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Recombinant Human Platelet-Activating Factor-Acetylhydrolase Inhibits Airway Inflammation and Hyperreactivity in Mouse Asthma Model

William R. Henderson, Jr.,* Jiangyang Lu,†† Karen M. Poole,‡ Gregory N. Dietsch,† and Emil Y. Chi††

Numerous in vitro and in vivo studies in both animal models and human asthmatics have implicated platelet-activating factor (PAF) as an important inflammatory mediator in asthma. In a murine asthma model, we examined the anti-inflammatory activities of recombinant human PAF-acetylhydrolase (rPAF-AH), which converts PAF to biologically inactive lyso-PAF. In this model, mice sensitized to OVA by i.p. and intranasal (i.n.) routes are challenged with the allergen by i.n. administration. The OVA challenge elicits an eosinophil infiltration into the lungs with widespread mucus occlusion of the airways and results in bronchial hyperreactivity. The administration of rPAF-AH had a marked effect on late-phase pulmonary inflammation, which included a significant reduction in airway eosinophil infiltration, mucus hypersecretion, and airway hyperreactivity in response to methacholine challenge. These studies demonstrate that elevating plasma levels of PAF-AH through the administration of rPAF-AH is effective in blocking the late-phase pulmonary inflammation that occurs in this murine allergen-challenge asthma model. These results suggest that rPAF-AH may have therapeutic effects in patients with allergic airway inflammation. The Journal of Immunology, 2000, 164: 3360–3367.

Platelet-activating factor (PAF)†† (1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphocholine) is a potent proinflammatory lipid mediator with diverse biologic activities relevant to the pathogenesis of asthma (1, 2). In both laboratory species and humans, PAF administration mimics many of the immunologic and physiologic events associated with asthma. The instillation of PAF into the airways of animals leads to the recruitment of eosinophils into the lungs producing an intense, prolonged bronchial inflammation (3). In animals, PAF administration induces other physiologic events frequently associated with asthma including bronchoconstriction (4), mucus secretion (5, 6), increased vascular permeability (7–10), and nonspecific bronchial hyperresponsiveness (11, 12).

In normal human volunteer subjects, inhalation of aerosolized PAF produces many of these same effects including dose-dependent bronchoconstriction and nonspecific airway hyperresponsiveness (13). Studies with asthmatic subjects have found that plasma PAF levels increase after allergen challenge (14) and are significantly elevated during asthmatic attacks compared with basal levels measured in the absence of symptoms (15). Higher concentrations of PAF are found in sputum (16) and bronchoalveolar lavage (BAL) fluid from asthmatic patients than from subjects without asthma (17). Patients with asthma also have increased PAF receptor mRNA levels in their lungs (18).

The potent biologic activities of PAF require that the mediator’s synthesis and subsequent degradation be tightly regulated. In plasma, the conversion of PAF to biologically inactive lyso-PAF is catalyzed by a 43-kDa protein known as PAF-acetylhydrolase (PAF-AH) (19). The plasma form of human PAF-AH has been cloned and expressed as a recombinant protein (rPAF-AH) (19, 20). PAF-AH is a group VII phospholipase A2 that hydrolyzes the sn-2 bond of PAF, limiting its half-life to a few minutes (19, 21). Biochemical studies have demonstrated that rPAF-AH has substrate specificity and enzymatic activity comparable to that of native plasma PAF-AH (20). rPAF-AH suppresses PAF-induced inflammation in vivo. After i.v. administration, rPAF-AH inhibits PAF-induced pleurisy and paw edema in rats (20). A genetically linked plasma PAF-AH deficiency has been found in 4% of the Japanese population (22). This deficiency is attributable to a missense mutation in exon 9 of the plasma PAF-AH gene (V279F) in which phenylalanine replaces valine 279 (23). The V279F mutation causes a complete loss of PAF-AH enzyme activity (23). In the Japanese population, the prevalence of the V279F mutation is higher in asthmatic than in control subjects; patients homozygous for this mutation are more likely to develop the most severe form of asthma (24).

