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Relationship Between Interleukin-1β and Platelet-activating Factor in the Pathogenesis of Acute Immune Complex Alveolitis in the Rat

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Intrapulmonary interleukin-1β (IL-1β) participates in the pathogenesis of acute IgG immune-complex alveolitis through a mechanism involving neutrophil recruitment. We have examined the relationship between intrapulmonary IL-1β and locally produced platelet-activating factor (PAF) in the development of acute alveolitis. Instillation of IgG anti-bovine albumin into the lungs of rats, followed immediately by intravenous infusion of bovine serum albumin (BSA), resulted in acute neutrophil-mediated lung injury. Development of IgG immune-complex lung injury was accompanied by three- and five-fold increases in bronchoalveolar lavage (BAL) fluid and whole lung PAF levels, respectively. Intratracheal administration of the PAF antagonists, WEB-2086 (Boehringer) or L-652,731 (Merck, Sharpe, and Dohme, Rahway, NJ), reduced pulmonary vascular leakage. Neutralization of intrapulmonary IL-1 activity with anti-IL-1β antibodies reduced pulmonary vascular permeability and whole lung PAF levels. Morphometric analysis and whole lung myeloperoxidase measurements revealed a differential effect between the PAF antagonists and anti-IL-1β with respect to pulmonary neutrophil recruitment. Intratracheal instillation of anti-IL-1β retarded net pulmonary neutrophil recruitment while the PAF antagonists retarded migration of neutrophils from the interstitial/vascular compartments into the alveolar compartment. Intratracheal instillation of anti-IL-1β plus L-652,731 resulted in reduction in lung vascular permeability and retarded net pulmonary neutrophil recruitment. No additive effect was observed. Stimulation of isolated mouse alveolar macrophages with recombinant murine IL-1β or IL-1α resulted in rapid, dose-dependent, and cell concentration-dependent increases in PAF secretion. These data suggest that intrapulmonary IL-1β amplifies local PAF production and that IL-1β and PAF modulate different aspects of pulmonary neutrophil recruitment. (Am J Pathol 1992, 141:551–560)

The formation of complement-fixing immune complexes in tissue triggers a cascade of humoral and cellular responses that can ultimately lead to tissue damage.[1] Studies in the rat IgG immune complex-induced alveolitis model have revealed that acute lung injury is complement-dependent and largely neutrophil-mediated.[2] Recent studies indicate that pulmonary neutrophil recruitment is dependent on the local production of both tumor necrosis factor (TNF) and interleukin 1β (IL-1).[3,4] While numerous *in vitro* and *in vivo* studies have implicated these cytokines in leukocyte recruitment, the relative contributions of various potential mediator pathways have not been clearly delineated. For instance, studies using complement-depleted rats,[3] and complement-deficient mice[5] have revealed a nearly absolute requirement for the potent chemotactic peptide, C5a. *In vivo* and *in vitro* studies suggest that oxidants, neutrophil chemotactic factor (NCF or interleukin-8), and chemotactic lipids such as leukotriene B4, may also be important, although direct cause-and-effect data for NCF/IL-8 are lacking.[1,6–8] Platelet-activating factor (PAF) also appears to play a role in pulmonary neutrophil recruitment in this model.[9,10]

There are several pathways through which TNF and/or IL-1 may modulate pulmonary neutrophil recruitment. *In vitro* studies have revealed that endothelium exposed to TNF or IL-1 expresses and secretes increased levels of NCF/IL-8.[7] Endothelial activation with TNF or IL-1 leads to the upregulation of several adhesion molecules that interact with neutrophils, including endothelial-leukocyte

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adhesion molecule-1 (ELAM-1).[11–14] Platelet-activating factor can be synthesized and secreted by a variety of cell types that play key roles in the pathogenesis of acute inflammation.[15] For instance, incubation of isolated resident alveolar macrophages with TNF results in PAF secretion.[9] Likewise, stimulation of monocytes, neutrophils, vascular endothelial cells, and rat peritoneal macrophages with TNF or IL-1 results in PAF production.[16–20] In the present study, we have examined the role of locally produced IL-1 in the regulation of intrapulmonary PAF production and we have examined the roles of IL-1 and PAF in pulmonary neutrophil recruitment. The data suggest that both IL-1 and PAF both play major roles in pulmonary neutrophil recruitment, but that they act at discrete steps in this complex process.

Materials and Methods

Materials

WEB-2086 BS was provided by Dr. Hubert Heuer and Ms. Peggy Ganong (Boehringer-Ingelheim Pharmaceuticals, Inc., Ridgefield, CT). L-652,731 (trans-2,5-bis; 3,4,5-trimethoxyphenyl tetrahydrofuran) and its cis-isomer, L-652,763, were from Drs. John C. Chabala and Phil Davies (Merck, Sharpe, and Dohme, Rahway, NJ). Stock solutions of L-652,731 and L-652,763 (0.4 mg/ml sterile saline) were prepared for each use in 1.0% dimethylsulfoxide (Sigma Chemical Company, St. Louis, MO). Final concentrations of dimethylsulfoxide never exceeded 0.1% in any *in vitro* or *in vivo* experiment. PAF was from Sigma. 1-0-[^{3}H]hexadecyl-2-acetylglycero-3-phosphocholine (59 Ci/mmole) (^{3}H-PAF) and 1-0-[^{3}H]alkyl-2-sn-glycerol-3-phosphocholine (45 Ci/mmole) (lyso-^{3}H-PAF) were purchased from New England Nuclear (Boston, MA). Recombinant murine IL-1α and IL-1β (10^8 units/mg) were from Genzyme (Boston, MA).

