Provocation of Pulmonary Vascular Endothelial Injury in Rabbits by Human Recombinant Interleukin-1 β

SIMEON E. GOLDBLUM,^{1*} KOKICHI YONEDA,² DONALD A. COHEN,³ AND CRAIG J. MCCLAIN⁴

Department of Medicine, Veterans Administration Medical Center, University of Maryland School of Medicine, Baltimore, Maryland 21201,¹ and Departments of Medicine⁴ and Pathology,² Veterans Administration Medical Center, and Department of Microbiology and Immunology,³ College of Medicine, University of Kentucky, Lexington, Kentucky 40536

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Interleukin-1 (IL-1) mediates components of the acute-phase response, stimulates granulocyte metabolism, and induces endothelial cell surface changes. We studied the effects of human recombinant IL-1 β (rIL-1 β) or rIL-1 α on circulating granulocytes, their sequestration within the pulmonary microvasculature, pulmonary edema formation, and changes in pulmonary vascular permeability to ¹²⁵I-labeled albumin. rIL-1 β administration induced significant ($P < 0.03$) but transient granulocytopenia followed by significant ($P < 0.04$) neutrophilia and significant ($P < 0.04$) pulmonary leukostasis compared with saline-infused rabbits. Rabbits preinfused with ¹²⁵I-labeled rabbit serum albumin and administered saline, rIL-1 β , or rIL-1 α were sacrificed, and lung wet/dry weight ratios and bronchoalveolar lavage fluid and plasma ¹²⁵I activities determined. Both rIL-1 β and rIL-1 α increased lung wet/dry weight ratios ($P < 0.025$ and $P < 0.01$, respectively) compared with saline controls. rIL-1 β increased bronchoalveolar lavage fluid/plasma ¹²⁵I radioactivity ratios ($P < 0.025$). Electron microscopic analysis of lung sections obtained from rIL-1 β -infused animals demonstrated endothelial injury, perivascular edema, and extravasation of an ultrastructural permeability tracer. The observation that human rIL-1 can evoke acute pulmonary vascular endothelial injury and lung edema in rabbits supports the hypothesis that IL-1 may play a role in the pathogenesis of the adult respiratory distress syndrome.

The pathogenesis of acute lung injury and the adult respiratory distress syndrome (ARDS) remains unclear (35). Elevation of circulating interleukin-1 (IL-1) bioactivity has been demonstrated in some of the very same clinical settings associated with acute lung injury, including septicemia and endotoxemia as well as head trauma (26, 45, 46). In our laboratory we have shown serially increasing IL-1 bioactivity in bronchoalveolar lavage fluid (BALF) from rats using an established model of monocrotaline-induced lung injury (11). Recently, increased immunoreactive IL-1 has been recovered in the BALF of ARDS patients (T. M. Siler, J. E. Swierkosz, and R. O. Webster, *Am. Rev. Respir. Dis.* 135: A258, 1987). Therefore, IL-1 is present within the relevant body compartment during the host response to acute lung injury and ARDS. IL-1 produces biological effects on two likely target tissues in acute lung injury: circulating granulocytes (16, 20, 39) and the endothelium (1, 3, 4). This cytokine reportedly induces neutrophil degranulation (20) and stimulates neutrophil oxygen-dependent metabolism (39). In a recent report, recombinant IL-1 (rIL-1) was shown to have no effect on neutrophil intracellular calcium or function (10). IL-1 also can act directly on the endothelium increasing its adhesiveness for granulocytes (3, 5, 34) and its synthesis of both platelet-activating factor (PAF) (4) and the vasodilatory arachidonate metabolite, prostaglandin E₂ (1). These biological properties make IL-1 a candidate mediator of granulocyte-dependent acute lung injury. As a first test of the direct involvement of IL-1 in acute lung injury, we showed that a single-bolus infusion of a semipurified 10,000- to 30,000-molecular-weight murine P388D₁ macrophage-derived fraction containing IL-1 activity induces early profound but transient granulocytopenia and sustained pulmonary leukostasis in rabbits (12). This monokine preparation did not