Despite the findings linking PAF to the etiology of asthma, clinical trials of potent PAF receptor antagonists in the treatment of asthma have been disappointing. A novel alternate approach to limiting PAF’s effects in asthma is elevating endogenous plasma PAF-AH levels by the administration of rPAF-AH. To test the therapeutic potential of rPAF-AH in asthma, we examined the effect of exogenously administered rPAF-AH in a murine model of...
allergen-induced asthma (25, 26). In this model, airway inflammation was induced in OVA-sensitized mice by intranasal (i.n.) OVA challenge administered on 3 consecutive days. Twenty-four hours after the last i.n. OVA challenge, airway inflammation and hyperreactivity to methacholine were evaluated. We found that rPAF-AH treatment inhibits mucus hypersecretion, eosinophil influx into the lungs, and airway hyperreactivity to methacholine after allergen challenge.

**Materials and Methods**

**Animals**

Female BALB/c mice (6–8 wk of age; D&K, Seattle, WA) were used in all experiments. All animal study protocols were approved by the University of Washington Animal Care Committee.

**Allergen induction of eosinophil infiltration**

To induce eosinophil infiltration into murine airway tissue, mice were sensitized and later challenged with OVA (Pierce, Rockford, IL) as the allergen. Mice were immunized with OVA (100 μg) complexed with aluminum potassium sulfate in a 0.2-ml volume, administered by i.p. injection on days 0 and 14 and 0.05 ml normal saline by the i.n. route on days 25, 26, and 27. Mice were anesthetized with 0.2–0.3 ml of ketamine (6.5 mg/ml) and xylazine (0.44 mg/ml) diluted in normal saline. The OVA, rPAF-AH (100 μg)/OVA, and rPAF-AH (200 μg)/OVA groups all received 100 μg OVA in 0.05 ml normal saline by the i.n. route on day 14 and 50 μg of OVA in 0.05 ml normal saline by the i.n. route on days 25, 26, and 27. The control group received normal saline with aluminum potassium sulfate by the i.p. route on days 0 and 14 and 0.05 ml normal saline by the i.n. route on days 14, 25, 26, and 27.

**Administration of rPAF-AH**

The rPAF-AH (100 μg)/OVA group received a rPAF-AH dosage of 100 μg/day, whereas the rPAF-AH (200 μg)/OVA group received a dosage of 200 μg/day. The initial rPAF-AH dose was administered by i.p. injection 30 min before challenge with OVA on day 25. Previous pharmacokinetic studies indicated that the plasma half-life of rPAF-AH in the mouse was in the range of 6–8 h. Therefore, to maintain plasma rPAF-AH levels greater than 5 μg/ml during the course of the allergen challenge, mice received additional rPAF-AH dosages on days 26 and 27 by the i.p. route 30 min before OVA treatment. rPAF-AH was supplied by ICOS (Bothell, WA).

**Pulmonary function testing**

On day 28, −24 h after the last i.n. administration of either normal saline or OVA, pulmonary mechanics in response to an i.v. infusion of methacholine in the two-chamber whole-body plethysmograph; the dead space of the system was 0.025 ml. The following minute ventilation maintained normal arterial blood gases: tidal volume, 0.2 ml/20 gm; frequency, 120 breaths/min; and positive-end expiratory pressure, 2.5–3.0 cm H2O (29). Copper mesh in the plethysmograph served as a heat sink for rapid gas compression during each tidal breath. Because the plethysmograph is a closed system, a change in box volume (Vt) measured by a sensitive transducer (±0.7 cm H2O) represented the change in box volume (∆Vt = ∆Pbox). Pbox is equivalent to lung pleural pressure. The system was calibrated by delivering a known volume of 0.2 ml of air at a frequency of −120/min such that a calibration factor was equal to 0.2 ml/∆Pbox. Lung volume was equal to ∆Pbox with each breath multiplied by the calibration factor. Ambient pressure swings and temperature increases in the first chamber were offset as previously described (25). Pressure movements at the opening of the tracheal tube (P0) were measured by another transducer referenced to Pbox to determine transpulmonary pressure (P0 = Pbox − P0). After initial inflation to a P0 of 30–35 cm H2O, the lungs were inflated at least once 1–2 min before each measurement to prevent partial collapse. An analogue-to-digital data acquisition system (Strawberry Tree, Sunnyvale, CA) was employed to sample P0 and Pbox at 5 ms intervals with a smoothing function employed to dampen background noise as previously described (25). Flow was calculated by the change in volume from ∆Pbox point-to-point/5 ms.

