

Nasal neutrophilia and eosinophilia induced by challenge with platelet activating factor

Alberto Tedeschi, MD, Giuseppe Palumbo, MD, Nunzio Milazzo, MD, and Antonio Miadonna, MD Milan, Italy

Background: It has been demonstrated that *in vitro* platelet activating factor-acether (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine; PAF) has the capacity to attract eosinophils and neutrophils. We investigated whether the same applies when human nasal airways are stimulated with PAF.

Methods: Symptom scores and cytologic changes in nasal lavage fluids were evaluated in 10 atopic and 10 nonatopic subjects after nasal challenge with PAF, its precursor and metabolite, 1-O-hexadecyl-sn-glycero-3-phosphorylcholine (lyso-PAF), or saline solution.

Results: Nasal obstruction was reported by all the atopic subjects and seven of the 10 nonatopic subjects after nasal challenge with PAF; other symptoms such as rhinorrhea, itching, and sneezing were generally mild. PAF induced neutrophilia, which appeared after 30 minutes in atopic subjects and after 1 hour in nonatopic subjects, and peaked at 3 hours in both. Less neutrophilia was found 3 hours after stimulation with lyso-PAF in both groups of subjects. PAF also induced eosinophilia, which appeared after 30 minutes in atopic subjects and only after 3 hours in nonatopic subjects. An increase in eosinophil counts was observed 3 hours after lyso-PAF stimulation in atopic but not in nonatopic subjects.

Conclusion: PAF can attract neutrophils and eosinophils into human nasal airways; however, the recruitment of inflammatory cells is more rapid in atopic than in nonatopic subjects, suggesting a different degree of responsiveness to PAF challenge in the two groups of subjects. (*J ALLERGY CLIN IMMUNOL* 1994;93:526-33.)

Key words: Allergic inflammation, eosinophils, lyso-PAF, nasal airways, neutrophils, platelet activating factor

Platelet activating factor-acether (PAF) is now recognized as a mediator of inflammatory and allergic reactions.¹ Several clinical and experimental findings indicate that PAF may contribute to the pathogenesis of respiratory allergy. PAF is released from most inflammatory cells after immunologic activation and is characterized by powerful biologic activity,² including the capacity to induce airway microvascular leakage and bronchoconstriction,³ bronchial inflammation, and bronchial hyperresponsiveness.⁴⁻⁷ PAF is also a potent chemoattractant and activator for eosinophils and neutrophils,⁸⁻¹⁰ two cell types that are

Abbreviations used

Lyso-PAF: 1-O-Hexadecyl-sn-glycero-3-phosphorylcholine; precursor and metabolite of PAF

PAF: Platelet activating factor-acether
1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine

supposed to be involved in allergic inflammation, particularly in the late phase reactions in the upper and lower airways.¹¹⁻¹⁸ Deficiency of serum PAF acetylhydrolase, the enzyme that inactivates PAF, is associated with severe respiratory symptoms in children with asthma.¹⁹

It has been demonstrated that PAF and its precursor and metabolite (lyso-PAF) are released in human nasal airways²⁰ together with histamine, peptide leukotrienes, and prostaglandin D₂ after antigen challenge in patients with allergic rhinitis.^{21,22} The evaluation of the effects of PAF on

From the Department of Internal Medicine, Infectious Diseases and Immunopathology, University of Milan, Ospedale Policlinico.

Received for publication Feb. 2, 1993; revised June 4, 1993; accepted June 7, 1993.

Reprint requests: A. Miadonna, MD, Pad. Granelli, Ospedale Policlinico, Via Sforza, 35, I-20122 Milan, Italy.

Copyright © 1994 by Mosby-Year Book, Inc.

0091-6749/94 \$3.00 + 0 1/1/49189

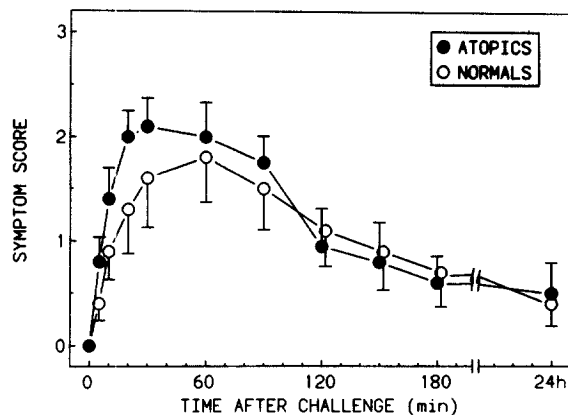


FIG. 1. Time course of nasal obstruction after nasal stimulation with 500 nmol of PAF in 10 atopic and 10 normal subjects. Results are expressed as means $\pm$ SEM. Symptom scores were significantly higher in atopic than in normal subjects ($p < 0.04$).