contain detectable tumor necrosis factor (TNF) or C5 bioactivity and contained only subthreshold quantities of endotoxin. Divided-dose infusions of this same monokine preparation provoked increased pulmonary edema formation and increased pulmonary extravasation of radiolabeled albumin (13). Electron microscopic studies of lung sections obtained from monokine-challenged animals demonstrated endothelial cell injury, perivascular edema, and extravasation of an ultrastructural permeability tracer. In addition to IL-1, our endotoxin-induced monokine preparation contained multiple factors. To establish whether IL-1 itself contributes to the pathogenesis of acute lung injury, we studied the *in vivo* effects of highly purified recombinant human IL-1 on circulating granulocyte counts, granulocyte sequestration within the pulmonary microvasculature, lung edema formation, and pulmonary vascular permeability.

MATERIALS AND METHODS

Human rIL-1 preparations. Human rIL-1 α was generously provided by Peter T. Lomedico (Hoffman-La Roche Inc., Roche Research Center, Nutley, N.J.). This protein was purified from *Escherichia coli* transfected with the gene for IL-1 α and consists of the carboxy-terminal 154 amino acids of the 271-amino-acid human IL-1 α precursor (24). The rIL-1 α was prepared in 30 mM Tris hydrochloride-400 M NaCl buffer and had a specific activity of 5×10^6 U/mg of protein. The preparation contained 50 pg of endotoxin by the *Limulus* amoebocyte lysate assay (M. A. Bioproducts, Walkersville, Md.) per 10^5 U of IL-1 activity.

Human rIL-1 β (2) was generously provided by Cistron Technology (Pine Brook, N.J.). The rIL-1 β preparation had a specific activity of 10^6 U/mg of protein and contained 1.4 ng of endotoxin per 10^5 U of IL-1.

IL-1 assay. IL-1 bioactivity was determined by the standard comitogenic murine thymocyte proliferation assay as

* Corresponding author.

previously described (12, 13). The rIL-1 preparations were standardized for infusion on the basis of IL-1 bioactivity. IL-1 activity was defined as a ratio of the dilution of each preparation required to induce 50% of the maximal thymocyte proliferation relative to the dilution required for 50% maximal proliferation of a laboratory standard of murine IL-1 prepared from the P388D₁ cell line (arbitrarily defined as 100 U/ml) (12, 13).

Rabbit model. New Zealand White rabbits (4 kg each) were restrained, and 2.5-cm, 22-gauge Teflon catheters (Quick-Cath; Travenol Laboratories, Inc., Deerfield, Ill.) were inserted into a central ear artery and a marginal ear vein. Catheter patency was maintained by saline flushing. Rabbits were injected intravenously with either single-bolus infusions of 25, 100, 200, or 500 U of rIL-1 β or with 500 U of rIL-1 (100 ng of rIL-1 α or 500 ng of rIL-1 β per rabbit) in five equivalent 100-U bolus infusions. Control animals were infused with equivalent volumes of sterile, nonpyrogenic normal saline (Travenol Laboratories, Deerfield, Ill.). The divided-dose infusions were administered at 0, 30, 60, 90, and 120 min. Whole blood was drawn into EDTA-treated tubes immediately before and after single-bolus infusions. Total leukocyte counts were determined with a model F Coulter counter (Coulter Electronics Inc., Hialeah, Fla.), and absolute granulocyte counts were determined by using different counts of Wright-stained peripheral smears. Blood granulocytes were expressed as the percentage of the preinjection baseline value. At 5 h animals were sacrificed with 2 g sodium pentobarbital (Butler Co., Columbus, Ohio) by the intravenous route, and their lungs were evaluated for pulmonary leukostasis, edema formation, and changes in pulmonary vascular permeability. To control for endotoxin contamination in studies of lung water and changes in pulmonary vascular permeability, an additional six animals were each similarly administered five equivalent 2.0-ng bolus infusions of *E. coli* O111:B4 lipopolysaccharide (Difco Laboratories, Detroit, Mich.) for a total of 10.0 ng per animal.