Data from seven consecutive breaths collected three times during the first 10 min were used to calculate basal pulmonary function. Methacholine was then infused by hand delivery into the jugular vein over 10 s at a concentration of 120 μg/kg after 10 min of ventilation. Dynamic compliance (Cdyn) was determined for both the control period and during the peak response to methacholine challenge. Tracheal tube resistance (0.63 cm H2O × ml−1 × s) was subtracted from all airway resistance measurements. Cdyn was calculated as the change in tidal volume (∆Vt) divided by the difference between P0 at end-inspiration and end-expiration when flow is zero (∆P0 = ∆Vt/∆Pbox), the inflation of recruitment at which each mouse was exsufflated by cardiac puncture; plasma samples were collected from all mice in each experimental group and stored at −70°C until assay of rPAF-AH.

**Bronchoalveolar lavage**

After tying off the left lung of the mainstem bronchus, the right lung was lavaged three times with 0.4 ml of normal saline. The three BAL fluid samples collected from each animal were pooled, and the number of cells in a 0.05-ml aliquot was determined using a hemocytometer. The remaining sample was centrifuged at 4°C for 10 min at 200 × g, and the supernatant was stored at −70°C until assay of rPAF-AH and IL-5 levels. The cell pellet was resuspended in normal saline containing 10% BSA, and BAL fluid cell smears were made on glass slides. To stain eosinophils, dried slides were stained with Diff-Quik’s diluting fluid (0.05% aqueous dye in 1% ethyl alcohol, 5% acetic acid) for 2 min. Slides were washed in water for 0.5 min, and counterstained with 0.07% methylene blue for 2 min.

**Lung histology**

The trachea and left lung (upper and lower lobes) were collected and fixed in Carnoy’s solution at 20°C for 15 h. After embedding in paraffin, the tissues were cut into 5-μm sections. Eosinophils were stained in the lung tissue with Diff-Quik’s solution as described above. The number of eosinophils per unit airway area (2200 μm2) was determined by morphometric analysis as previously described (25, 30). Airway mucus was identified by the following staining methods: hematoxylin and eosin, methylene blue, mucicarmine, toluidine blue, alcian blue, and alcian blue/periodic acid–Schiff (PAS) reaction as previously described (25). Mucin was stained with mucicarmine solution. Mucin and sialic acid-rich nonsulfated mucosubstances were stained metachromatically with toluidine blue (pH 4.5) and acidic mucin, and sulfated mucosubstances were stained with alcian blue (pH 2.5) and PAS reaction (25).

Oclusion of the airway diameter by mucus was assessed on a semi-quantitative scale ranging from 0 to 5+ (25). For each mouse, 10 airway sections randomly distributed throughout the left lung were assessed for mucus occlusion by morphometric analysis by individuals blinded to the protocol design. Each airway section was assigned a score for airway diameter occlusion by mucus based on the following criteria: 0, no mucus; 1, <10% occlusion; 2, 10–30% occlusion; 3, 30–50% occlusion; 4, 50–80% occlusion; 5, >80% occlusion (25). Mucus occlusion was assessed morphometrically in 8–10 airways randomly distributed throughout the lungs of each mouse.

