

26. Frigas E, Gleich GJ. The eosinophil and pathophysiology of asthma. *J ALLERGY CLIN IMMUNOL* 1986;77:527-37.
27. Hodges MK, Weller PF, Gerard NP, Ackerman SJ, Drazen JM. Heterogeneity of leukotriene C4 production by eosinophils from asthmatic and from normal subjects. *Am Rev Respir Dis* 1988;138:799-804.
28. Djukanovic R, Wilson JW, Britton KM, et al. Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunocytochemistry. *Am Rev Respir Dis* 1990;142:863-71.
29. Bousquet J, Chané P, Lacoste JY, Barneon G, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
30. Frick WE, Sedgwick JB, Busse WW. The appearance of hypodense eosinophils in antigen-dependent late phase asthma. *Am Rev Respir Dis* 1989;139:685-8.

Increased plasma platelet-activating factor in children with acute asthmatic attacks and decreased in vivo and in vitro production of platelet-activating factor after immunotherapy

Kue-Hsiung Hsieh, MD, and Cheng-Koon Ng, BS

Taipei, Taiwan, Republic of China

Background: To explore the possible role of platelet-activating factor (PAF) in the pathogenesis of bronchial asthma, circulating PAF and in vitro production of PAF were studied.

Methods: Radioimmunoassay kits were used in 15 children with acute asthmatic attacks, in 25 newly diagnosed asthmatic children, in 25 good and 18 poor responders to immunotherapy, and in 18 healthy controls.

Results: The results demonstrated the following: (1) PAF was present in the blood of healthy controls. (2) New patients had much higher circulating PAF than did healthy controls ($p < 0.005$), and the circulating PAF decreased after immunotherapy in good ($p < 0.005$) but not in poor responders. (3) The circulating PAF increased up to 20 times that of healthy controls during acute asthmatic attacks. (4) The spontaneous and allergen-stimulated secretion of PAF were markedly increased in new patients and decreased to normal after successful immunotherapy ($p < 0.005$). (5) No increased spontaneous and allergen-stimulated production of PAF was found during acute attacks, but granulocytes from those patients still produced the greatest amount of PAF when stimulated with calcium ionophore A23187. (6) Although a major portion of allergen-induced PAF was secreted, less than 10% of ionophore-induced PAF was secreted.

Conclusion: The findings that the circulating PAF increased markedly during acute asthmatic attacks and the enhanced in vivo and in vitro productions of PAF decreased to normal after successful immunotherapy strongly suggest that PAF may be involved in the pathogenesis of bronchial asthma. (*J ALLERGY CLIN IMMUNOL* 1993;91:650-7.)

Key words: Platelet-activating factor, asthma, immunotherapy, children

From the Division of Allergy, Immunology and Rheumatology, Department of Pediatrics and Graduate Institute of Microbiology and Immunology, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China.

Supported by a grant from the National Science Council of the Republic of China, grant no. NSC-77-0412-B-002-139.

Received for publication Jan. 14, 1991.

Revised April 2, 1992.

Accepted for publication Oct. 26, 1992.

Reprint requests: Kue-Hsiung Hsieh, MD, Department of Pediatrics, National Taiwan University Hospital, 7 Chung-Shan South Rd., Taipei, 10016 Taiwan, Republic of China.

Copyright © 1993 Mosby-Year Book, Inc.

0091-6749/93 \$1.00 + .10 1/1/43651

Platelet-activating factor (PAF-acether) is a potent phospholipid mediator involved in a wide variety of diseases.^{1, 2} Animal experiments have demonstrated that PAF can produce bronchoconstriction,^{3, 4} increase vascular permeability,⁵ induce airway hyperreactivity,⁶ recruit inflammatory cells, especially eosinophils, into the lungs,^{5, 7} and attenuate the airway allergic response.^{8, 9} Several lines of evidence from recent studies strongly suggest that PAF may also be an important mediator of the inflammatory events associated with asthma in human beings: (1) Plasma platelet factor 4 was increased during antigen-induced airway reaction in asthmatic subjects¹⁰; (2) Platelet products were detected in the lavage fluid of asthmatic subjects¹¹; (3) PAF was detected in sputum of asthmatic subjects¹²; (4) Intratracheal administration of PAF caused bronchospasm in decerebrated humans¹³; (5) Aerosolized PAF was capable of inducing bronchoconstriction and bronchial hyperresponsiveness in both healthy subjects and asthmatic patients¹⁴⁻¹⁶; (6) PAF antagonist, BN52021 and BN52063, was able to suppress the immediate response to inhaled allergen challenge in asthmatic patients via aerosol and oral route,^{16, 17} and (7) An open trial of Ginkgo-Biloba extract, a PAF antagonist, could improve severity of chronic asthma and decrease blood and sputum eosinophilia and serum IgE after treatment for 1 year.¹⁸ Thus PAF may play a certain role, in conjunction with other mediators, in the pathogenesis of bronchial asthma.

