased neutrophil activation and increased endothelial permeability (4, 22, 23, 25, 38, 40). However, I/R of the lungs has not been demonstrated to upregulate ICAM-1 expression. In this report, we determined the time course profile of I/R-induced ICAM-1 expression in the lungs. We showed that ischemia alone upregulated ICAM-1 protein and that I/R had a biphasic effect on ICAM-1 protein expression, an initial decrease at I30R60, and a late-phase increase at I30R180. SOD and neutrophil depletion prevented the early downregulation of ICAM-1, suggesting that superoxide generation and neutrophil activation play roles in this ICAM-1 downregulation. The upregulation of ICAM-1 protein level by I30R180 temporally coincided with the increased microvascular leakage index and tissue GSSG/GSSG+GSH in the lungs. Additionally, ICAM-1 MAb reversed the I/R-induced increase in microvascular leakage and BAL fluid GSSG/GSSG+GSH in the lungs. Our results confirm but extend previous studies (6, 20, 31) by showing that lung I/R upregulates ICAM-1 expression that contributes to the I/R injury and tissue oxidant stress in the lungs. We do not have direct evidence supporting that the upregulated ICAM-1 is mainly responsible for the I/R lung injury. ICAM-1 MAb blocks the biological action of both the constitutive and the inducible form of ICAM-1. However, as judged from our Western blot data (compare C210 with I30R180 in Fig. 1B), the constitutive ICAM-1 constitutes <40% of the total membrane-bound ICAM-1 protein detected at the time point of I30R180. This would suggest that the inhibitory effects of ICAM-1 MAb on the I30R180-induced increase in lung microvascular leakage and tissue oxidant stress were at least partially, if not mainly, mediated through blocking the biological action of the inducible form of ICAM-1. Thus upregulation of ICAM-1 expression is an important mechanism of lung I/R-induced increase in microvascular leakage and tissue oxidant stress, although the constitutive ICAM-1 may also play an important role.

We showed for the first time that I/R up- or downregulated ICAM-1 depending on the duration of reperfusion. ICAM-1 was downregulated after 60 min of reperfusion but was upregulated after 180 min of reperfusion. Because neutrophil endothelial adhesion mediated by the interaction between endothelial ICAM-1 and neutrophil β2-integrins is a critical initial step in neutrophil activation and tissue injury, our data provide a molecular basis for the early reported observation that neutrophil mediates the late-phase but not the early-phase I/R lung injury (9, 28).

We showed that I30R60 decreased ICAM-1 protein but not ICAM-1 mRNA, indicating that the ICAM-1 reduction after I30R60 is not a result of reduced new ICAM-1 synthesis but rather a loss of the existing ICAM-1 protein on endothelial cell surface. This could be the result of an increased ICAM-1 turnover (degradation) or an increased ICAM-1 release from the endothelial cell surface (shedding) or both. I/R has been shown to cause the shedding of several endothelium-specific markers, including angiotensin-converting enzyme (1) and factor VIII (16). Clarification of which mechanism (shedding or degradation) mediates the I/R-induced ICAM-1 downregulation warrants further investigation. The prevention of I/R-induced ICAM-1 downregulation by SOD and neutrophil depletion sug-
gests that superoxide derived from activated neutrophil (and other leukocytes) plays a role in this down-regulation. ROS have been shown to induce ICAM-1 expression in cultured endothelial cells (32). Our study is the first to report that ROS is also involved in the ICAM-1 downregulation. Other factors may also contribute to the reduced ICAM-1 protein after short periods of I/R. It has been shown that inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α cause ICAM-1 shedding (10). It is possible that lung I/R stimulates tumor necrosis factor-α production, which in turn causes endothelial damage leading to ICAM-1 shedding.