**Immunocytochemistry (ICC)**

Esterase-positive macrophages in the lung sections were detected by the α-naphthyl acetate histochemical method (31). The phenotype of the lung esterase-positive macrophages was assessed by ICC. The number of CD11b- and CD11c-positive macrophages per unit airway area (2200 μm2) in perivascular, periairway, and alveolar regions of the lungs was determined by morphometric analysis as previously described (25, 30). The lung sections were incubated for 30–60 min at room temperature with the primary Ab, either rat IgG1 Ab against mouse CD11b/CD18 (Mac-1) (100 μg/0.5 ml, clone M1/70 (9)) purchased from Boehringer Mannheim, (Indianapolis, IN) or hamster IgG1 Ab against mouse CD11c (>1 mg/ml, secreted from hybridoma cell line N418CB8224; American Type Culture Collection, Manassas, VA; and kindly provided by Dr. Andrew G. Farr, University of Pittsburgh, PA). After washes, the sections were then incubated for 15–45 min at room temperature with HRP solution of the ABC Elite Kit (Vector Laboratories, Burlingame, CA) and were counterstained with 2% aqueous methyl green, dehydrated in a series of ethanol concentrations up to 100%, cleared in xylene, and mounted with Permount (Fisher Scientific, Pittsburgh, PA).
FIGURE 1. rPAF-AH reduces the number of eosinophils in the BAL fluid after OVA challenge. BAL fluid was obtained from saline-treated mice (Saline; n = 10), OVA-sensitized/challenged mice (OVA; n = 10), OVA-sensitized/challenged mice treated with rPAF-AH at a dosage of 100 µg/day (rPAF-AH (100 µg/OVA; n = 9)), or OVA-sensitized/challenged mice pretreated with rPAF-AH at a dosage of 200 µg/day (rPAF-AH (200 µg)/OVA; n = 4). The number of total cells (A) and percentage (B) and number (C) of eosinophils present in BAL fluid from each group are presented as the mean ± SE. The p values between OVA vs either rPAF-AH (100 µg)/OVA or rPAF-AH (200 µg)/OVA were determined using a Student’s two-tailed t test.

Assay of IL-5 in BAL fluid

Levels of IL-5 in the BAL fluid were determined by ELISA using EM IL-5 kit (Endogen, Woburn, MA). The IL-5 kit has a sensitivity of <5 pg/ml. Dilutions of the BAL fluid were made starting at 1:2. IL-5 standards, ranging from 20 to 320 pg/ml, were also assayed. A standard polynomial equation (y = a + bc + cx²) was used to create a standard curve for the IL-5 standards. Concentrations of IL-5 in the BAL fluid were calculated from the line equation and the sample dilution factor.

Assay of rPAF-AH levels in plasma and BAL fluid

Levels of rPAF-AH in the plasma and BAL fluid were determined by a standard enzyme immunoassay (EIA) method described below. The EIA is specific for human PAF-AH and does not detect endogenous murine PAF-AH, which is present in the plasma and possibly in the BAL fluid samples. For the EIA, Immulon 4 “C” plates (Dynex Technologies, Chantilly, VA) were coated with 125 µl of the rPAF-AH-specific mAb 90G11D diluted to 3.0 µg/ml; after removing the coating solution, wells were blocked. Murine plasma and BAL fluid samples were plated in triplicate at 100 µl/well into 90G11D-coated plates. After incubation at 37°C for 30 min, plates were washed. 100 µl volume of a second rPAF-AH-specific mAb, biotin-labeled 90F2D, was added to each well, and plates were incubated for 30 min at 37°C. After extensive washing, 100 µl of Strepavidin-HRP (Pierce) was added to each well, and absorbance at 450/630 nm was read on an EL 312e Bio-kinetic microplate reader (Bio-Tek, Winooski, VT) within 30 min of adding the sample dilution factor. Data analysis was performed by calculating the mean A450 for each of the standards, test samples, and assay control samples. A standard polynomial equation (y = a + bc + cx²) was used to create a standard curve for the rPAF-AH standards. Concentrations of rPAF-AH in the sample specimens were calculated using the line equation, the sample mean A450, and the sample dilution factor.

Statistical analysis

The data are presented as the mean ± SE of the mean. A Student’s two-tailed t test was used to compare data for BAL fluid eosinophil counts and airway mucus between the different experimental groups. For the evaluation of pulmonary mechanics, a Fisher protected least significant difference test was used to compare values for Cbm between experimental groups. A one-way ANOVA (Dunn’s method) was used to compare IL-5 levels in the BAL fluid between the experimental groups. Differences were considered statistically significant for p values <0.05.