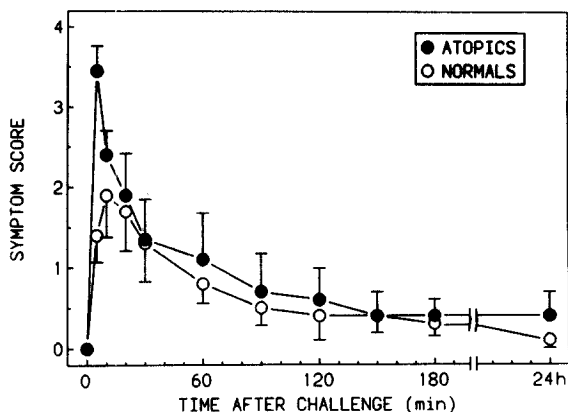


FIG. 2. Time course of nasal symptoms (itching, sneezing, rhinorrhea) other than nasal obstruction after nasal challenge with 500 nmol of PAF in 10 atopic and 10 normal subjects. Results are expressed as means $\pm$ SEM.

human nasal airways may provide further insight into the pathogenetic mechanisms of allergic rhinitis. We previously showed that PAF insufflation in the nose can provoke an increase in nasal airway resistance associated with clinical symptoms such as nasal obstruction, itching, rhinorrhea, and sneezing.²³

The aim of this study was to investigate the effects of PAF stimulation on nasal cytology, because PAF has the ability to attract both neutrophils and eosinophils, two cell types that may be important in allergic inflammation. We performed nasal lavages and evaluated the cellular components before and after insufflation of PAF, lyso-PAF, and saline solution. The results obtained after nasal insufflation of saline solution were used as control values.

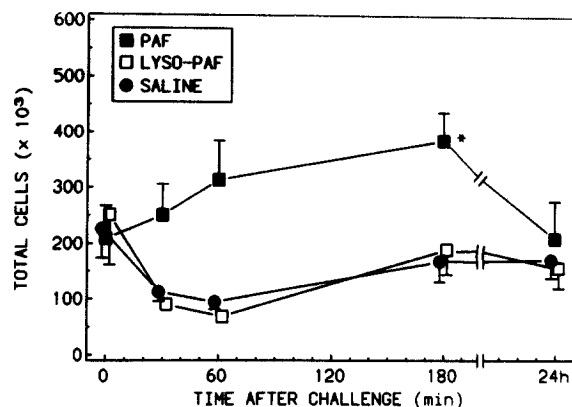


FIG. 3. Time course of total cells in nasal lavage fluids collected after PAF (500 nmol), lyso-PAF (500 nmol), or saline solution (0.9% wt/vol NaCl solution) challenge in 10 atopic subjects. Results are expressed as means $\pm$ SEM * $p < 0.006$.

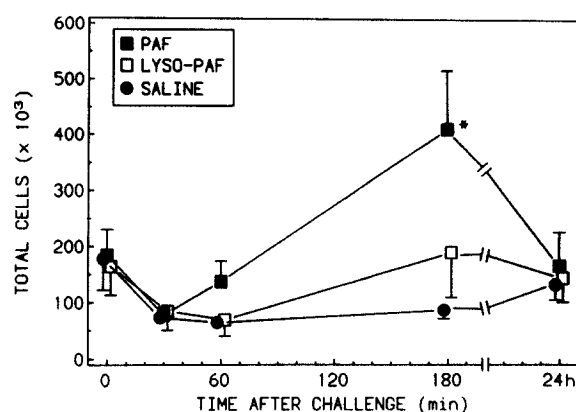


FIG. 4. Time course of total cells in nasal lavage fluids collected after PAF (500 nmol), lyso-PAF (500 nmol), or saline challenge in 10 normal subjects. Results are expressed as means $\pm$ SEM * $p < 0.05$.

TABLE I. Characteristics of the patients with seasonal allergic rhinitis included in study

Subject No.	Age (yr)	Sex	Allergen
1	34	M	GP
2	32	M	GP
3	30	F	PJ
4	28	M	GP
5	31	F	PJ
6	25	M	BP
7	19	F	GP
8	37	M	GP
9	36	M	PJ
10	30	F	GP
Mean (SEM)	30.2 (1.6)		

BP, birch pollen; GP, grass pollen; PJ, *Parietaria judaica*.