Evaluation for pulmonary leukostasis. (i) Histological studies. Lungs were histologically evaluated as previously described (12–14). Briefly, lungs were deflated by atmospheric pressure, and the right lung was fixed in situ with 10% formaldehyde at constant hydrostatic perfusion pressure (20 cm of H₂O) for 30 min. The lung was then dissected from the thoracic cavity and immersed in the same fixative for an additional 24 h. Multiple 5.0- $\pm$ 1.0- μ m lung sections were obtained, mounted on slides, and stained for evaluation in blind fashion. By using a microscope grid, granulocyte sequestration was quantified by counting alveolar septal wall granulocytes per high-power field in 25 randomly chosen fields, using no more than 5 fields per tissue section. Microscopic fields containing bronchial structures, larger vessels, or pleura were not counted.

(ii) Extraction of lung tissue for MPO activity. Lung myeloperoxidase (MPO) activity was extracted as previously described (12–14). The left lung from each animal was washed with sterile, nonpyrogenic normal saline, blotted dry of surface blood, weighed, and homogenized in 0.5% hexadecyltrimethylammonium bromide (HTAB) in 50 mM phosphate buffer, pH 6.0 (5.0 ml of HTAB per g of tissue), on ice using the Virtis model 45 homogenizer (The Virtis Co., Inc., Gardner, N.Y.) at a setting of 40 for 10 s. The homogenate was centrifuged at 40,000 $\times$ g for 10 min at 4°C, the supernatant was decanted, and the pellet was suspended in HTAB. The pellet-HTAB suspension was freeze-thawed (20 min at –70°C), homogenized with a Tekmar Tissuemizer at a setting of 50 for 60 s, and sonified with a Branson W185 cell

disruptor (Heat Systems Ultrasonics, Inc., Plainview, N.Y.) at 65 W for 3 s intervals with intervening rests for a total of 1.8 min of sonication. The lung suspension was centrifuged at 40,000 $\times$ g for 5 min at 4°C, and the supernatant was collected. The pellet was suspended in HTAB. This whole extraction procedure, which was performed on ice, was repeated two times for a total of three extractions per lung.

(iii) MPO assay. MPO in supernatants was assayed using the standard spectrophotometric technique with *O*-dianisidine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.) and 0.0005% hydrogen peroxide (Mallinckrodt, Paris, Ky.) as the substrate (14). The absorbance change at 460 nm was measured over 2 min in the thermoregulated flow cell (25°C) of a Spectronic 2000 Spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.).

Gravimetric assessment of pulmonary edema formation. The ratio of blood-inclusive wet weight to blood-inclusive dry weight was used as a gravimetric index of pulmonary edema as previously described (12, 13). Rabbits were infused via the marginal ear vein either with the rIL-1 preparation or with equivalent volumes of the saline vehicle. At 5 h, animals were killed with an overdose of intravenous sodium pentobarbital, their lungs were immediately excised, and the lung wet weight was determined to the nearest 1.0 mg. The specimen was allowed to dry at room temperature until a stable dry weight was obtained. Pulmonary edema formation was expressed as the lung wet/dry weight ratio.

Assessment of changes in pulmonary vascular permeability. Animals were assessed for alterations in pulmonary vascular permeability to radiolabeled albumin by the method of Henson and co-workers (21), with minor modifications as previously described (13). Rabbit serum albumin (Sigma) was radiolabeled with Na¹²⁵I (New England Nuclear Corp., Boston, Mass.) and chloramine T Iodobeads (Pierce Chemical Co., St. Louis, Mo.). The ¹²⁵I-radiolabeled rabbit serum albumin was passed through a Sephadex G25 column, the exclusion volume was collected, and ¹²⁵I specific activity was determined. Pairs of rabbits were simultaneously infused with ¹²⁵I-labeled rabbit serum albumin (3.0 μ Ci per rabbit). Thirty minutes later, one rabbit was administered rIL-1, and the other animal received equivalent volumes of saline. At 5 h, blood was obtained, the animals were sacrificed, and ¹²⁵I activities per milliliter of plasma were determined. The lungs were lavaged with 20 ml of saline, the BALF was centrifuged (1,000 $\times$ g, 15 min), and ¹²⁵I activities in the supernatants were measured with a gamma counter (Packard Instrument Co., Inc., Downer's Grove, Ill.). Changes in permeability to ¹²⁵I-labeled albumin across the alveolar-capillary barrier into the intra-alveolar compartment were each separately expressed as the ratio of counts per minute per milliliter of BALF to that per milliliter of plasma. In all cases the recovery of BALF was $\geq$ 65%, and there were no differences between control and experimental groups.