Since its introduction in 1911,¹⁹ immunotherapy or hyposensitization has been shown to be effective in treating some patients with respiratory allergy.^{20, 21} Studies on the working mechanisms of immunotherapy have focused almost completely on the alteration of immunologic status,²²⁻²⁸ but only a few studies have focused on allergy mediators.²⁹⁻³¹ This project was therefore conducted to study the changes of *in vivo* and *in vitro* production of PAF after long-term immunotherapy in asthmatic children. In addition, patients with acute asthmatic attacks were also studied.

MATERIAL AND METHODS

Study populations

Fifteen children with acute asthmatic attacks, 25 newly diagnosed asthmatic children, and 29 good responders and 18 poor responders to immunotherapy were enrolled into this study. Eighteen healthy laboratory personnel with negative skin tests to mites were included as controls. This study has been approved by the Human Research Committee of this hospital, and informed consents were obtained from all study subjects. All the patients were sensitive only to mite (*Dermatophagoides pteronyssinus*) in terms of a positive history of exposure, had a greater than 2+ skin test (wheal >10 mm by intracutaneous route, with use of mite

Abbreviations used

PAF:	Platelet-activating factor
HPLC:	High-performance liquid chromatography
RIA:	Radioimmunoassay

extract at a concentration of 10^{-6} gm/ml), and had a positive RAST (Pharmacia Diagnostics, Uppsala, Sweden). The four patient groups were comparable regarding the sex, age, skin testing, total serum IgE, and mite RAST. Immunotherapy was started with weekly injections of 10^{-6} gm/ml of *D. pteronyssinus*, and the dose was increased gradually until a maximal tolerated dose was reached (usually within 6 months) and then was maintained at 4-week intervals. All the patients had been receiving immunotherapy for more than 2 years (mean, 2.5 years). The total dose of crude mite allergen given ranged from 89.5 μ g to 173.5 μ g. No steroid was taken for at least 2 weeks, and bronchodilators were withheld for at least 24 hours before blood samples were taken. The effectiveness of immunotherapy was judged by comparing the sum of the frequency of asthma and the amount of medications consumed (total doses of two agonists, theophylline and steroids) in the year before immunotherapy with that in the second year after immunotherapy. The result was considered to be excellent if the decrease of both parameters was greater than 90%, much improved with a 75% to 90% decrease, improved for a 50% to 75% decrease, slightly improved for a 25% to 50% decrease, and poor for a decrease of 25% or less.³² In this study good responders consisted of those patients showing an improvement greater than 75%, whereas poor responders were those showing an improvement less than 25%.

Preparation of plasmas

To inactivate the serum PAF acetylhydrolase, 1.0 ml of 0.5N HCl were added immediately after 10 ml of heparinized blood were collected. The blood-HCl mixture (pH, 5.8) was centrifuged at 1000 g for 10 minutes at 4° C, and the plasma was collected and stored at -70° C until testing.

Preparation of granulocyte suspension

Diluted heparinized whole blood was layered over Ficoll-Hypaque (Pharmacia) density gradient and centrifuged at 800 g for 30 minutes. After aspiration of mononuclear cells (MNCs) from the interface layer, the pelleted red blood cells were resuspended in 6% dextran in normal saline solution and were stood at room temperature for 1 hour. The leukocyte-rich portion was aspirated, and the contaminating red blood cells were lysed by cold distilled water. The granulocytes were washed three times with Hank's balanced salt solution and then adjusted to a concentration of 5×10^6 cells per milliliter in working buffer. The working buffer consisted of 0.25% bovine serum albumin (Sigma fraction V fatty acid-free) (Sigma Chemical Co., St. Louis, Mo.) in 10 mmol/L Tris, pH 7.2, with Ca^{++} (1 mmol/L). The viability of cells was more than 96% by trypan blue test, and the final granulocyte suspension contained $95\% \pm 2\%$ polymorphonuclear neutrophils and less than 0.2% basophils.