TABLE II. Neutrophil counts in nasal lavage fluids collected from 10 normal and 10 atopic subjects after nasal challenge with PAF (500 nmol), lyso-PAF (500 nmol), or saline solution

	Neutrophils in normal subjects ($\times 10^3$)				
	Baseline	30 min	1 hr	3 hr	24 hr
PAF	73.7 $\pm$ 30.9	36.1 $\pm$ 9.7	104.6 $\pm$ 34.6*	353.1 $\pm$ 109.5*	105.9 $\pm$ 58.6
Lyso-PAF	61.9 $\pm$ 31.0	15.4 $\pm$ 3.8	13.0 $\pm$ 3.1	110.4 $\pm$ 44.5*	65.8 $\pm$ 21.2
Saline solution	73.8 $\pm$ 36.4	14.0 $\pm$ 4.0	12.8 $\pm$ 7.2	18.3 $\pm$ 11.0	42.4 $\pm$ 1.6

Results are expressed as mean(s) $\pm$ SEM.

*Significantly different from control value after insufflation of saline solution at $p < 0.05$.

† Significantly different from control value after insufflation of saline solution at $p < 0.01$.

METHODS

Subjects and study design

Ten patients with seasonal allergic rhinitis (six men, four women; 19 to 37 years of age) and 10 healthy subjects (five men, five women; 18 to 34 years of age) were selected for this study. The characteristics of the allergic patients are reported in Table I. Allergic rhinitis was diagnosed by clinical history and skin prick tests. Our study was performed in the winter, when the patients were free of symptoms and not taking any medication. Informed consent was obtained from each subject before the study.

Each volunteer underwent nasal stimulation with PAF, lyso-PAF, and saline solution. Symptom scores were recorded, and nasal lavages were performed at baseline and at different postchallenge times to evaluate changes in nasal cytology. The results obtained after nasal insufflation of saline solution were used as controls.

Nasal challenge and nasal lavage procedure

PAF (1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphorylcholine, purchased from Bachem, Bubendorf, Switzerland) and lyso-PAF (1-O-hexadecyl-*sn*-glycero-3-phosphorylcholine, Bachem) were dissolved in 99.8% ethanol (Analyticals, Carlo Erba-Farmitalia, Milan, Italy; final concentration, 1%) and diluted in 0.9% wt/vol NaCl solution (Sifra, Verona, Italy) containing 0.25% wt/vol of human serum albumin (Institut Mérieux, Lyon, France). PAF and lyso-PAF solutions were divided into small amounts and frozen at -20°C . The solution was thawed just before nasal challenge. The biologic activity of the PAF solution was checked at regular intervals by intradermal injections in volunteers. PAF, lyso-PAF, or saline solution was nebulized into the nose by means of a disposable metered-dose nebulizer calibrated to deliver $100 \pm 10 \mu\text{l}$ per puff. The size of the particles delivered by the nebulizer was 1 to $1.5 \mu\text{m}$. The dose of 500 nmol of PAF was chosen because we had previously observed that this could provoke nasal symptoms in most subjects.²³ Before challenge the subjects waited 30 minutes to allow the nasal mucosa to become acclimatized.

To evaluate changes in nasal cytology after PAF, lyso-PAF, and saline solution insufflation, nasal lavage fluids were collected with a technique previously described.²² Briefly, 5 ml of 0.9% wt/vol NaCl solution was injected into each nostril. After 10 seconds the subject expelled the mixture of mucus and saline solution, which was collected into conical polypropylene tubes (Falcon, Becton Dickinson Labware, Oxnard, Calif.). Recovery of nasal lavage fluid ranged between 50% and 80% of the starting volume (10 ml for each washing). Nasal lavages were performed before and 30 minutes, 60 minutes, 3 hours and 24 hours after nasal stimulation. Nasal fluids were shaken to disperse mucus, and aliquots were collected for total cell counts, which were performed in duplicate in a Fuchs-Rosenthal chamber (Firma Walter Schrenck GmbH, Hoffheim am Taunus, Germany) after staining with Turk liquid (50 μl sample and 450 μl Turk liquid, prepared by Farmacia Ospedale Policlinico, Milan, Italy). Eosinophil count was performed by Randolph staining (solutions provided by Farmacia Ospedale Policlinico).²⁴ The lavage samples were then centrifuged at 1000 g for 10 minutes at 4°C . The supernatant of each sample was aspirated, and the cell pellet was resuspended in 1 ml of 0.9% NaCl solution with 0.1% human serum albumin. Two cytocentrifuged slides for each sample were prepared (100 μl with 2 to 4×10^4 cells for each slide); centrifugation was carried out at 500 rpm for 10 minutes in a Shandon cytocentrifuge (Shandon Southern Products, Ltd., Runcorn, Cheshire, U.K.). Cytocentrifuged slides were stained by May-Grünwald-Giemsa stain (provided by Farmacia Ospedale Policlinico) and examined by light microscopy under oil immersion. At least 200 to 300 cells were examined in each slide. The cells were classified as epithelial cells, neutrophils, eosinophils, and mononuclear cells (the latter category included lymphocytes, monocytes, and mononuclear cells of unclear origin). The reader was blinded as to whether the subject had received PAF, lyso-PAF, or saline solution.