Electron microscopy. In three rIL-1 β -infused and three saline-injected animals, 10 ml of 15% (wt/vol) oyster glycogen (Sigma) in sterile nonpyrogenic normal saline was administered intravenously 10 min before sacrifice. The oyster glycogen had no detectable endotoxin by the *Limulus* amoebocyte lysate assay. The lungs were immediately excised, and a catheter was advanced into a main stem bronchus. A fixative solution (1% formaldehyde and 2.5% glutaraldehyde in 0.15 M phosphate buffer [pH 7.3]) was infused through the catheter at 20 cm of H₂O pressure to full lung expansion. The bronchus was ligated, and the expanded lung was submerged into the same fixative for 2 h at 4°C. The tissue was then

FIG. 1. Changes in circulating granulocytes. Mean percent changes from the preinfusion baseline of blood granulocytes in animals after single-bolus infusions of either saline (four animals) or 25 U (seven animals), 100 U (five animals), 200 U (three animals), or 500 U (five animals) of rIL-1 β . Each symbol represents the mean ($\pm$ SEM) percent change of blood granulocytes.

diced into small pieces, postfixed in 1% osmium tetroxide in 0.15 M phosphate buffer for 1 h, washed in the same buffer, and then dehydrated through graded ethanols and embedded in epoxy resin. Thin sections cut with a diamond knife were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

Statistical methods. Parameters that were repeatedly measured over time (circulating granulocytes) were analyzed by using an analysis of variance procedure for a split-plot design in which animal groups represented the whole plot factor, and time represented the split-plot or repeated-measures factor. For parameters that were measured once, i.e., after sacrifice (granulocytes per high-power field, lung MPO, lung wet/dry weight ratios, BALF/plasma 125 I activity ratios), an analysis of variance was used to compare the mean responses between groups.

RESULTS

Effects of human rIL-1 on circulating granulocytes. Rabbits were administered 25 U ($n = 7$), 100 U ($n = 5$), 200 U ($n = 3$), or 500 U ($n = 5$) of rIL-1 β in single-bolus infusions and compared with four rabbits similarly infused with equivalent volumes of saline (Fig. 1). The mean ($\pm$ standard error of the mean [SEM]) pretreatment baseline granulocyte counts per cubic milliliter in the control ($3,028 \pm 570$) and the experimental groups ($1,663 \pm 114$, $1,926 \pm 292$, $1,755 \pm 252$, and $1,379 \pm 192$) were not significantly different. Animals infused with 200 or 500 U of rIL-1 β displayed significant ($P < 0.03$) but transient granulocytopenia compared with saline-infused controls at 0.5 h after infusion. Moreover, significant ($P < 0.04$) neutrophilia was evident for each of the four doses of rIL-1 β at 1 h after infusion. In contrast, saline-infused animals displayed no significant granulocytopenia or neutrophilia.

Evaluation for pulmonary leukostasis. The animals described above were all sacrificed at 5 h, and their lungs were evaluated histologically and biochemically for pulmonary leukostasis (Fig. 2). Lungs were obtained from six saline-infused animals and animals infused with 200 U ($n = 3$) or 500 U ($n = 5$) of rIL-1 β in single-bolus infusions. Lungs obtained from animals administered 200 U of rIL-1 β had

FIG. 2. Changes in pulmonary leukostasis. (A) Mean ($\pm$ SEM) alveolar septal wall granulocytes per high-power field and (B) mean ($\pm$ SEM) myeloperoxidase activity per gram of tissue in lungs obtained from six animals infused with saline, three animals infused with 200 U of rIL-1 β , and five animals infused with 500 U of rIL-1 β .