Rabbit IgG rich in antibody directed against BSA was used to induce lung injury. Briefly, IgG was prepared from hyperimmune serum by precipitation with saturated ammonium sulfate (50%), followed by dialysis against 0.005 mol/l phosphate-buffered saline (PBS) and passage through a DEAE-cellulose column.[3] The IgG anti-BSA and BSA (low endotoxin; ICN Biomedicals, Costa Mesa, CA) preparations contained <0.02 ng/ml and 0.012 ng/ml of endotoxin activity, respectively, as estimated using the Limulus amoebocyte lysate assay (E-toxate; Sigma, St. Louis, MO).

As previously described,[4] anti-IL-1β was produced by immunization of rabbits with recombinant murine IL-1β (Genzyme, Boston, MA). The resulting antiserum was affinity purified using a protein A Sepharose column (Sigma, St. Louis, MO). Anti-IL-1 serum was diluted 1:1 with PBS (100 mmol/l phosphate, pH 8.0, 150 mmol/l NaCl) and applied slowly to the PBS-washed column. After extensive washing with PBS, the column was stripped with 100 mmol/l sodium acetate buffer, pH 3.0. Fractions (1 ml) were collected in tubes containing 50 μl of 1 mol/l Tris buffer, pH 8.0. The affinity purified IgG fraction was then dialyzed against PBS and tested for specificity against IL-1.

Animal Model of Immune Complex Alveolitis

Male Long-Evans pathogen-free rats (250–350 g; Charles River Breeding Laboratories, Inc., Wilmington, MA) were used for all studies. Intraperitoneal injections of ketamine (5.0 mg/100 g body weight) and sodium pentobarbital (5 mg/100 g body weight) were given for sedation and anesthesia. Immune complex lung injury was induced as previously described.[2] Antibody solutions (250 μg of anti-BSA unless otherwise specified), mixtures of antibody, and anti-IL-1β or nonspecific rabbit IgG, or mixtures of antibody and PAF antagonists were instilled into the lungs via a tracheal cannula. In all cases, a final volume of 300 μl was instilled into the lungs. Antigen (BSA, 10 mg) was injected intravenously. Where indicated, ^{125}I-labeled aliquots of nonspecific γ-globulin (rabbit) (1–2 μg) were added to antigen preparations. Rats were sacrificed at the indicated times, BAL fluid was harvested, lung PAF was extracted, and lung myeloperoxidase (MPO) activity was determined. Pulmonary injury was quantitated by permeability measurements as well as by morphometric analysis. Permeability indices were calculated by comparing the leakage of ^{125}I-labeled bovine γ-globulin from the circulation into the lung to the ^{125}I-labeled colloid in 1 ml of blood as previously described.[2] Intrapulmonary IL-1β neutralization was carried out as previously described.[4]

Tissue Collection for PAF Measurements

PAF measurements in rat plasma, whole lung homogenates, and BAL fluids were carried out as previously described.[9] Blood was drawn from the inferior vena cava into ice-chilled test tubes containing 0.45 ml of citric acid to inhibit the action of plasma acetylhydrolase.[21] Samples were centrifuged at 550*g* (1600 rpm) for 2 minutes. One-milliliter aliquots of the plasma were removed and placed into 18 ml of chloroform/methanol/water (1:2:0.8 (v/v/v)). Whole lung lavages with normal saline were carried out via a tracheal cannula. Five milliliters of saline were alternatively instilled into, and aspirated via the trachea and the recovered lavage fluid was placed into a tube containing 30 ml of ice-chilled methanol. This procedure was repeated four times (recovering at least 16 ml of fluid/rat). Heart and lungs were quickly removed *en bloc* and residual blood in the lung was removed by per-

fusing 10 ml of normal saline through the pulmonary artery. The lungs were then removed, placed in 25 ml of ice-cold methanol, and homogenized in a model SDT 1810 Tissumizer (Tekmar Company, Cincinnati, OH).

Plasma, BAL fluid, and lung homogenates were subjected twice to Bligh-Dyer total lipid extraction.[22] Extracted samples were subjected to thin-layer chromatography on silica get plates (Silica Gel G, 250 μm, 20 × 20 cm, Fisher Scientific, Pittsburgh, PA) using a chloroform: methanol:water; (65:25:6, (v/v/v)) solvent system.[23] The zones that comigrated with standard PAF ([^{3}H]-PAF) were scraped, repeatedly eluted with chloroform:methanol:water, (1:2:8, [v/v/v]) and dried under nitrogen. Aliquots were reconstituted in Tyrode's BSA with Ca^{2+}, and assayed for PAF activity. Samples used to assess *in vivo* PAF degradation were handled the same way except that [^{3}H]-PAF recovered from thin-layer chromatography plates was added to scintillation fluid and counted.[9] Previous studies have shown that greater than 90% of exogenous [^{3}H] PAF is degraded within 2 hours after instillation into the lungs of rats with evolving IgG immune complex alveolitis.[9]

Collection of Alveolar Macrophages and BAL Fluid

Alveolar macrophages (82 to 96% pure) were recovered from normal mice using a previously described modification of the method of Ward et al.[24] BAL contents (from rats) for cytokine measurements were collected using 5 ml of 37°C, serum-free RPMI 1640 medium. At least 90% of the administered fluid was always recovered, centrifuged (400*g;* 7 minutes) to remove cells, and stored at 20°C before analysis.