Symptom registration

The subjects were asked to record the severity of rhinorrhea, itching, sneezing, and nasal obstruction

Neutrophils in atopic subjects ($\times 10^3$)

Baseline	30 min	1 hr	3 hr	24 hr
66.6 $\pm$ 22.2	149.8 $\pm$ 35.4*	224.9 $\pm$ 64.6*	335.3 $\pm$ 51.3†	90.9 $\pm$ 21.0
63.8 $\pm$ 30.2	24.8 $\pm$ 13.5	20.8 $\pm$ 3.0	142.0 $\pm$ 41.5*	67.6 $\pm$ 17.8
101.7 $\pm$ 34.6	31.5 $\pm$ 8.6	20.0 $\pm$ 6.7	38.1 $\pm$ 14.9	52.0 $\pm$ 16.1

after nasal insufflation of PAF, lyso-PAF, or saline solution. Rhinorrhea, itching, and nasal obstruction were classified on an arbitrary scale from 0 to 4 (none = 0, mild = 1, moderate = 2, intense = 3, very intense = 4). Sneezing was classified according to number of sneezes (no sneezes = 0, one to five sneezes = 1, six to 10 sneezes = 2, 11 to 15 sneezes = 3, >15 sneezes = 4). Nasal obstruction, which was the main symptom caused by PAF insufflation, was reported separately, whereas the scores for the other symptoms were combined.

Statistical analysis

Results were expressed as means $\pm$ SEM. The two-tailed Student *t* test for paired data was used to analyze the cytologic changes in the nasal lavages. (Prechallenge values were compared with postchallenge values obtained at different times; moreover, the values obtained after PAF or lyso-PAF challenge were compared with the values obtained after insufflation of saline solution.) Pearson's correlation coefficient was used to analyze the correlation between cells and symptoms. Values of *p* lower than 0.05 were considered significant.

RESULTS

Symptoms

Nasal obstruction was the main symptom observed after nasal challenge with 500 nmol of PAF; it was reported by 10 of 10 atopic and seven of 10 nonatopic subjects. Maximum obstruction was reported 30 to 90 minutes after PAF insufflation; this subsided gradually, with three atopic and three normal subjects still reporting a mild nasal blockage after 24 hours after challenge (Fig. 1). Other symptoms caused by PAF were generally mild and were represented by rhinorrhea (10/10 atopic, 8/10 normal subjects), itching (3/10 atopic, 8/10 normal subjects), and sneezing (3/10 atopic, 5/10 normal subjects). These symptoms reached the maximum score 5 to 10 minutes after challenge and then subsided gradually (Fig. 2). Symptom scores were higher in atopic than in normal subjects (*p* < 0.04 for nasal obstruction and *p* = 0.07 for symptoms other than nasal ob-

struction). Nasal insufflation of lyso-PAF (500 nmol) or saline solution did not provoke any nasal symptoms.

Cytologic changes in nasal lavage fluids after insufflation of PAF, lyso-PAF, and saline solution

Total cell counts in basal nasal lavages (mean $\pm$ SEM) were $208.9 \times 10^3 \pm 58.1 \times 10^3$ in atopic subjects and $184.2 \times 10^3 \pm 45.9 \times 10^3$ in normal subjects, so that the difference between the two kinds of subjects was not statistically significant. A significant increase in total cell counts when compared with baseline values was found in the nasal lavages collected 3 hours after PAF insufflation, but not after lyso-PAF or saline solution insufflation both in atopic (*p* < 0.006) and nonatopic subjects (*p* < 0.05). Moreover, the time course of cell counts after PAF stimulation was different from that observed after saline insufflation, which was supposed to represent the spontaneous rate of desquamation and/or leukocyte migration. Both atopic and nonatopic subjects showed higher total cell counts after PAF challenge than after saline insufflation (atopic subjects: *p* < 0.05 at 1 hour and *p* < 0.02 after 3 hours; nonatopic subjects: *p* < 0.05 at 1 hour and *p* < 0.02 at 3 hours). After an initial decrease in the first hour after saline insufflation, total cell counts after 24 hours returned to the initial value. The time course of total cell counts after lyso-PAF insufflation was not significantly different from that observed after saline insufflation, with similar behavior in atopic and nonatopic subjects (Figs. 3 and 4).