FIG. 3. Pulmonary edema formation. Mean wet/dry lung weight ratios ($\pm$ SEM) in 10 saline-infused animals, 6 animals infused with rIL-1 β , 6 animals infused with rIL-1 α , and 6 animals infused with endotoxin.

significantly increased mean alveolar septal wall granulocytes per high-power field ($P < 0.03$) compared with saline-infused controls. Lungs obtained from animals administered 500 U of rIL-1 β had both significantly increased granulocytes per high-power field ($P < 0.005$) and increased MPO activity per gram of lung tissue ($P < 0.04$) compared with saline-infused controls.

Effect of human rIL-1 on pulmonary edema formation. The six animals infused with 500 U of rIL-1 β in divided-dose infusions had significantly ($P < 0.025$) increased mean lung wet/dry weight ratios compared with 10 saline-injected controls (Fig. 3). The six animals similarly infused with rIL-1 α also had significantly ($P < 0.01$) increased mean lung wet/dry weight ratios compared with controls. No significant difference in lung wet/dry weight ratios could be detected between animals infused with the two different human rIL-1 forms, rIL-1 β and rIL-1 α . The mean ($\pm$ SEM) lung wet/dry weight ratio of the six endotoxin-challenged animals was not significantly different from that of the saline-injected controls (Fig. 3).

Effect of human rIL-1 on pulmonary vascular permeability. Animals were preinfused with 125 I-labeled albumin and subsequently administered either 500 U of rIL-1 β (6 animals), 500 U of rIL-1 α (6 animals), or equivalent volumes of saline (10 animals) in divided-dose infusions. Animals were sacrificed at 5 h after the first rIL-1 infusion, and 125 I activities in BALF and plasma were determined. Changes in pulmonary extravasation of 125 I-labeled albumin into the intra-alveolar compartment were expressed as counts per minute per milliliter in BALF divided by that in plasma (Fig. 4). Animals infused with rIL-1 β had significantly increased BALF/plasma 125 I activity ratios ($P < 0.025$) compared with saline-injected controls, whereas animals infused with rIL-1 α ($P < 0.07$) did not. There were no significant differences in BALF/plasma 125 I activity ratios between animals infused with the two different human recombinant forms of IL-1. When all 12 animals infused with either rIL-1 β or rIL-1 α were grouped together, both lung wet/dry weight ratios ($P < 0.005$) and BALF/plasma 125 I activity ratios ($P < 0.05$), were significantly increased compared with the saline-injected controls. The mean ($\pm$ SEM) BALF/plasma 125 I activity ratio of the six endotoxin-challenged animals was not significantly different from that of the saline-injected controls (Fig. 4).

Electron microscopic studies. In three control animals that were injected with saline, the pulmonary microvasculature displayed normal morphology. Glycogen particles, which

FIG. 4. Changes in pulmonary extravasation of 125 I-labeled albumin into the intra-alveolar compartment. Mean BALF/plasma 125 I radioactivity ratios ($\pm$ SEM) in 10 saline-infused animals, 6 animals infused with rIL-1 β , 6 animals infused with rIL-1 α , and 6 animals infused with endotoxin.

are recognized as electron-dense dots, were confined to the intravascular space. In animals injected with 500 U of rIL-1 β in five divided-dose infusions, the most pronounced changes were present in the pulmonary venules, with lesser changes in capillaries. Pulmonary arterioles were devoid of any morphological changes. The venules showed widening of the wall with electron-lucent spaces separating smooth muscle cells and bundles of collagen fibers from each other, indicating interstitial edema (Fig. 5). The endothelial cells displayed intracellular and subcellular blebs. In about half of the venules examined by electron microscopy (7 of 15 venules in three animals), definite gap formation was observed in endothelial cells (Fig. 6). These gaps were composed of a space made by an apparent separation of endothelial cells from each other; endothelial cells on both sides of a gap were morphologically intact. On higher magnification, glycogen particles were seen within the intravascular space, in the gap, and in the interstitium of the venular wall, indicating leakage of macromolecules (Fig. 7). The capillary endothelium displayed some blebs, but no gap formation could be appreciated. In representative areas of endothelial injury, pulmonary leukostasis was not consistently evident.

