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INTERLEUKIN-1β-INDUCED NEUTROPHIL RECRUITMENT AND ACUTE LUNG INJURY IN HAMSTERS

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Abstract—Administering recombinant interleukin-1β (IL-1β) intratracheally caused lung neutrophil accumulation and lung injury in hamsters. The percentage of leukocytes that were neutrophils increased progressively in lavages from lungs of hamsters given 25, 50, or 100 ng IL-1β intratracheally 2 h before. Lung injury, reflected by increased lung lavage protein concentrations and lung lavage hemoglobin concentrations, increased 2 h after administering 100 ng IL-1β. Lung injury, reflected by lung wet weight/body weight ratios, followed similar patterns, with significant increases occurring 2 h after insufflating 50 or 100 ng IL-1. Our results indicate that increased concentrations of IL-1β in lung airways can cause neutrophil recruitment and lung injury in hamsters. This mechanism may contribute to the development of lung neutrophil accumulation and lung injury that characterizes ARDS patients who have increased airway levels of IL-1β.

INTRODUCTION

The acute respiratory distress syndrome (ARDS) is a rapidly developing pulmonary injury characterized by noncardiogenic pulmonary edema, severe hypoxemia, and increased lung neutrophils (1). The pathogenesis of ARDS is unknown but recent findings suggest a role for interleukin-1 (IL-1), a potent modulatory protein with diverse proinflammatory properties including the ability to stimulate neutrophil recruitment (2, 3). The possible contribution of IL-1 to the development of ARDS has been suggested by two findings: First, alveolar macrophages recovered from individuals with ARDS make more IL-1 than alveolar

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macrophages from normal individuals (4). Second, ARDS patients have increased concentrations of IL-1 in their lung lavages compared to control subjects and lung lavage IL-1 levels correlate with lung lavage protein concentrations, which are also increased in ARDS patients (5, 6).

Animal and in vitro observations also support a participation of IL-1 in the development of acute lung injury. First, administering IL-1 causes shock and lung injury (7–11). Second, IL-1 can enhance neutrophil chemotaxis, adherence, and O_2 radical release in vitro (12–18).

The potential relationship between increased IL-1 levels, lung neutrophil influx, and lung injury prompted us to assess the nature of this interaction directly by insufflating IL-1β into the lungs of hamsters. Our results indicate that giving IL-1β intratracheally rapidly causes lung neutrophil recruitment and lung injury in hamsters.

METHODS AND MATERIALS

Description of Experimental Protocol. Male Golden Syrian hamsters (Sasco, Omaha, Nebraska) weighing 100–150 g were anesthetized by giving a combination of ketamine (200 mg/kg) and Rompun (20 mg/kg), intramuscularly (9). After each hamster reached a surgical plane of anesthesia, the trachea was surgically isolated and a blunt 18-gauge needle covered with PE-160 tubing was inserted intratracheally. The tracheal catheter was then secured with a 2-0 silk suture. Subsequently, 25, 50, or 100 ng IL-1β (R&D Systems, Inc., Minneapolis, Minnesota) in 500 μl phosphate-buffered saline (PBS) containing 0.1% human serum albumin (HSA) or 500 μl PBS containing HSA (vehicle) was administered intratracheally. Lungs were then lavaged with 3 cc of saline, of which about 80% was recovered. Hamsters were sacrificed immediately after lung lavage.

Measurement of Lung Inflammation and Lung Injury. After each experiment, lavage samples were centrifuged to pellet the leukocytes and the volume decanted to 1.0 ml. All leukocytes were then resuspended and counted using a Coulter counter. Slides were made and Wright-stained to determine the percentages of neutrophils. A Bio-Rad competitive dye-binding assay was used to measure protein samples. Hemoglobin concentrations were determined on each sample using a plasma hemoglobin kit (Sigma, St. Louis, Missouri). The ratios of lung wet weight/body weight (milligrams per gram) were also determined by standard technique using lungs removed at various times and perfused blood-free before analysis (9).

Analysis of Statistical Significance. Analysis of variance (ANOVA) was used to analyze the data. Values achieving a $P < 0.05$ were considered significantly different.