IL-1 Assay of Lung Lavage Fluid and Macrophage Culture Medium

IL-1 levels were measured by means of thymocyte coproliferation assay, as modified from the procedure of Mizel et al.[25] Briefly, 5 × 10^5 murine (C3H/HEJ) thymocytes in 0.1 ml of complete RPMI 1640 containing 2.5 μg/ml of phytohemagglutinin (Burroughs Welcome, Research Triangle Park, NC) were added to each well of a 96-well microtiter plate. Equal volumes of $\log_2$ dilutions of sample were added to the wells in triplicate. After 66 hours of incubation at 37°C in 5% CO_2/95% air, the thymocytes were pulsed with 0.5 μCi [^{3}H]thymidine (6.9 Ci/nmol; ICN National Biochemicals, Irvine, CA) and collected 6 hours later on glass fiber strips using an automatic cell harvester. [^{3}H]Thymidine incorporation was determined by using a liquid scintillation counter. For standardization, data are expressed as U/ml based on a recombinant human IL-1β standard (The Upjohn Co., Kalamazoo, MI) assayed concomitantly with unknown samples. Assay specificity was conferred by the ability of anti-IL-1α plus anti-IL-1β antibodies to neutralize IL-1 activity.[4]

PAF Assay

PAF activity was determined by release of [^{3}H]-serotonin from platelets. Human platelets were isolated as described by Henson.[26] Platelet-rich plasma was removed, pooled, and incubated at 37°C with 1 μCi/ml of [^{3}H]serotonin binoxalate (New England Nuclear, Boston, MA) for 15 minutes. The platelets were sedimented at 2,500 × *g* for 15 minutes and washed first in Tyrode's gel with Ca^{2+} with EGTA and then in Tyrode's gel without Ca^{2+}. [^{3}H]Serotonin-labeled platelets (1.5 to 2.5 × 10^8) were finally incubated (37°C, 15 minutes) in duplicate with stimuli in a final volume of 0.4 ml Tyrode's gel with Ca^{2+}. The reaction was stopped by centrifuging at 12,000*g* in a microfuge-12 (Beckman Instruments, Inc., Fullerton, CA) for 30 seconds. Serotonin release was measured by counting an aliquot of the supernatant fluid in a liquid scintillating counter. Serotonin release was expressed as cpm [^{3}H]serotonin release. Incubation of platelets with increasing concentration of PAF (10^{-7} to 10^{-5} mol/l) resulted in dose-dependent serotonin release. Tyrode's gel without Ca^{2+}, Tyrode's gel without Ca^{2+} with EDTA, Tyrode's gel with Ca^{2+}, Tyrode's BSA without Ca^{2+}, and Tyrode's BSA with Ca^{2+} were prepared as described by Lynch et al.[27] Assay specificity and tissue sample PAF activity were confirmed by the capacity for L-652,731 (50 μmol/l) to block serotonin release from labeled platelets.[9]

Whole Lung Myeloperoxidase Activities

Whole lung myeloperoxidase activities were assayed as a means of quantitating pulmonary neutrophil influx.[3] Whole lungs were homogenized with a Polytron homogenizer (4 × 10 seconds at a setting of 4) using 6 ml of homogenization buffer. The homogenization buffer (50 mmol/l phosphate, pH 6.0) contained 0.5% hexadecyltrimethyl ammonium bromide (Sigma, St. Louis, MO) and 5 mmol/l EDTA. Homogenized samples were then sonicated (3 × 10 seconds at a setting of 5) and centrifuged (3,000*g;* 30 minutes) at 4°C. Myeloperoxidase activity in supernatants was assayed by measuring the change in A460 resulting from decomposition of H_2O_2 in the presence of o-dianisidine.[28]

Morphometric Analysis of PMN Recruitment

Transmission electron micrographs were prepared from whole lungs fixed in 4% glutaraldehyde under con-

stant pressure inflation (25 cm H_2O), washed in 0.1 mol/l cacodylate buffer (pH 7.3) and embedded in plastic. Sections 1 μm thick were stained with toluidine blue. As previously described,[9] morphometric analysis was carried out by a board-certified pathologist (J.S.W.) who was blinded to sample origin. "Total PMN/40X HPF" refers to all neutrophils including alveolar, interstitial, and intravascular. In many cases, intravascular PMN could not be distinguished from interstitial PMN. Alveolar PMN refers to cells located entirely within alveoli. For each condition, four samples were examined. In each sample, 60 randomly selected 40× microscopic fields were analyzed.

Secretion of PAF by Mouse Alveolar Macrophages

Mouse alveolar macrophages were plated in 1.7 × 1.6 cm flat bottom tissue culture wells (Linbro, McLean, VA) in 0.5 ml of RPMI 1640 medium-BSA (2.5 g/l) and allowed to adhere for 1 hour at 37°C, 5% CO_2 before addition of mouse IL-1α or IL-1β.[9] Cell concentrations and incubation times were varied as described in Results. At the end of prescribed incubations (37°C, 5% CO_2), culture supernatants were aspirated and subjected to the [^{3}H]serotonin release PAF assay. Platelet-activating factor release was quantified by comparison of [^{3}H]serotonin release triggered by culture supernatants with release triggered by known concentrations of PAF.[9] The assay was sensitive to 2 fmol/ml of PAF.