DISCUSSION

Pulmonary leukostasis, acute lung injury, and ARDS have all been described in a variety of stressful experimental and clinical settings (11–14, 17, 21, 25, 28, 32, 35–38, 41, 42). These stressful but diverse stimuli to the host can all elicit the acute phase response. One established mediator of multiple components of the acute-phase response is the cytokine IL-1 (6). Appropriate to the diversity of acute stress, in vitro monocyte production of IL-1 can be triggered by a wide range of stimuli (6). Multiple experimental models of acute injury have been developed to study the pathogenesis of ARDS. These models have included endotoxin (17) and thrombin (41) challenge in sheep, complement activation in rats (42), and administration of phorbol myristate acetate (37), platelet-activating factor (28), or arachidonate metabolites (38) to rabbits. More recently, infusion of recombinant TNF α (rTNF α) into rats has been shown to induce lung injury (43). One endogenous mediator that could be central to all of these diverse processes leading to pulmonary

FIG. 5. Low-magnification electron micrograph of a pulmonary venule from an rIL-1 β -treated rabbit. Notice endothelial blebs (arrowheads) and interstitial edema (*) in the wall. V, vascular space; I, interstitium. (Uranyl acetate and lead citrate stain; magnification, $\times 3,500$).

vascular endothelial injury is the cytokine IL-1. Endotoxin (6, 23, 40), thrombin (40), C5 cleavage products (15), phorbol myristate acetate (22), and rTNF α (7, 30) can all induce intracellular synthesis and release of IL-1. In fact, endotoxin (23), thrombin (40), and rTNF α (30) have been shown to induce IL-1 production at the endothelial cell surface. On the other hand, IL-1 has been shown to induce endothelial

biosynthesis of both platelet-activating factor (4) and arachidonate metabolites (1). We have previously demonstrated that a semi-purified 10,000 to 30,000-molecular-weight P388D₁ murine macrophage-derived monokine fraction containing IL-1 activity induces pulmonary vascular endothelial injury and lung edema in rabbits (13). Because the active fraction of this monokine preparation was compatible with

FIG. 6. Electron micrograph of a venular endothelium from an rIL-1 β -treated rabbit. Notice gap in the endothelium (arrowhead). V, Vascular space; I, interstitium. (Uranyl acetate and lead citrate stain; magnification $\times 8,000$.)

FIG. 7. Higher magnification of Fig. 6. Notice glycogen particles in the gap and in the interstitium (arrowhead). V, Vascular space; I, interstitium. (Uranyl acetate and lead citrate stain; magnification, $\times 28,000$.)

IL-1, we studied the effects of purified recombinant human IL-1 α and IL-1 β in our rabbit model.

In our present studies, we demonstrated that a single-bolus infusion of rIL-1 β induced transient granulocytopenia followed by a rebound neutrophilia as well as granulocyte sequestration within the pulmonary microvasculature (rIL-1 α was not tested). Divided-dose infusions of human rIL-1 induced pulmonary vascular endothelial injury and lung edema in rabbits. Divided-dose infusions were used because the intravascular half-life of IL-1 is reportedly <2 min (19) and its *in vivo* effects can be markedly enhanced by divided-dose administration (44). First, using a gravimetric index of lung water (lung wet/dry weight ratio), we demonstrated that infusions of pure human rIL-1 α and rIL-1 β significantly increased pulmonary edema formation. In animals preinfused with ^{125}I -labeled albumin, rIL-1 β significantly increased pulmonary vascular endothelial permeability to ^{125}I -labeled albumin (BALF/plasma ^{125}I activity ratios) over the 5-h study period. Although rIL-1 α did increase the lung leak ($P < 0.07$), this increase did not achieve statistical significance. Thus after rIL-1 infusion, albumin with a molecular weight of 60,000 traversed the alveolar-capillary barrier into the intra-alveolar compartment. No significant difference could be demonstrated for either pulmonary edema formation or changes in pulmonary vascular permeability between animals infused with rIL-1 α and those infused with rIL-1 β . We then utilized electron microscopic studies of lungs obtained from rIL-1 β -challenged animals preterminally infused with the electron-dense ultrastructural permeability tracer glycogen. These studies demonstrated endothelial injury in the form of bleb formation and extravasation of glycogen across the endothelial barrier, indicating increased permeability. The glycogen tracer could be demonstrated in transit through open gaps in the venular endothelium. Unlike radio-labeled albumin, glycogen with an average molecular weight of 2.7×10^6 was confined to the pulmonary interstitium, where electron lucency indicated perivascular edema. Rep-