Results

rPAF-AH levels in the plasma and BAL fluid

On day 28, plasma and BAL fluid samples were collected 24 h after administration of the final rPAF-AH dosage and were assayed for rPAF-AH levels. In an effort to maintain plasma rPAF-AH levels above 5 µg/ml (~10-fold over the endogenous PAF-AH level in plasma), for the duration of the allergen challenge, a dosage of 100 µg/day was selected for intervention in this murine asthma model. We also determined whether increasing the rPAF-AH dosage to 10 mg/kg/day (200 µg/mouse/day) had additional effects in this model. In the rPAF-AH (100 µg)/OVA group, which received a rPAF-AH dosage of 100 µg/day on days 25, 26, and 27, the mean plasma concentration was 5.7 ± 0.7 µg/ml on day 28. Increasing the rPAF-AH dosage to 200 µg/day in the rPAF-AH (200 µg)/OVA group resulted in mean plasma rPAF-AH levels of 7.4 ± 2.8 µg/ml on day 28. BAL fluid samples from the rPAF-AH (100 µg)/OVA and rPAF-AH (200 µg)/OVA groups did not have detectable levels of rPAF-AH. As expected, rPAF-AH was not detected in any plasma (or BAL fluid) samples collected from the saline and OVA groups.

Effect of rPAF-AH on eosinophil and macrophage recruitment into the lungs

Twenty-four hours after the final i.n. OVA challenge, BAL was performed on the right lung of all animals from each experimental group. The mean number of eosinophils in BAL fluid collected from the saline group was 0.01 ± 0.003 × 10⁶ cells (Fig. 1C). After OVA treatment, the number of eosinophils in the BAL fluid from the OVA group increased 285-fold to 2.85 ± 0.68 × 10⁵ (saline group vs OVA group; p = 0.0005). Pretreatment with either 100 µg/day or 200 µg/day of rPAF-AH reduced eosinophil infiltration into the BAL fluid by 74% (p = 0.0095; rPAF-AH (100 µg)/OVA vs OVA) and by 80% (p = 0.05; rPAF-AH (200 µg)/OVA vs OVA), respectively (Fig. 1C). rPAF-AH (200 µg/day) also significantly reduced eosinophil infiltration into the lung parenchyma and alveoli of OVA-sensitized/challenged mice (Fig. 2. C vs B; Fig. 3; Fig. 4, B vs A; and Fig. 5, B vs A).

Despite the reduction in lung eosinophils, the total number of cells recovered in the BAL fluid was similar in OVA-treated mice in the absence or presence of rPAF-AH at a dosage of either 100 µg/day or 200 µg/day (Fig. 1A). rPAF-AH treatment induced a significant increase in macrophages in the lungs of the OVA-treated mice (Fig. 4, B vs A; Fig. 5, B vs A; and Fig. 6). The number of esterase-positive macrophages was increased in the perivascular, periairway, and alveolar areas of the lungs of the rPAF-AH-treated mice (Fig. 6). ICC of the lung tissue was performed to phenotype the lung macrophages. A significant increase in both CD11b- and CD11c-positive macrophages in each of these lung regions was seen in OVA-sensitized/challenged mice.
treated with rPAF-AH (200 μg/day) compared with OVA treatment alone (Fig. 6).

**Effect of rPAF-AH on airway mucus release**

A marked increase in mucus-secreting airway cells and airway mucus as detected histochemically was observed in OVA-treated mice compared with saline controls (Fig. 2, B vs A; Fig. 3; and Fig. 7A). rPAF-AH (200 μg/day) significantly reduced the percentage of airway cells staining positive for mucus glycoproteins in the lungs of the OVA-treated mice (Fig. 3 and Fig. 7, B vs A). Daily i.p. administration of rPAF-AH during allergen challenge also reduced occlusion of the airways resulting from mucus secretion (Fig. 3 and Fig. 7, B vs A). In the OVA group, a mean score of 3.38 ± 0.30 was seen representing 60–80% occlusion of the airways by mucus (0–5+ scale) were determined by morphometric analysis; 10 lung sections per mouse were examined. *, p < 0.05, rPAF-AH/OVA vs OVA.

**FIGURE 2.** Effect of rPAF-AH on airway inflammation in OVA-treated mice. Lung tissue (upper and lower lobes of left lung) of saline-treated mice (A) and OVA-sensitized/challenged mice in the absence (B) or presence (C) of rPAF-AH treatment (200 μg/day) was obtained, stained with hematoxylin and eosin, and examined by light microscopy. A, In the saline-treated control mice, inflammatory cells are absent in the lung parenchyma and alveoli (A). Mucus release is not present in the airway (AW) lumen. B, Airway (AW) mucus plugging is observed in the OVA-sensitized/challenged mice. An intense leukocytic infiltration of the lung parenchyma (arrows) and alveoli (A) by eosinophils and other inflammatory cells is evident. C, rPAF-AH treatment reduced the inflammatory cell infiltration of the parenchyma (arrows) and alveoli (A) and airway (AW) mucus release in the OVA-treated mice. Bar = 100 μm.