Results

Intrapulmonary PAF Production in Immune Complex-induced Alveolitis

Induction of IgG immune complex alveolitis resulted in a threefold increase in bronchoalveolar lavage (BAL) fluid PAF accompanied by a more than five-fold increase in whole lung PAF activity (Figure 1). As previously reported,[9] more than 90% of exogenous [^{3}H] PAF instilled at time zero into the lungs of rats with IgG immune complex alveolitis was degraded within 2 hours (data not shown). Whole blood samples collected 1, 2, and 4 hours after initiation of lung injury contained 3.1 ± 0.6, 2.9 ± 1.8, and 6.4 ± 2.4 ng/ml of PAF, respectively. These values are comparable to PAF levels determined in normal rat plasma (3.6 ± 2.6 ng/ml; mean ± SEM, n = 3 for each value). These data indicate that immune complex alveolitis is accompanied by local PAF production.

Role of PAF in Acute Immune Complex Alveolitis

Intratracheal administration (at time 0) of the PAF antagonists, L-652,731 and WEB-2086, resulted in dose-dependent decreases in immune complex-induced pulmonary vascular leakage (Figure 2). Intratracheal instillation of L-652,763 or vehicle alone had no suppressive effect on pulmonary vascular leakage. L-652,763 is the *cis*-isomer of L-652,731 and has 100- to 1000-fold less PAF receptor antagonist activity than L-652,731.[29] As previously reported,[9] parenteral administration of WEB-2086 (4.5 mg/kg; 5 divided doses) did not reduce immune complex-induced vascular leakage (data not shown). These data suggest that intrapulmonary PAF plays a role in the development of vascular leakage in evolving immune complex alveolitis. The lack of effect by intravenously administered WEB-2086 suggests that the pulmonary vascular leakage associated with acute alveolitis is not mediated by intravascular PAF.

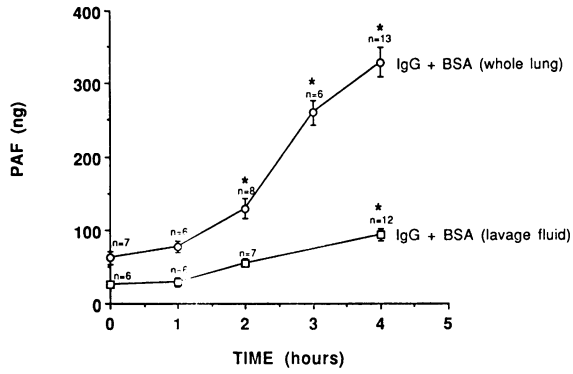

Figure 1. *Intrapulmonary PAF production (BAL fluid and whole lung) during the development of IgG immune complex-induced lung injury. At the indicated times, rats were sacrificed and the lungs were lavaged with saline (20 ml) or homogenized as described in Methods. The time course data represent the cumulative means ± SEM of two experiments except at the 4-hour timepoint when four separate experiments were carried out. In a single experiment, triplicate negative controls (intratracheal nonspecific IgG alone [250 μg] and intravenous BSA alone [10 mg]) were included. Intratracheal nonspecific IgG alone resulted in 36 ± 19 and 28 ± 16 ng of PAF for whole lungs and BAL fluid, respectively. Intravenous infusion of BSA alone resulted in 14 ± 7 and 9 ± 8 ng of PAF for whole lungs and BAL fluid, respectively. The total number of rats employed for each variable is indicated in parentheses. Data were analyzed by one-way analysis of variance with significant differences assigned for P < .05 (41). *Indicates significant difference versus rats that received both anti-BSA and BSA at time zero. BAL fluid PAF is expressed as total PAF retrieved where at least 80% of the lavage fluid (16 ml) was retrieved.*

Disparate Roles for PAF and IL-1β in Pulmonary Neutrophil Recruitment

Pulmonary neutrophil recruitment was quantitated by whole lung myeloperoxidase measurements. Since lung myeloperoxidase measurements cannot delineate the distribution of neutrophils among the pulmonary vascular, interstitial, and alveolar compartments, morphometric analysis was also employed. Intratracheal administration of L-652,731 or WEB-2086 reduced immune complex-mediated pulmonary vascular leakage (Figure 2) but did not reduce whole lung myeloperoxidase activity (Table

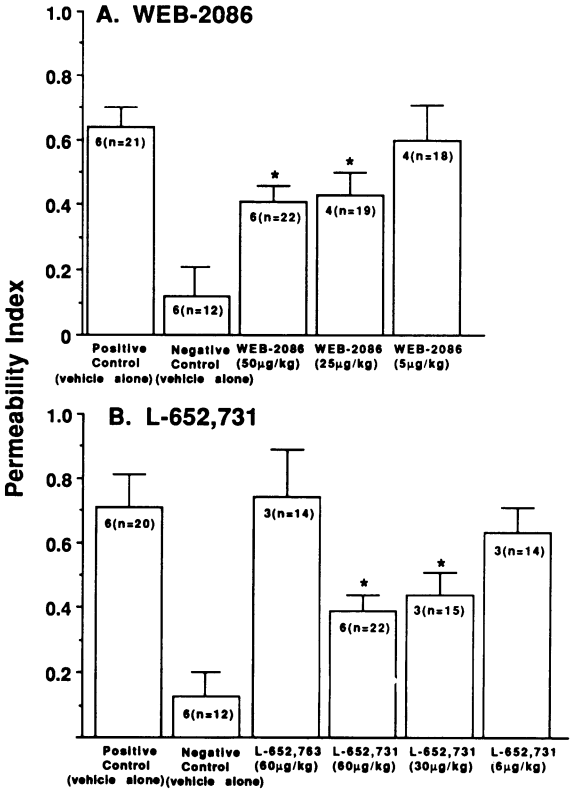

Figure 2. *Intratracheal WEB-2086 and L-652,731 block development of increased pulmonary vascular permeability associated with immune complex alveolitis. Rats were sacrificed 4 hours after the induction of lung injury. The data for WEB-2086 (Frame A) and L-652,731 (Frame B) represent the means ± SEM of three to six experiments. The number of experiments and the cumulative number of rats is indicated within each bar. Data were analyzed by one-way analysis of variance with significance assigned for $P < .05$ (41). *Indicates significant difference versus positive control (IgG: BSA treated with vehicle alone or L-652,763) rats. Negative control rats received intratracheal IgG plus vehicle alone and intravenous saline instead of BSA.*