resentative areas of endothelial injury were not consistently leukostatic. No epithelial cell injury could be seen. No injury or leakage was seen in saline-injected control animals. Utilizing several techniques, we have thus demonstrated that rIL-1 infused into rabbits causes an alveolar-capillary leak with pulmonary edema.

In our previous studies, we utilized a murine monokine preparation containing IL-1 bioactivity (13). Divided-dose infusions of 50 U of IL-1 activity induced lung injury in rabbits. In the present studies, 500 U of human rIL-1 was required. It is not clear whether this discrepancy in dose requirements is due to a species difference between murine and human IL-1 or differences between the native and recombinant molecules or whether another murine macrophage-derived mediator(s) or comediator(s) is involved. The rIL-1 α and rIL-1 β preparations contained 50 pg and 1.4 ng of endotoxin per 10^5 U of IL-1 activity, respectively. For each 4-kg rabbit infused with 500 U of rIL-1, this translates into a maximum of 7 pg of endotoxin per an animal or 1.75 pg per kg of body weight. A paucity of data is available regarding endotoxin-induced pulmonary vascular endothelial injury in rabbits. In 1969, McGrath et al. showed that an intracardiac injection of endotoxin (2.8 to 4.2 mg/kg of body weight) into rabbits produced endothelial injury in mesenteric arteries (27). In rabbits pretreated with [^3H]thymidine, Gaynor has shown that an endotoxin challenge of 40 to 80 $\mu\text{g/kg}$ of body weight induces a threefold increase in pulmonary capillary endothelial incorporation of [^3H]thymidine (i.e., DNA synthesis) compared with saline-injected controls (9). This increased [^3H]thymidine incorporation by the endothelium correlates with endothelial injury and repair. Therefore, in rabbits a 2×10^9 -fold higher dose of endotoxin than that used in the present studies was required to induce mesenteric vascular endothelial injury, and an $\sim 8 \times 10^7$ -fold higher dose was required to induce pulmonary capillary damage. To more rigorously exclude endotoxin, we challenged animals with 10 ng per animal or 2.5 ng per kg of body weight, a

7,000-fold increase over the maximum endotoxin contained in our rIL-1 preparations. No significant increase in either pulmonary edema formation or pulmonary vascular permeability could be demonstrated. It is therefore unlikely that the rIL-1-induced endothelial injury described in the present studies can be ascribed solely to endotoxin contamination. These findings do not exclude the possibility of a synergistic action between rIL-1 and endotoxin.