The changes in the cellular composition of the nasal lavages are reported in Tables II, III, and IV. Nasal stimulation with PAF induced neutrophilia in the noses of both allergic and normal subjects; the increase in neutrophils appeared 30 minutes after challenge in atopic subjects and 1 hour after stimulation in nonatopic subjects, and persisted until 3 hours after challenge in both

TABLE III. Eosinophil counts in nasal lavage fluids collected from 10 normal and 10 atopic subjects after nasal challenge with PAF (500 nmol), lyso-PAF (500 nmol), or saline solution

	Eosinophils in normal subjects ($\times 10^3$)				
	Baseline	30 min	1 hr	3 hr	24 hr
PAF	0.11 $\pm$ 0.10	0.70 $\pm$ 0.49	1.56 $\pm$ 0.53*	10.01 $\pm$ 3.50*	1.77 $\pm$ 1.23
Lyso-PAF	1.52 $\pm$ 1.39	0.48 $\pm$ 0.48	0.0 $\pm$ 0.0	0.67 $\pm$ 0.67	2.50 $\pm$ 2.23
Saline solution	0.26 $\pm$ 0.26	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

The results are expressed as mean(s) $\pm$ SEM.

*Significantly different from control value after insufflation of saline solution at $p < 0.05$.

†Significantly different from control value after insufflation of saline solution at $p < 0.01$.

groups. An increase in neutrophils was also found 3 hours after lyso-PAF insufflation in both groups. PAF insufflation also induced an increase in eosinophil count, which appeared 30 minutes after challenge in atopic subjects and was delayed until 3 hours after stimulation in nonatopic subjects. A significant increase in eosinophil counts was found 3 hours after lyso-PAF challenge in atopic but not in nonatopic subjects. The behavior of epithelial cells was inverse to that of neutrophils, with the counts dropping in the first 3 hours after PAF challenge. Reduced counts were also found 3 hours after lyso-PAF challenge in atopic subjects. Saline insufflation did not significantly influence the cellular composition of nasal lavage fluids.

No correlation was found between neutrophil counts and symptom scores at any time after PAF challenge in either normal or atopic subjects. The only significant correlation was observed between the eosinophil counts 3 hours after PAF challenge and the scores for nasal obstruction in normal subjects ($r = +0.79$, $p < 0.01$).

DISCUSSION

These results indicate that nasal challenge with PAF but not with lyso-PAF can induce nasal symptoms such as obstruction, itching, sneezing, and rhinorrhea in both atopic and nonatopic subjects. PAF challenge is followed by nasal neutrophilia and to a lesser extent eosinophilia, with more rapid recruitment of inflammatory cells in atopic than in nonatopic subjects. Although the activity of lyso-PAF is weaker and slower than that of PAF, nasal challenge with this phospholipid also causes a significant increase in neutrophil counts and, in atopic subjects only, eosinophil counts in the nasal lavages. Insufflation of saline solution neither causes symptoms nor changes the cellular composition of the nasal lavages.

As observed previously,²³ nasal obstruction was the main symptom reported by the patients after PAF challenge; this is probably caused by a thickening of the nasal mucosa, resulting from an increase in the volume of the capacitance vessels and an increase in interstitial fluid, and is not strictly related to mucosal infiltration by inflammatory cells. No correlation was found between neutrophil counts and symptom scores at any time after PAF challenge in either atopic or nonatopic subjects. Indeed, increased numbers of neutrophils and eosinophils were found in the nasal lavages collected 3 hours after PAF challenge, when nasal symptoms were no longer apparent in most subjects. Furthermore, the increased numbers of neutrophils found after lyso-PAF insufflation were not associated with any symptom. It is noteworthy that the atopic subjects reported higher scores than the normal subjects; this could be related to enhanced nasal responsiveness in the subjects with allergic rhinitis.²⁵

On a molar basis, PAF is less powerful than leukotrienes, prostaglandin D₂, and histamine in provoking nasal symptoms.^{22,26,27} However, PAF has a broad range of biologic activities, which could be important in the pathogenesis of allergic inflammation of respiratory airways. PAF is able to induce in vitro neutrophil and eosinophil chemoattraction and adhesion to the vascular endothelium^{28,29}; therefore, it is reasonable to suppose that the increase in the numbers of neutrophils and eosinophils in nasal lavages after PAF insufflation is the result of PAF-induced neutrophil and eosinophil adhesion to the vascular endothelium and subsequent accumulation in the nasal mucosa. From our results PAF appears to be a weaker chemoattractant for eosinophils than for neutrophils. This appears to contradict the in vitro results,⁸ which show that PAF is the most powerful eosinophilotactic agent. However,

Eosinophils in atopic subjects ($\times 10^3$)				
Baseline	30 min	1 hr	3 hr	24 hr
1.35 ± 1.35	$11.66 \pm 2.67^\dagger$	$9.12 \pm 2.57^\dagger$	$6.93 \pm 1.50^\dagger$	3.39 ± 1.37
0.10 ± 0.10	1.87 ± 1.87	0.60 ± 0.54	$4.81 \pm 1.97^*$	0.08 ± 0.08
1.63 ± 1.43	0.08 ± 0.08	0.31 ± 0.31	0.85 ± 0.59	0.88 ± 0.84

neutrophils are far more numerous than eosinophils in the peripheral blood and respiratory airways of normal and symptom-free allergic subjects and therefore are likely to be attracted in larger numbers after PAF challenge. Furthermore, Wardlaw et al.³⁰ found that inhaling PAF leads to the activation of circulating neutrophils with the subsequent infiltration of the bronchial airways and lung spaces in human beings.