1). Morphometric analysis of lung sections from L-652,731-treated, WEB-2086-treated, and positive control rats also revealed no new differences in total lung neutrophil content (Table 1). However, morphometric analysis of intrapulmonary neutrophil distribution revealed dose-dependent reductions in alveolar compartment neutrophils in rats that received intratracheal L-652,731 or WEB-2086 (Table 1). Addition of L-652,731 (50 μmol/l) to 100 μl aliquots of positive control lung homogenate had no effect on myeloperoxidase activity (data not shown). Bronchoalveolar lavage contents from L-652,731 and WEB-2086-treated rats contained fewer neutrophils than lavage fluid from positive control animals (Table 2).

In contrast, intratracheal administration of anti-IL-1β antibodies resulted in a marked reduction in whole lung myeloperoxidase activity accompanied by reductions in both alveolar compartment and interstitial/vascular compartment neutrophils (Table 1). As shown in Table 2, there was a marked reduction in BAL neutrophils retrieved from rats that received anti-IL-1β compared to controls. These data suggest that locally produced PAF plays a role in translocation of neutrophils from the vascular/interstitial compartment into the alveolar compartment, while intrapulmonary IL-1β plays a role in recruitment of neutrophils from the vascular space into the lungs.

Interleukin-1β-Triggered PAF Production in Immune Complex Alveolitis

Previous studies have shown that full pulmonary neutrophil recruitment requires local IL-1β activity.[4] Intratracheal instillation of anti–IL-1β markedly reduced intrapulmonary IL-1 activity (31 ± 6 U/ml BAL fluid in positive controls; 6 ± 4 U/ml BAL fluid in rats that received anti-IL-1β) as well as whole lung and BAL fluid PAF levels measured at 4 hours (Figure 3). These data suggest that intrapulmonary PAF elaboration is dependent on local IL-1β activity.

Effect of Simultaneous IL-1β and PAF Blockade in Immune Complex Alveolitis

As expected, intratracheal administration of anti-IL-1β antibodies plus L-652,731 resulted in a marked reduction in immune complex-mediated pulmonary vascular leakage accompanied by a reduction in whole lung myeloperoxidase activity (Table 3). Comparisons of lung injury, whole lung myeloperoxidase activity, and pulmonary neutrophil recruitment by morphometric analysis between rats that received intratracheal anti-IL-1β plus L-652,731 and either anti-IL-1β alone or L-652,731 alone, revealed no additive interactions (Table 3). In other words, the combination of intratracheal anti-IL-1β plus L-652,731 did not reduce lung vascular permeability or neutrophil recruitment further than in rats that received anti-IL-1β alone. This observation is consistent with the premise that IL-1β is absolutely required for full development of lung injury and neutrophil recruitment, that local PAF production is dependent on IL-1β, and that PAF plays a role in translocation of neutrophils from the vascular/interstitial space into the alveolar compartment.

PAF Secretion by IL-1β–Stimulated Alveolar Macrophages

Mouse alveolar macrophages incubated with recombinant murine IL-1α and IL-1β secreted PAF in a cell concentration-dependent, time-dependent, and dose-dependent manner (Figure 4).

Table 1. *Pulmonary Neutrophil Recruitment in IgG Immune-complex Alveolitis:* Whole Lung MPO and Morphometric Analysis*

Group	MPO† activity	Neutrophil influx: Sum PMN‡/ 40× HPF	*P* value‖	Neutrophil influx: Alveolar PMN/ 40 × HPF	*P* value‖
A. IT anti-BSA; IV BSA (positive control)	.421 ± 0.05	96.5 ± 4.8	—	69.5 ± 2.3	—
B. IT anti-BSA + L652,763 IV BSA (positive control)	.396 ± 0.07	89.6 ± 7.2	—	70.5 ± 4.8	—
C. IT anti-BSA; IV Saline (negative control)	.010 ± 0.06	5.3 ± 1.9	$P < .05$ vs. A,B	3.5 ± 1.4	—
D. IT anti-BSA + L652,731 (60 μg/kg); IV BSA	.388 ± 0.15	92.5 ± 6.6	NS vs. A,B	52.8 ± 3.6	$P < .05$ vs. A,B
E. IT anti-BSA + WEB-2086 (50 μg/kg); IV BSA	.406 ± 0.17	90.9 ± 4.9	NS vs. A,B	48.2 ± 2.6	$P < .05$ vs. A,B
F. IT L652,731 (60 μg/kg) alone; IV BSA	not done	5.8 ± 2.2	—	not done	—
G. IT WEB-2086 (50 μg/kg) alone; IV BSA	not done	7.5 ± 3.3	—	not done	—
H. IT anti-BSA + nonspecific IgG (80 μg); IV BSA (positive control)	.418 ± 0.08	90.6 ± 6.2	—	73.6 ± 4.5	—
I. IT anti-BSA + anti-IL-1β (80 μg); IV BSA	.189 ± 0.15	40.8 ± 3.9	$P < .05$ vs. H	32.6 ± 4.7	$P < .05$ vs. H

* IgG immune-complex alveolitis in the presence or absence of PAF antagonists was induced as described in the Methods section. IT, intratracheal; IV, intravenous.