The mechanism(s) of IL-1-induced lung injury may be complex. IL-1 produces multiple biological effects on granulocytes (16, 20, 39) and has direct effects on the endothelium (1, 3, 4). Furthermore, IL-1 induces pulmonary leukostasis with strategic localization of granulocytes in close proximity to the pulmonary microvascular endothelial surface (12). A role for granulocytes as effector cells has been postulated in multiple experimental systems of pulmonary vascular endothelial injury and in the pathogenesis of ARDS (17, 37, 41, 42). Even in the absence of granulocyte effector cells, IL-1 can act directly on the endothelium (1, 3, 4). This cytokine recognizes and binds to endothelial cell receptors (8) and induces surface expression of several antigens (3, 5) and mediators (4) which may influence granulocyte-endothelial cell interaction and endothelial integrity. IL-1 increases endothelial adhesiveness for granulocytes (3, 34) which can be blocked by a monoclonal antibody (H4/18) that recognizes an IL-1-induced endothelial surface protein (3, 5) or with a monoclonal antibody directed against a granulocyte surface antigen, the CDw18 complex (34). IL-1 also stimulates biosynthesis and cell surface expression of procoagulant activity in human endothelial cell monolayers and suppresses the protein C pathway, an antithrombotic mechanism (31). IL-1 induces human endothelial cell synthesis of plasminogen activator inhibitor (29) and PAF (4), an established mediator of acute lung injury (28, 48). The increased expression of PAF and procoagulant activity combined with inhibition of plasminogen activator may shift the balance at the endothelial surface toward a prothrombotic state. These IL-1-induced events within the pulmonary microvasculature may promote granulocyte-endothelial cell interactions, intrapulmonary vasculocclusive events, and lung injury. In addition, IL-1 stimulates endothelial production of the arachidonate metabolite prostaglandin E₂ (1). Henson and co-workers have found that intrabronchial installation of C5 fragments only in concert with prostaglandin E₂ or hypoxia could induce injury (18), and that lung injury induced by intravascular complement activation together with hypoxia can be blocked by the cyclooxygenase inhibitor meclofenamate in rabbits (21). Therefore, IL-1 may act singularly or in concert with other endogenous mediators to induce acute lung injury. There is now preliminary evidence that IL-1 can increase lung water without altering vascular pressures in the isolated perfused rat lung (C. T. Schnitzlein-Bick, J. W. Barnard, J. E. Roepke, R. A. Rhoades, *Am. Rev. Respir. Dis.* 135:A258, 1987). This suggests that hemodynamic alterations are not essential to IL-1-induced lung injury. Recently, ARDS has been described in profoundly neutropenic subjects (25, 32). It is possible that IL-1 contributes directly to the pathogenesis of ARDS even in profoundly neutropenic patients.

Several other endogenous mediators including C5 cleavage products (18, 21) TNF α (33, 43), arachidonate metabolites, (36, 38), and PAF (28, 48) may not relate to IL-1 biosynthesis and its biological effects, but may also directly contribute to IL-1-induced pulmonary vascular endothelial injury in vivo. In rabbits, systemic complement activation alone has been shown to not increase pulmonary extravasa-

tion of ¹²⁵I-labeled rabbit serum albumin at 4 h compared with saline-injected controls (18, 21, 47). When complement activation is combined with hypoxia, there is an approximately eightfold increase in pulmonary vascular permeability that can be blocked by cyclooxygenase inhibition. In a similar rabbit model we have shown that rTNF α increases both lung water (151%) and pulmonary extravasation of ¹²⁵I-labeled rabbit serum albumin (376%) compared with saline controls at 5 h (S. E. Goldblum, B. Hennig, M. Jay, K. Yoneda, and C. J. McClain, *J. Leuk. Biol.* 42:A31, 1987). Recently Okusawa et al. demonstrated that either rIL-1 or rTNF α in low doses induced minimal lung injury, whereas coadministration of the two cytokines induced pulmonary hemorrhage and leukostasis with massive extravasation of protein (33). Infusion of arachidonic acid into rabbits causes platelet thrombi in the pulmonary microvasculature, with death within minutes (38). Shasby et al. demonstrated that arachidonate only in the presence of granulocytes increases pulmonary vascular permeability in isolated perfused rabbit lungs (36). Infusion of PAF into rabbits causes immediate neutropenia and thrombocytopenia, with pulmonary sequestration of these elements (28). These rabbits also display increased lung water without extravasation of ¹²⁵I-labeled albumin into the BALF at 1 h after PAF infusion (48). Whether this interstitial pulmonary edema evolves into alveolar edema was not determined. Therefore, these four endogenous mediator systems may work in concert with IL-1 to produce pulmonary vascular endothelial injury.

In summary, the present studies demonstrate that human rIL-1 β can increase lung water and protein flux from the pulmonary intravascular compartment into the intra-alveolar space in unanesthetized rabbits. rIL-1 β compromised pulmonary vascular endothelial integrity and promoted extravasation of an ultrastructural permeability tracer with perivascular edema. We conclude that IL-1 β , an endogenous cytokine that is an integral part of the host response to acute stress and inflammation, can induce pulmonary vascular endothelial injury and lung edema in rabbits. Since increased immunoreactive IL-1 has been recovered in the BALF of ARDS patients, our findings suggest that IL-1 may play a role in the pathogenesis of acute lung injury and ARDS.

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