It is noteworthy that the atopic subjects showed a more rapid accumulation of eosinophils and neutrophils than the normal subjects, indicating a different degree of responsiveness in the two kinds of subjects to nasal challenge with PAF. A difference in the response to intradermal PAF injection was also observed by Henocq and Vargaftig,³¹ who found greater accumulation of eosinophils in atopic subjects.

The changes in the percentages of epithelial cells found in nasal lavage fluids were inverse to the changes in neutrophils. After an initial decrease in the first hour after PAF challenge, the percentage of epithelial cells returned after 24 hours to the initial value. This behavior is probably the consequence of the initial mucosal infiltration by neutrophils and the subsequent inactivation of PAF.

An increase in the percentage of neutrophils was also observed after insufflation of lyso-PAF both in atopic and in nonatopic subjects, although the effects of this phospholipid were weaker and slower than in the case of PAF. Furthermore, an increase in eosinophils was found in the nasal lavages collected 3 hours after challenge in atopic but not in normal subjects. Lyso-PAF is a precursor and a metabolite of PAF, and is generally considered to be biologically inactive; however, lyso-PAF is able to induce bronchoalveolar eosinophilia in nonhuman primates,³² and it is therefore not surprising to find that it can induce biologic changes in human beings as well. The changes in nasal cytology that we observed after

lyso-PAF insufflation could be its direct consequence; alternatively, these changes could be the result of an *in vivo* acetylation and transformation into PAF. In fact, it has been demonstrated that PAF can be produced by cultured bacteria supplemented with lyso-PAF.³³ In eukaryotic cells the key enzyme in PAF formation is an acetyltransferase that acetylates lyso-PAF into PAF. PAF formation is conditional on the amount of lyso-PAF; hence, PAF production is strictly dependent on lyso-PAF availability.³⁴ On the basis of our results we hypothesize that bacteria or inflammatory cells present in the nose can produce PAF when supplemented with exogenous lyso-PAF. This hypothesis is in line with our results, which show that the effect of lyso-PAF on the cellular composition of the nasal lavages is slower than that of PAF, because an increase in the percentage of neutrophils was found only 3 hours after challenge. This delay could be due to either the requirement for lyso-PAF acetylation or the relative weakness of the direct impact of this compound when compared with PAF.

It has been suggested that the recruitment of neutrophils in atopic subjects after antigen challenge is nonspecific. For example, bronchial challenge with saline solution in atopic subjects has been reported to result in the local accumulation of neutrophils.³⁵ However, other authors^{12, 36, 37} have demonstrated that antigen challenge in the bronchi, the nose, or the skin causes the recruitment of large numbers of neutrophils in atopic patients but not in nonatopic control subjects. In our experiments nasal challenge with saline solution had no effect on the cellular composition of the nasal lavages in both atopic and nonatopic subjects; moreover, the total number of cells decreased in the nasal fluids collected until 3 hours after saline insufflation, probably because the cells present on the mucosal surface were washed away by the repeated lavages. Therefore we con-

TABLE IV. Epithelial cell counts in nasal lavage fluids collected from 10 normal and 10 atopic subjects after nasal challenge with PAF (500 nmol), lyso-PAF (500 nmol), or saline solution

	Cell counts in normal subjects ($\times 10^3$)				
	Baseline	30 min	1 hr	3 hr	24 hr
PAF	109.2 $\pm$ 24.4	35.2 $\pm$ 7.4	31.2 $\pm$ 11.1	46.0 $\pm$ 17.0	49.7 $\pm$ 10.5*
Lyso-PAF	90.3 $\pm$ 40.5	61.0 $\pm$ 34.3	55.8 $\pm$ 27.9	77.9 $\pm$ 36.8	76.2 $\pm$ 24.2
Saline solution	101.0 $\pm$ 21.2	58.7 $\pm$ 10.6	51.2 $\pm$ 9.2	62.2 $\pm$ 10	80.7 $\pm$ 19.0

Results are expressed as mean(s) $\pm$ SEM.

*Significantly different from control value after insufflation of saline solution at $p < 0.05$.

clude that changes in nasal cytology provoked by PAF and lyso-PAF in nasal cytology are not nonspecific.