† MPO activity (whole lung homogenates) is expressed as ΔO.D. (A_{460}) per minute resulting from decomposition of H_2O_2 in the presence of o-dianisidine.[28]

‡ Sum PMN equals the total of neutrophils contained in the alveolar, interstitial, and vascular compartments.

‖ One-way analysis of variance.[41] NS, not significant at $P < 0.05$.

Alveolar PMN (neutrophils contained within the alveolar compartment).

Discussion

Recent studies suggest that locally produced IL-1 and PAF are important mediators of acute immune complex-induced lung injury in rats.[4,9,10] Both IL-1β and TNF are required for full recruitment of neutrophils into the lungs of rats with evolving alveolitis.[3,4] Locally produced PAF also appears to play a role in the development of this injury since intratracheal instillation of highly specific PAF receptor antagonists can attenuate immune complex-induced lung injury.[9,10] This study examined the regulatory role of locally produced IL-1 in intrapulmonary PAF production and analyzed the respective roles of IL-1 and PAF in pulmonary neutrophil recruitment. The data confirm that both IL-1 and PAF are involved in acute IgG immune complex-induced alveolitis. Physiologic concentrations of IL-1α and IL-1β trigger PAF secretion by isolated murine alveolar macrophages. In addition, the data suggest that locally produced IL-1 can trigger the intrapulmonary elaboration of PAF and that IL-1 and PAF modulate different aspects of pulmonary neutrophil recruitment.

IgG immune complex deposition resulted in a pronounced increase in whole lung and BAL fluid PAF levels. Previous studies suggest that intrapulmonary PAF production is anatomically compartmentalized and that it is not the result of pulmonary neutrophil recruitment *per se*.[9] Chang et al have observed that intratracheal instillation of calcium ionophore A23187 into isolated-perfused rat lungs results in a rapid rise in BAL fluid PAF levels.[30] These investigators suggested that injured endothelial cells or other indigenous lung cells may be responsible for PAF production. We recently observed that neutrophil depletion does not result in a significant decrease in whole lung PAF levels after immune complex deposition.[9] Finally, this study and previous studies indicate that isolated alveolar macrophages stimulated with either IL-1α, IL-1β, or TNF can produce physiologically relevant concentrations of PAF activity.[9] These observations do not exclude the possibility that recruited neutrophils may contribute to the total pool of whole lung PAF activity but they strongly suggest that recruited neutrophils are not the sole or predominant source. In fact, *in vitro* studies indicate that several cell types present within an inflammatory milieu can produce PAF in response to IL-1 or TNF.[16–20] The present study provides corroborating *in vivo* evidence that IL-1 mediates local PAF production in a well-characterized model of acute lung injury. It should be emphasized that both whole lung and BAL fluid "levels" are static measurements and that there is strong evidence that locally produced PAF is rapidly catabolized through a variety of pathways.[16,31–37] We have previ-

Table 2. *Retrieved Neutrophils in Lung Lavages from Rats Treated with PAF Antagonists*

Group	Total BAL neutrophils* (x ± SEM; 6/group)	
A. IT anti-BSA; IV BSA (positive control)	$18.6 \pm 2.3 \times 10^{-6}$	—
B. IT anti-BSA + L652,763: IV BSA (positive control)	$17.9 \pm 3.2 \times 10^{-6}$	—
C. IT anti-BSA; IV saline (negative control)	$.09 \pm .03 \times 10^{-6}$	$P < .05$ vs. A,B†
D. IT anti-BSA + L652,731 (60 μg/kg); IV BSA	$11.8 \pm 2.6 \times 10^{-6}$	$P < .05$ vs. A,B†
E. IT anti-BSA + WEB-2086 (50 μg/kg); IV BSA	$10.7 \pm 1.8 \times 10^{-6}$	$P < .05$ vs. A,B†
F. IT anti-BSA + nonspecific IgG (80 μg); IV BSA	$22.7 \pm 4.2 \times 10^{-6}$	—
G. IT anti-BSA + anti-IL-1β (80 μg); IV BSA	$9.9 \pm 3.5 \times 10^{-6}$	$P < .05$ vs. F‡

* Total lung lavage neutrophils retrieved with exhaustive 30-ml lavage procedures.

† Data were analyzed by one-way analysis of variance with significant differences assigned where $P < .05$.

‡ Data were analyzed by two-tailed Student's *t*-test with significance assigned where $P < .05$.[41]

ously observed that more than 90% of exogenous [^{3}H] PAF is degraded within the lungs within 2 hours after initiation of IgG immune complex alveolitis.[9]