**FIGURE 3.** rPAF-AH blocks airway eosinophil infiltration and mucus release in OVA-treated mice. Lung tissue was obtained from saline-treated mice (Saline; n = 10) and OVA-sensitized/challenged mice in the absence (OVA; n = 10) or presence (rPAF-AH (200 μg)/OVA; n = 8) of rPAF-AH treatment at a dosage of 200 μg/day. The number of eosinophils per unit area (2200 μm²), percentage of airway cells positive for mucus glycoproteins by alcian blue/PAS staining, and occlusion of airway diameter by mucus (0–5+ scale) were determined by morphometric analysis; 10 lung sections per mouse were examined. *, p < 0.05, rPAF-AH/OVA vs OVA.

**Effect of rPAF-AH on IL-5**

In the saline group, the mean BAL fluid IL-5 levels were 0.5 ± 0.2 pg/ml (Fig. 8). In the OVA group, a 52-fold increase in BAL fluid IL-5 levels were seen with a mean concentration of 22.2 ± 3.9 pg/ml. In the rPAF-AH (100 μg)/OVA group, the rPAF-AH dosage of 100 μg/day had no effect on IL-5 levels in the BAL fluid with a mean of 26.1 ± 5.8 pg/ml. However, in the rPAF-AH (200 μg)/OVA group, mean BAL fluid levels of IL-5 decreased to 9.7 ± 3.1 pg/ml, a 56.3% reduction compared with the OVA-treated group; however, this effect was not statistically significant compared with the OVA-treated group (rPAF-AH (200 μg)/OVA group vs OVA group; p = 0.1).
Effect of rPAF-AH on allergen-induced airway hyperreactivity to methacholine

Airway reactivity was evaluated on day 28, which was 24 h after the third i.n. challenge. In the OVA group, airway hyperreactivity was seen after challenge with methacholine with a significant decrease in $C_{dyn}$ compared with the saline group (saline group vs OVA group; $p < 0.001$) (Fig. 9). rPAF-AH at 100 $\mu$g/day (rPAF-AH (100 $\mu$g)/OVA group) given before OVA on days 25, 26, and 27 did not significantly reduce bronchial hyperresponsiveness to methacholine in the OVA-sensitized and challenged mice. In contrast, rPAF-AH at 200 $\mu$g/day significantly decreased the methacholine-induced lung response (rPAF-AH (200 $\mu$g)/OVA group vs OVA group; $p = 0.0438$).

Discussion

The study aim was to determine whether the administration of rPAF-AH reduced airway inflammation and hyperreactivity in a murine model of allergen-induced asthma. In this model, mice sensitized to OVA were exposed to i.n. OVA challenge for three consecutive days. This allergen challenge leads to airway hyperresponsiveness to methacholine, extensive eosinophil infiltration of the lung, and occlusion of the airways by mucus hypersecretion; these effects were significantly reduced by treatment with rPAF-AH.

Plasma levels of rPAF-AH greater than 5 $\mu$g/ml were achieved 24 h after the final rPAF-AH administration at both 100 $\mu$g/day and 200 $\mu$g/day dosages. The 100 $\mu$g/day dosage resulted in mean plasma rPAF-AH levels of 5.7 $\pm$ 0.7, whereas plasma rPAF-AH levels of 7.4 $\pm$ 2.8 $\mu$g/ml were achieved with the 200 $\mu$g/day dosage. These plasma rPAF-AH levels are sufficient to block PAF-mediated inflammation in other animal models (20). rPAF-AH was not detected in BAL fluid collected from mice treated with rPAF-AH, indicating that extravasation of the enzyme into the alveolar space did not occur in the model. This suggests that degradation of PAF by rPAF-AH, which reduces eosinophil infiltration and mucus secretion in the airways, occurs within the blood vessels. Although rPAF-AH treatment at a dosage of 100 $\mu$g/day did not reduce airway hyperresponsiveness or affect IL-5 levels in BAL fluid, eosinophil infiltration into the lungs was inhibited. When the dosage was increased to 200 $\mu$g/day, reduction in BAL fluid IL-5 levels and airway hyperreactivity to methacholine was observed.