Unlike in other organs, the major site for neutrophil emigration in the lungs is the pulmonary capillary (7, 14, 19). The diameters of most capillary segments are similar to or even smaller than neutrophils (7). Neutrophils must deform to pass through the capillary bed (7, 14, 19). This geometric constraint can become an important factor controlling neutrophil retention in the lungs without utilizing the ICAM-1-dependent mechanism, particularly under low blood flow conditions such as seen in the isolated blood-perfused lung preparation used in this study. This could explain why we saw a large number of neutrophils sequestered in the control lungs subjected to continuous perfusion without I/R. This also explains why lungs in the control (C210), I30R180 (MOPC-21), and I30R180 plus anti-ICAM-1 antibody (1A29) groups showed a similar MPO activity. The inability of ICAM-1 MAb to reduce lung MPO activity should not be taken as evidence against the involvement of ICAM-1 in the I/R-induced lung injury. First, ICAM-1 MAb completely reversed the I/R-induced increase in microvascular leakage; second, this MPO activity cannot distinguish the I/R-activated neutrophil from inactivated neutrophils that have been retained in the lungs by ICAM-1-independent mechanisms as discussed above, we could not see a reduction in MPO activity in ICAM-1 MAb-treated lungs (1A29 group). This explanation is consistent with our observation that lungs in control (C210), I30R180 (MOPC-21), and I30R180 plus ICAM-1 MAb (1A29) groups showed a similar MPO activity.

The inability of ICAM-1 MAb to inhibit the I/R-induced increase in BAL fluid neutrophil count suggests that ICAM-1 is not important for I/R-induced neutrophil emigration into bronchoalveolar space. Consistent with our result, anti-CD18 antibody has been reported to fail to block neutrophil emigration into the alveoli or parenchyma of the lungs in response to I/R, to intrabronchial S. pneumoniae, or to C5a complement fragment (8, 18, 37). Moreover, mice with ICAM-1 or CD18 null mutation display normal neutrophil emigration into the lungs in response to various inflammatory stimuli (2, 30). These results indicate that neutrophil emigration through the pulmonary microvasculature can occur through ICAM-1- and CD18-independent pathways.

The contrast effect of ICAM-1 MAb on pulmonary microvascular leakage and BAL fluid neutrophil count indicates dissociation between neutrophil migration and increase in pulmonary microvascular leakage. This dissociation is not necessarily evidence against the role of neutrophil endothelial interaction in I/R-induced endothelial injury. It has been demonstrated on cultured endothelial cells that increased endothelial cell permeability induced by activated polymorphonuclear neutrophil is linked to initial CD11/CD18-dependent adhesion and is independent of subsequent neutrophil transendothelial migration (12, 13). Thus it is possible that the initial neutrophil adhesion to endothelial cells and increase in pulmonary vascular permeability are mediated by ICAM-1 and CD11/CD18 interaction, whereas the later neutrophil emigration is largely ICAM-1 independent. An alternative explanation is that ICAM-1 functions as a signaling molecule that mediates or initiates a signal cascade, leading to endothelial injury and tissue oxidant stress in response to I/R, but the same signaling pathway is not crucial for the I/R-induced neutrophil emigration into bronchoalveolar space.

We showed that I30R0 dramatically increased membrane ICAM-1 protein level, indicating that ischemia alone is sufficient to upregulate ICAM-1 expression. These data provide further evidence for the early reports that ischemia alone is sufficient to cause neutrophil activation and to induce pulmonary microvascular endothelial injury in this normoxic I/R lung injury model (1, 11, 29). We do not have a good explanation for the reduced membrane ICAM-1 level in lungs continuously perfused for 90, 150, and 120 min but suggest that it may be related to the transit I/R during the procedure of setting up the preparation.

In summary, we showed that lung ischemia increased membrane ICAM-1 protein level and that I/R of the lungs caused a biphasic change in ICAM-1 expression with an initial decrease at I30R60 and a late-phase increase at I30R180. The initial decrease in ICAM-1 protein was prevented by pretreatment with superoxide scavenger SOD and neutrophil depletion, suggesting that superoxide derived from neutrophils (and other leukocytes) plays a role in the early ICAM-1 downregulation. The late-phase ICAM-1 upregulation temporally coincided with the I/R-induced increase in pulmonary microvascular leakage. Additionally, ICAM-1 MAb prevented I/R-induced increase in pulmonary microvascular leakage index, suggesting that upregulation of ICAM-1 expression is an important factor contributing
to I/R endothelial injury in the lungs. The biphasic change in ICAM-1 expression explains the reperfusion duration-dependent variation in the neutrophil dependence of I/R lung injury.

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