As previously reported, intrapulmonary IL-1β and PAF each play pivotal roles in the evolution of acute immune complex-induced lung unjury in the rat.[4,9,10] However, the data summarized in Tables 1, 2, and 3 suggest that these two mediators are involved in different aspects of pulmonary neutrophil recruitment. Neutralization of intrapulmonary IL-1β resulted in reduced recruitment of neutrophils into the alveolar compartment as well as decreased net pulmonary neutrophil sequestration. This observation is consistent with actions attributed to IL-1 based on *in vitro* studies. Several groups have observed that IL-1–treated endothelial cell monolayers exhibit increased adhesiveness for neutrophils through a protein synthesis-dependent mechanism.[11–14] One can envision a scenario in which reduced IL-1 activity results in a less "sticky" endothelium and reduced efficiency of pulmonary neutrophil sequestration. In contrast, neutralization of intrapulmonary PAF activity (with L-652,731 or WEB-2086) did not affect total lung neutrophil content yet had a moderate effect on emigration of neutrophils from the vascular/interstitial compartment into the alveolar compartment. This observation is consistent with the neutrophil chemotactic activity attributed to PAF.[15] In this scenario, neutrophil to endothelial cell adhesive interactions would be normal or near normal yet efficient transmigration of neutrophils from the vascular/interstitial space into the alveolar space would not occur in the absence of PAF. An experiment, revealing that anti-IL-1β plus L-652,731 exerts an effect on lung injury and neutrophil recruitment similar to anti-IL-1β alone (Table 3), suggests that IL-1β and PAF possess actions that are related. While this particular experiment does not prove that IL-1β and PAF function at different points in pulmonary neutrophil recruitment, the data are consistent with the interpretation that IL-1 mediates pulmonary neutrophil sequestration and that PAF mediates translocation of neutrophils from the vascular and/or interstitial space into the alveolar space. Although this study suggests that PAF influences transmigration of neutrophils into the alveolar compartment, the potential role of PAF in neutrophil-endothelial cell adhesive interactions cannot be dismissed. Zimmerman et al have shown that PAF may play a role in early protein synthesis-independent, neutrophil-endothelial adhesive interactions.[38] If the PAF-mediated neutrophil to endothelial cell adhesive interactions are quantitatively much less than later occurring protein synthesis-dependent adhesive interactions, the methods used in this study to track neutrophil recruitment would probably be too insensitive to detect such interactions. Nevertheless, it appears that IL-1 and PAF play distinctive roles in pulmonary neutrophil recruitment in acute immune complex-induced alveolitis in the rat.

Several investigators have provided data that suggest that cytokines such as IL-1 and TNF can trigger local PAF production which in turn plays a role in tissue injury. For instance, Rubin and Rosenbaum[39] have observed that the PAF receptor antagonist, SRI 63-441, can block both

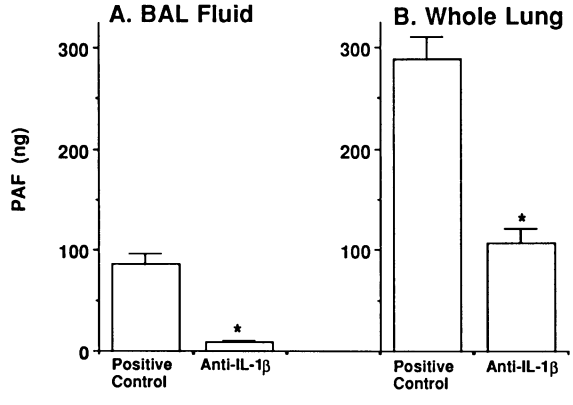

Figure 3. *Intratracheal anti-IL-1β (80 μg) reduces BAL fluid and whole lung PAF measured 4 hours after initiation of immune-complex lung injury. These data represent the means ± SEM of two experiments in which three rats were used in each group. Positive control rats received nonspecific rabbit IgG (80 μg) instead of anti-IL-1β. Paired sets of data were analyzed using a two-tailed Student's t-test with significant differences (*) assigned where $P < .05$.[41] Baseline (time 0) whole lung PAF levels and BAL fluid levels were less than 80 ng and 40 ng, respectively (data not shown).*

Table 3. *Lung Injury* and Neutrophil Recruitment: Effect of Anti L-1β Plus L-652,721*

Group	Permeability index	*P* value‖	MPO† activity	Neutrophil influx				
				P value‖	Sum PMN‡/ 40× HPF	*P* value‖	Alveolar PMN/ 40 × HPF	*P* value‖
A. IT anti-BSA; IV BSA (positive control)	.59 ± .08	—	.406 ± .10	—	114.2 ± 8.8	—	64.9 ± 3.3	—
B. IT anti-BSA; IV Saline (negative control)	.12 ± .08	—	.016 ± .06	—	6.2 ± 2.2	—	4.2 ± 1.6	—
C. IT anti-BSA + L652,763 (60 μg/kg); IV BSA (positive control)	.56 ± .11	—	.419 ± .12	—	106.8 ± 7.5	—	66.7 ± 4.2	—
D. IT anti-BSA + L652,731 (60 μg/kg); IV BSA	.38 ± .06	$P < .05$ vs. A,C	.399 ± .14	NS vs. A,C	108.2 ± 8.9	NS vs. A,C	48.6 ± 4.1	$P < .05$ vs. C
E. IT anti-BSA + non-spec IgG (80 μg); IV BSA (positive control)	.61 ± .14	—	.429 ± 14	—	100.6 ± 7.3	—	70.3 ± 2.9	—
F. IT anti-BSA + anti IL-1β (80 μg); IV BSA	.29 ± .10	$P < .05$ vs. E	.215 ± .08	$P < .05$ vs. E	52.2 ± 6.0	$P < .05$ vs. A,E	38.5 ± 4.7	$P < .05$ vs. E
G. IT anti-BSA + anti IL-1β (80 μg) plus L-652,731 (60 μg/kg); IV BSA	.31 ± .09	NS vs. D,F $P < .05$ vs. C,E	.194 ± .12	$P < .05$ vs. C,E NS vs. F	48.6 ± 5.8	$P < .05$ vs. A,C,E NS vs. F	36.2 ± 5.2	NS vs. F