PAF is a potent chemotactic and chemokinetic factor for eosinophils (32). PAF promotes eosinophil recruitment to sites of allergic inflammation through activation of the eosinophil adhesion molecules, the $\beta_1$ integrin very late Ag-4 ($\alpha_\beta_1$; CD49d/CD29),...
and the β2 integrin CD11/CD18, which interact with their respective ligands on endothelial cells, VCAM-1, and ICAM-1 (33). The increased adherence of eosinophils to cultured HUVEC has been shown in vitro when the eosinophils are activated by PAF (34). Eosinophil activation by PAF also induces the selective transendothelial migration of eosinophils across unstimulated HUVEC (35). In an in vitro model of human eosinophil transmigration through basement membrane components, both an eosinophil-activating cytokine (e.g., IL-5) and a specific chemoattractant (e.g., PAF) are required for the eosinophil transmigration (36). Thus, inactivation of PAF by rPAF-AH may interfere with a variety of PAF-induced mechanisms of eosinophil recruitment including reduction in IL-5, which primes eosinophils for migration into allergic inflammatory sites.

Unexpectedly, increased numbers of CD11b- and CD11c-positive macrophages were recovered in the lung parenchyma and alveoli of the rPAF-AH-treated allergic mice. The CD11c immunostaining of these cells suggests that they are either dendritic cells or activated macrophages (37, 38). The airways of patients with

![FIGURE 6. rPAF-AH increases CD11b- and CD11c-positive macrophages in OVA-treated mice. Lung tissue was obtained from saline-treated mice (Saline, n = 6) and OVA-sensitized/challenged mice in the absence (OVA; n = 8) and presence (rPAF-AH/OVA; n = 8) of rPAF-AH treatment at a dosage of 200 µg/day. The numbers of esterase-, CD11b-, and CD11c-positive macrophages per unit area (2200 µm²) in perivascular, periairway, and alveolar areas of the lung tissue were determined by morphometric analysis; 10 lung sections per mouse were examined. *p < 0.05 is shown for rPAF-AH/OVA vs OVA.](image)

![FIGURE 7. Effect of rPAF-AH on airway mucus in OVA-treated mice. Lung tissue was obtained from saline-treated mice (Saline, n = 6) and OVA-sensitized/challenged mice in the absence (OVA; n = 8) and presence (rPAF-AH/OVA; n = 8) of rPAF-AH treatment at a dosage of 200 µg/day. Lung sections were stained with alcian blue/PAS; 10 sections per mouse were examined. A, In OVA-sensitized/challenged mice, the bronchiolar epithelium contains many mucus-positive cells (arrows). Mucus plugging of the airway (AW) lumen is noted. B, Fewer cells are positive for mucus (arrows) in the epithelium, and mucus release is greatly reduced in the airway (AW) of OVA-sensitized/challenged mice treated with rPAF-AH compared with mice treated with OVA alone. Bar = 100 µm.](image)

![FIGURE 8. Effect of rPAF-AH on IL-5 levels in BAL fluid of OVA-treated mice. IL-5 levels (in pg/ml; mean ± SE) were determined in the BAL fluid obtained on day 28 from saline-treated mice (Saline; n = 12) and OVA-sensitized/challenged mice in the absence (OVA; n = 12) or presence of rPAF-AH treatment at a dosage of either 100 µg/day (rPAF-AH (100 µg)/OVA; n = 8) or 200 µg/day (rPAF-AH (200 µg)/OVA; n = 4).](image)