RESULTS

Lavages from lungs of hamsters given 25, 50, or 100 ng IL-1β intratracheally 2 h before had increased percentages of leukocytes that were neutrophils compared to hamsters given vehicle intratracheally (Figure 1). The percentage of lavage leukocytes that were neutrophils increased 23%, 37%, and 48% 2 h after administering 25, 50, or 100 ng IL-1β, respectively.

We next assessed lung injury in hamsters given increasing doses of IL-1β intratracheally. Lung lavage protein concentrations 2 h after giving 100 ng IL-1β intratracheally were increased ($P < 0.05$) compared to lung lavage protein concentrations for hamsters given vehicle intratracheally (Figure 2). Similarly, lung lavage hemoglobin concentrations from hamsters given 100 ng IL-1β 2 h before were increased compared to values obtained for vehicle-treated control hamsters (Figure 3). When compared to values obtained for corresponding control hamsters given vehicle intratracheally, lung wet weight to body weight ratios were significantly ($P < 0.05$) increased after 2 h in hamsters given 50 ng or 100 ng IL-1β intratracheally (Figure 4).

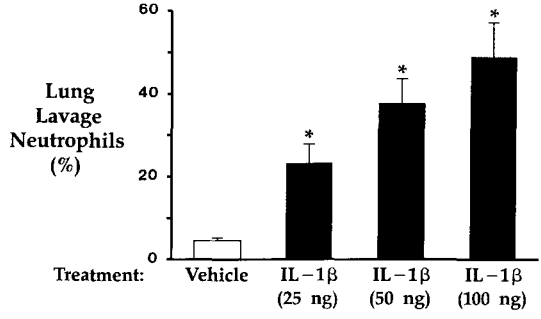

Fig. 1. Effect of IL-1β on lung lavage leukocyte percentages. The percentage of the total lung lavage leukocytes that were neutrophils increased (*$P < 0.05$) progressively in hamsters given increasing amounts of IL-1β intratracheally 2 h before. Each value is the mean ± *SE* of 8–10 individual determinations.

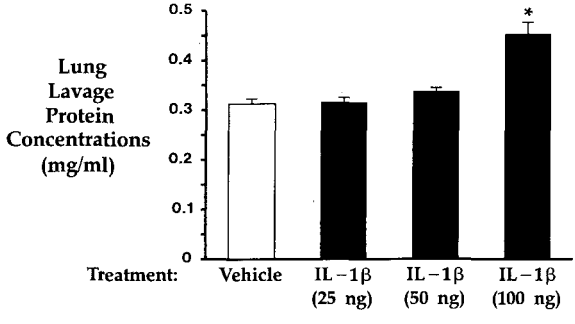

Fig. 2. Effect of IL-1β on lung lavage protein concentrations. Lung lavage protein concentrations were increased (*$P < 0.05$) in hamsters 2 h after intratracheal administration of 100 ng IL-1β compared to values obtained for hamsters given vehicle. Each value is the mean ± *SE* of five determinations.

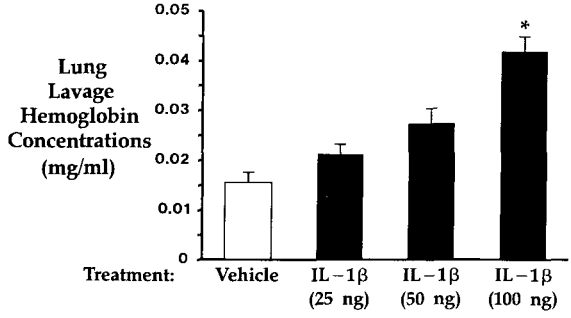

Fig. 3. Effect of IL-1β on lung lavage hemoglobin concentrations. Lung lavage hemoglobin concentrations were increased (*$P < 0.05$) in hamsters 2 h after intratracheal administration of 100 ng IL-1β compared to values obtained for hamsters given vehicle. Each value is the mean $\pm$ *SE* of five determinations.