* IgG immune-complex alveolitis was induced as described in the Methods section, IT, intratracheal; IV, intravenous.
† MPO activity (whole lung homogenates) is expressed as ΔO.D. (A460) per minute resulting form decomposition of H_2O_2, in the presence of o-dianisidine.[28]
‡ Sum PMN equals the total of neutrophils contained in the alveolar, interstitial, and vascular compartments.
‖ One-way analysis of variance.[41] NS, not significant at $P < 0.05$.
Alveolar PMN (neutrophils contained within the alveolar compartment)
These data represent the results of one experiment with 6 rats/group.

increases in microvascular permeability and leukocyte infiltration into the eyes of rabbits after the intravitreal injection of human IL-1α. Sun and Hsueh[23] have shown that PAF antagonists can block bowel necrosis induced in rodents with infusions of TNF. Finally, we have observed that neutralization of intrapulmonary TNF activity with anti-TNF antibodies results in reduced whole lung and BAL fluid PAF levels and a concomitant reduction in acute lung injury.[9]

Data presented in the present study (Figure 4) suggest that resident alveolar macrophages are a potential source of cytokine-triggered PAF production. The concentrations of PAF per cell and the time course characteristics of IL-1α and IL-1β-induced PAF secretion by alveolar macrophages are compatible with the *in vivo* BAL fluid and whole lung PAF activities measured in evolving immune complex alveolitis. Other cell types indigenous to the lung during evolving injury are also likely to produce PAF in response to cytokines, including IL-1β. As noted previously, monocytes, neutrophils, and vascular endothelial cells have been shown to produce PAF upon exposure to TNF or IL-1.[16–20] PAF itself may modulate cytokine production. For instance, Salem et al[40] have demonstrated that PAF upregulates IL-1 production by muramyl dipeptide-stimulated monocytes. One can envision a scenario in which a cytokine such as IL-1β induces the local production of PAF which in turn plays a role in neutrophil translocation and activation as well as a role in modulating IL-1β production.

The present study confirms that IL-1β and PAF play roles in the pathogenesis of acute IgG immune complex-induced alveolitis in the rat. The data further suggest that intrapulmonary PAF secretion is modulated by local IL-1 production and that IL-1 and PAF play distinctive, but related roles in pulmonary neutrophil recruitment in IgG immune complex-induced alveolitis.

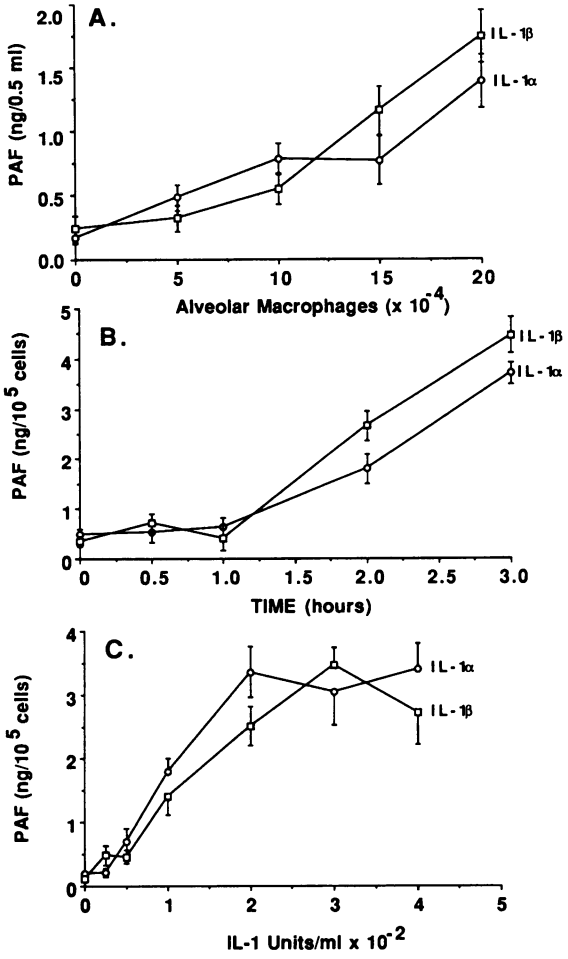

Figure 4. A: *PAF secretion by mouse alveolar macrophages stimulated with recombinant murine IL-1α (200 U/ml) and IL-1β (200 U/ml) as a function of cell concentration. Incubations were carried out in 0.5 ml of RPMI 1640-1% BSA for 3 hours at 37°C. Cells were incubated for 1 hour before addition of cytokine. IL-1-α and IL-1β-triggered PAF secretion could be completely blocked with anti-IL-1α (5 μg) and anti-IL-1β (5 μg), respectively (data not shown).* B: *PAF secretion by mouse alveolar macrophages (4 × 10⁵) stimulated with recombinant murine IL-1α (200 U/ml) and IL-1β (200 U/ml) as a function of time. Incubation conditions are described above. IL-1-α and IL-1β-triggered PAF secretion could be blocked at each timepoint with anti-IL-1α (5 μg) and anti-IL-1β (5 μg), respectively (data not shown).* C: *PAF secretion by mouse alveolar macrophages (4 × 10⁵) as a function of recombinant mouse IL-1α and IL-1β concentrations. Data are expressed as the cumulative means ± SEM for three experiments in which each variable was run in duplicate. Platelet-activating factor (ng/ml in frame A) and ng/10⁵ cells in frames B and C) values were interpolated from a standard curve generated with exogenous PAF in the [³H]serotonin release assay (Methods).*

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