Eosinophils and neutrophils have been found in the upper and lower respiratory airways during late antigen-induced reactions¹¹⁻¹⁶ and have the ability to release powerful inflammatory mediators on activation.³⁸⁻⁴¹ PAF can be released in the nose on antigen challenge, together with its precursor and metabolite lyso-PAF,²⁰ and it can attract neutrophils and eosinophils.⁸ This chemotactic activity may be relevant to the pathogenesis of allergic inflammation in respiratory airways. However, the role of neutrophils requires further evaluation.

We gratefully acknowledge the technical assistance of Mr. Maurizio Lorini.

REFERENCES

- Barnes PJ, Chung KF, Page CP. Platelet-activating factor as a mediator of allergic disease. *J ALLERGY CLIN IMMUNOL* 1988;81:919-34.
- Benveniste J, Arnoux B, eds. Platelet-activating factor and structurally related ether lipids. INSERM symposium No. 23, Amsterdam: Elsevier Science Publishers, 1983.
- Ewans TW, Chung KF, Rogers DF, Barnes PJ. Effect of platelet-activating factor on airway vascular permeability: possible mechanisms. *J Appl Physiol* 1987;63:479-84.
- Cuss FM, Dixon CMS, Barnes PJ. Effects of inhaled platelet-activating factor on pulmonary function and bronchial responsiveness in man. *Lancet* 1986;2:189-92.
- Benveniste J. A role for PAF-acether (platelet-activating factor) in bronchial hyperreactivity? *Am Rev Respir Dis* 1987;135:S2-S4.
- Rubin AE, Smith LJ, Patterson R. The bronchoconstrictor properties of platelet-activating factor in man. *Am Rev Respir Dis* 1987;136:1145-51.
- Mencia-Huerta JM, Hosford D, Braquet P. Acute and long-term pulmonary effects of platelet-activating factor. *Clin Exp Allergy* 1989;19:125-42.
- Wardlaw AJ, Moqbel R, Cromwell O, Kay AB. Platelet-activating factor: a potent chemotactic and chemokinetic factor for human eosinophils. *J Clin Invest* 1986;78:1701-6.
- Kroegel C, Yukawa T, Dent G, Venge P, Chung KF, Barnes PJ. Stimulation of degranulation from human eosinophils by platelet-activating factor. *J Immunol* 1989;142:3518-26.
- Shaw JO, Pinckard N, Ferrigni KS, McManus LM, Hanahan DJ. Activation of human neutrophils with 1-O-hexadecyl-octadecyl-2-acetyl-sn-glycerol-3-phosphorylcholine (platelet-activating factor). *J Immunol* 1981;127:1250-5.
- Pelikan Z, Pelikan-Filipek M. Cytologic changes in the nasal secretions during the late nasal response. *J ALLERGY CLIN IMMUNOL* 1989;83:1068-79.
- Bascom R, Pipkorn U, Lichtenstein LM, Naclerio RM. The influx of inflammatory cells into nasal washings during the late response to antigen challenge: effect of systemic steroid pretreatment. *Am Rev Respir Dis* 1988;138:406-12.
- Durham SR, Carroll M, Walsh GM, Kay AB. Leukocyte activation in allergen-induced late-phase asthmatic reactions. *N Engl J Med* 1984;311:1398-402.
- Fabbri LM, Boschetto P, Zocca E, et al. Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene diisocyanate. *Am Rev Respir Dis* 1987;136:36-42.
- Diaz D, Gonzalez MC, Galleguillos FR, et al. Leukocytes and mediators in bronchoalveolar lavage during allergen-induced late-phase asthmatic reactions. *Am Rev Respir Dis* 1989;139:1383-9.
- De Monchy JGR, Kauffman HF, Venge P, et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985;131:373-6.
- Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. *Clin Allergy* 1985;15:411-8.
- Frick WE, Ledwick JB, Busse WW. The appearance of hypodense eosinophils in antigen-dependent late phase asthma. *Am Rev Respir Dis* 1989;139:1401-6.
- Miwa M, Miyake T, Yamanaka T, et al. Characterization of serum platelet-activating factor (PAF) acetylhydrolase: correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. *J Clin Invest* 1988;82:1983-91.
- Miadonna A, Tedeschi A, Arnoux B, et al. Evidence of PAF-acether metabolic pathway activation in antigen challenge of upper respiratory airways. *Am Rev Respir Dis* 1989;140:142-7.
- Naclerio RM, Meyer HL, Kagey-Sobotka A, Adkinson NF, Meyers DA, Lichtenstein LM. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
- Miadonna A, Tedeschi A, Leggieri E, et al. Behavior and

Cell counts in atopic subjects ($\times 10^3$)