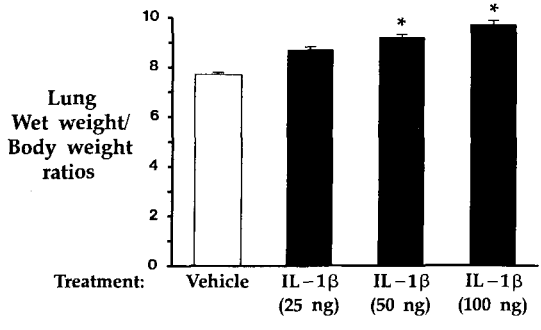

Fig. 4. Effect of IL-1β on lung wet weight-to-body weight ratios. Lung wet weight milligrams per gram to body weight milligram per gram ratios were increased (*$P < 0.05$) in hamsters 2 h after intratracheal administration of 50 or 100 ng IL-1β when compared to values obtained for hamsters given vehicle. Each value is the mean $\pm$ *SE* of 5 determinations.

DISCUSSION

Giving recombinant IL-1β intratracheally caused dose-dependent increases in lung inflammation in hamsters. Hamsters given increasing concentrations of IL-1β had progressively increasing percentages of neutrophils in their lung lavage leukocyte populations compared to lung lavages from hamsters given vehicle intratracheally. These findings indicate that increased airway concentrations of IL-1β can initiate an inflammatory lung process characterized by neutrophilia.

Giving IL-1β intratracheally to hamsters also produced lung injury. Lung damage was reflected by increased lung lavage protein concentrations, increased

lung lavage hemoglobin concentrations, and increased lung wet weight-to-body weight ratios. When considered together, these three assessments indicate an IL-1β induced lung insult involving transudation of protein, erythrocytes, and fluid from the blood into the lung. Consistent injury was observed 2 h after administering 100 ng IL-1β. These findings link increased airway IL-1β concentrations to the development of lung injury.

The mechanism responsible for lung inflammation and lung injury and the relationship between inflammation and injury following IL-1β administration intratracheally was not determined. However, recent studies indicate that giving IL-1α intratracheally to rats also causes lung neutrophil recruitment and the development of an acute oxidative lung injury (9). IL-1α-induced lung injury is dependent on neutrophils, since it is prevented by vinblastine-induced neutropenia (9), and oxygen radicals, and it is decreased by treatment with oxygen radical scavengers (9, 19). Oxygen radical scavenger intervention also reduces exhaled hydrogen peroxide (H_2O_2) concentrations and increased oxidized glutathione (GSSG) levels in lungs of rats given IL-1α intratracheally (9, 19).

Acute edematous lung injury also occurs in isolated rat lungs given IL-1α intratracheally and then perfused with human neutrophils (20). Lung injury occurs rapidly (< 1 h) and is dependent on oxidative mechanisms, since it did not develop in IL-1α-treated lungs perfused with human neutrophils heated in a way that eliminates their production of oxygen radicals, but not their chemotactic or adherence properties in vitro (20). Similar results were achieved when interleukin-8 (IL-8) was administered intratracheally in human neutrophil-perfused isolated rat lungs (21).

The significance of these findings is at least threefold. First, our results indicate that giving IL-1β intratracheally has similar effects to giving IL-1α intratracheally. Our findings indicate that treatment with interleukin-1 molecules (IL-1α or IL-1β) can cause lung neutrophil accumulation and leak in multiple species [rats (9), cows (11), and hamsters]. Although IL-1β and IL-1α share many properties, they are distinct gene products, and consequently, some differences may exist in their actions. Second, our observations provide another system for studying the mechanisms responsible for acute lung injury, such as that seen in patients with ARDS. This additional approach may be valuable since comparison of selected species differences in neutrophils (e.g., elastase activity) or other parameters between hamsters, rats, cows, and humans might provide useful insights. Third, the small amount of IL-1β given, 100 ng, is comparable to the concentrations of IL-1β recovered from the lung lavages of ARDS patients (5, 6). This latter observation further supports the premise that IL-1-mediated events contribute to the increased lung neutrophils and lung injury that characterize ARDS patients (1).

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