![FIGURE 9. Effect of rPAF-AH on pulmonary compliance. Lung compliance (Cdyn) was determined on day 28 in saline-treated mice (Saline) and in OVA-sensitized/challenged mice in the absence (OVA) or presence of rPAF-AH at a dosage of 100 µg/day (rPAF-AH (100 µg)/OVA) or 200 µg/day (rPAF-AH (200 µg)/OVA) as described in Fig. 1. *p < 0.05 vs OVA.](image)
asthma have an increased number of dendritic cells (39), and dendritic cells are found in lung parenchymal germinal centers after allergen challenge (40). The mechanism(s) and effects of the observed influx into the lungs of CD11b- and CD11c-positive mononuclear cells in our murine asthma model are unknown. Lung macrophages may play a protective role in limiting pulmonary inflammatory responses to allergens and other stimuli (41–46). Human alveolar macrophages after IgE-dependent activation suppress lymphocyte proliferation (42). Human monocyte-derived macrophages can ingest senescent leukocytes (46), and hydrogen peroxide-induced lung injury is reduced by alveolar macrophage antioxidants (44). In patients with the adult respiratory distress syndrome (ARDS), the number of alveolar macrophages in BAL fluid increases (both as a percentage of total cells and in absolute numbers) in survivors of ARDS (43).

Another potent anti-inflammatory effect exhibited by rPAF-AH treatment in this murine asthma model was blockade of airway mucus release. In rodent, feline, and human lung explants, in vitro administration of PAF induces mucus glycoprotein release (47–49). Similarly, in vivo administration of PAF by either inhaled or i.v. routes stimulates airway mucus release in ferrets (5, 6). A portion of PAF receptor-induced mucus glycoprotein secretion is mediated indirectly by release of leukotrienes, 5-lipoxygenase pathway products of arachidonic acid metabolism (48).

PAF induces bronchoconstriction directly by action on airway smooth muscle PAF receptors and indirectly by induction of other bronchoconstrictor molecules such as thromboxane A2 and cysteinyl leukotrienes (1, 50). In mice, PAF-induced airway hyperreactivity to acetylcholine is controlled by a single gene and is not dependent on PAF-induced hyperpermeability (51). Transgenic mice overexpressing the PAF receptor have increased airway responsiveness to methacholine compared with their littermate controls (52). Prior studies in animal models (50, 53) and in some studies of normal human volunteers have shown that PAF inhalation can increase bronchial hyperreactivity for prolonged periods (13, 54). However, other studies have failed to demonstrate increased airway hyperreactivity after PAF inhalation in normal (55, 56) or asthmatic subjects (57, 58). Although the PAF receptor antagonists WEB2086 (59) and U-K-74505 (60) do not reduce airway responsiveness after allergen challenge, the more potent PAF antagonist Y-24180 significantly reduces bronchial hyperresponsiveness in patients with asthma, as determined by improvement in the provocative concentration (PC) of methacholine, producing a 20% fall in the 1-s forced expiratory volume (FEV1) from control (PC20FEV1) values (61).

In our asthma model, airway reactivity was evaluated on day 28, which was 24 h after the third i.n. challenge of BALB/c mice with OVA. Mice in the OVA group demonstrated airway hyperreactivity when challenged with methacholine (i.e., C0.025 was significantly reduced compared with the control group). A dissociation between the effects of rPAF-AH on airway eosinophilia and hyperresponsiveness was observed. Although eosinophil influx into the lungs was inhibited comparably by the two doses of rPAF-AH employed, airway hyperreactivity was only affected by the highest dose (200 μg/day). These data showing a discordance between airway eosinophilia and hyperreactivity are consistent with our prior work demonstrating that 5-lipoxygenase and 5-lipoxygenase-activating protein inhibitors of leukotriene synthesis inhibit allergen-induced lung eosinophil recruitment without affecting bronchial hyperresponsiveness to methacholine (25). Similarly, inhibition of lung eosinophilia by anti-IL-5 mAb treatment fails to affect airway hyperreactivity in some (62) but not all murine asthma models (63). Using a CD49d mAb in our mouse model of asthma, we have found that local, intrapulmonary blockade of CD49d by i.n. administration of the Ab blocks all evidence of lung inflammation and airway hyperreactivity to methacholine (64). In contrast, systemic administration of CD49d mAb to block CD49d on circulating leukocytes prevents only airway eosinophilia and not bronchial hyperresponsiveness (64). Overall, these studies demonstrate that allergen-induced airway hyperreactivity may develop in the absence of eosinophilia.

Thus, PAF is likely an important mediator of both the airway hyperreactivity and late-phase inflammation that occur in this allergen-induced model of asthma. These data suggest that rPAF-AH treatment may reduce allergen-induced airway disease.

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References