Baseline	30 min	1 hr	3 hr	24 hr
140.7 $\pm$ 36.0	85.0 $\pm$ 19.1	75.3 $\pm$ 18.1	39.2 $\pm$ 14.0*	111.5 $\pm$ 43.0
122.6 $\pm$ 30.9	63.1 $\pm$ 10.7	46.3 $\pm$ 3.3*	35.1 $\pm$ 5.5*	88.3 $\pm$ 25.0
119.7 $\pm$ 22.5	80.0 $\pm$ 12.3	73.2 $\pm$ 10.9	127.7 $\pm$ 26.0	117.8 $\pm$ 21.2

- clinical relevance of histamine and leukotrienes C_4 and B_4 in grass pollen-induced rhinitis. *Am Rev Respir Dis* 1987; 136:357-62.
23. Leggieri E, Tedeschi A, Lorini M, Bianco A, Miadonna A. Study of the effects of PAF-acether on human nasal airways. *Allergy* 1991;46:466-71.
 24. Randolph TG. Differentiation and enumeration of eosinophils in the counting chamber with a glycol stain: a valuable technique in appraising ACTH dosage. *J Lab Clin Med* 1949;34:1696-701.
 25. Mullins RJ, Olson LG, Sutherland DC. Nasal histamine challenges in symptomatic allergic rhinitis. *J ALLERGY CLIN IMMUNOL* 1989;83:955-9.
 26. Miadonna A, Tedeschi A, Leggieri E, et al. Effetto della provocazione nasale con istamina, leucotriene C_4 e prostaglandina D_2 in pazienti affetti da rinite da pollini di graminacee. *Folia Allergol Immunol Clin* 1986;33:279-84.
 27. Bisgaard H, Olsson P, Bende M. Effect of leukotriene D_4 on nasal mucosal blood flow, nasal airway resistance and nasal secretion in humans. *Clin Allergy* 1986;16:289-97.
 28. Charo IF, Yuen C, Pere HD, Goldstein IM. Chemotactic peptides modulate adherence of human polymorphonuclear leukocytes to monolayers of cultured endothelial cells. *J Immunol* 1986;136:3412-9.
 29. Kimani G, Tonnesen MG, Henson PM. Stimulation of eosinophil adherence to human vascular endothelial cells in vitro by platelet-activating factor. *J Immunol* 1988;140: 3161-6.
 30. Wardlaw AJ, Chung KF, Moqbel R, et al. Effects of inhaled PAF in humans on circulating and bronchoalveolar lavage fluid neutrophils: relationship to bronchoconstriction and changes in airway responsiveness. *Am Rev Respir Dis* 1990;141:386-92.
 31. Henocq E, Vargaftig BB. Accumulation of eosinophils in response to intracutaneous PAF-acether and allergen in man. *Lancet* 1986;1:1378-9.
 32. Arnoux B, Denjean A, Page CP, Nolibe D, Morley J, Benveniste J. Accumulation of platelets and eosinophils in baboon lung after PAF-acether challenge: inhibition by ketotifen. *Am Rev Respir Dis* 1988;137:855-60.
 33. Denizot Y, Dassa E, Benveniste J, Thomas Y. PAF-acether production by *Escherichia coli*. *Biochem Biophys Res Commun* 1989;161:939-43.
 34. Ninio E, Mencia-Huerta JM, Heymans F, Benveniste J. Biosynthesis of platelet-activating factor (PAF-acether). I. Evidence for an acetyl-transferase activity in murine macrophages. *Biochim Biophys Acta* 1983;710:23-31.
 35. Liu M, Hubbard W, Proud D, et al. Late inflammation following antigen challenge of the airways in allergic asthmatics (Abstract). *J ALLERGY CLIN IMMUNOL* 1990;85: 263.
 36. Charlesworth EN, Kagey-Sobotka A, Schleimer RP, Norman PS, Lichtenstein LM. Prednisone inhibits the appearance of inflammatory mediators and the influx of eosinophils and basophils associated with the cutaneous late-phase response to allergen. *J Immunol* 1991;146:671-6.
 37. Dupuis R, Collins D, Koh YY, et al. Effect of antigen dose on the recruitment of inflammatory cells to the lung by segmental antigen challenge. *J ALLERGY CLIN IMMUNOL* 1992;89:850-7.
 38. Wright DG. The neutrophil as a secretory organ of host defense. In: Gallin JI, Fauci AS, eds. *Phagocytic cells: advances in host defense mechanisms*. New York: Raven Press, 1982;1:75-110.
 39. Larsen GL, Wilson MC, Marsh WR, Haslett C, Murphy KR. Neutrophils and late-phase reactions. In Kay AB, ed. *Allergy and inflammation*. London: Academic Press, 1987: 225-44.
 40. Kay AB. Eosinophils as effector cells in immunity and hypersensitivity disorders. *Clin Exp Immunol* 1985;62:1-12.
 41. Wardlaw AJ, Kay AB. The role of the eosinophils in the pathogenesis of asthma. *Allergy* 1987;42:321-35.