TABLE I. Specificity of PAF radioimmunoassay kit

Type of phospholipid	Amount added	PAF concentration measured by RIA
PAF	5000 pg	4940 pg
	2500 pg	2470 pg
Lyso-PAF	2500 pg	ND
Sphingomyelin	2500 pg	ND
Phosphatidylcholine	2500 pg	ND
Buffer		ND

All phospholipids purchased from Sigma Chemical Co., St. Louis, Mo.
ND, Not detected.

Preparation of crude mite extract

The crude mite extract (*D. pteronyssinus*) was prepared by the method of Nakada et al.³³ In brief, 1 gm of lyophilized mite powder was defatted with 100 ml ether for 48 hours then homogenized and stirred continuously in 100 ml phosphate buffer for 36 hours. After centrifugation (10,000 g), the supernatant was lyophilized and stored at -70°C until use. The protein content was measured by Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, Calif.). The same extract was used for skin testing, immunotherapy, and in vitro culture experiments.

In vitro production of PAF

To establish the dose-response and time course of PAF production, the granulocyte suspensions were incubated in working buffer alone, or stimulated with calcium ionophore A23187 (Sigma) at concentrations ranging from 0 to 10 $\mu\text{mol/L}$, or house dust mite at concentrations ranging from 0 to 40 $\mu\text{g/ml}$. The cells were incubated for 0, 5, 10, 15, 20, 25, and 30 minutes. At the end of incubation, supernatants and cell pellets were separated by centrifugation at 1000 g rpm at 4°C for 20 minutes and stored at -20°C until testing. The experimental condition was finally set as follows: 5×10^6 cells/ml, A23187 5 $\mu\text{mol/L}$, mite allergen 10 $\mu\text{g/ml}$, and incubation at 37°C for 10 minutes. Working buffer containing A23187 and working buffer containing mite antigen were included as controls in each experiment. The spontaneous PAF production was determined by incubating granulocytes in working buffer alone.

Extraction and measurement of PAF

To obtain the intracellular PAF, cell pellets (1×10^7) were resuspended in 2 ml working buffer and were frozen and thawed three times to lyse the cells and release intracellular PAF. PAF in cells, culture supernatants (2 ml), and plasma (obtained from 10 ml whole blood of each individual) were measured by radioimmunoassay, with PAF radioimmunoassay (RIA) kits purchased from DuPont Diagnostic Imaging Co. (Wilmington, Del.).

The extraction and measurement of PAF was done according to the procedure recommended by the manufacturer. In brief, tested specimens were passed through a pretreated (successively washed with 2 ml methanol, 2 ml water, and 5 ml 10% acetic acid) Bond-Elut C18 extraction column 200 mg (Analytichem International, Harbor City, Calif.).

The column was then washed with 10% acetic acid $\times 2$ and 2 ml of ethyl acetate $\times 3$ (to remove acetic acid, H_2O , and lipid). PAF was eluted from column C18 with 6 ml methanol, 2.4 ml water, and 3 ml of chloroform into a siliconized glass tube. An additional 3 ml of chloroform and 3 ml of water were added to the tube successively to obtain a final ratio of chloroform:methanol:water of 1:1:0.9. After being capped, the tube was shaken vigorously for 5 minutes. Finally, the bottom phase chloroform was removed and evaporated to dryness under a gentle stream of nitrogen and stored at -20°C until testing. Before assay, the PAF was reconstituted with 0.5 ml of working assay buffer and whirled in a vortex occasionally 15 minutes before measurement. All determinations were done in duplicate. During each extraction, a tube of internal standard of [^3H]PAF (0.5 nCi in 6 ml buffer; DuPont) was included. Moreover, to check the efficacy of extraction, 0.5 nCi PAF (DuPont) in 0.2 ml buffer were added to each sample. The recovery rate was $88.16\% \pm 1.78\%$ (average of 10 experiments).

The authenticity of PAF extracted from plasma, culture supernatants, and cell pellets was checked by HPLC (Beckman Instruments, Spinco Div., Palo Alto, Calif.), with an Ultrasphere C18 (4.6 mm $\times$ 25 cm) column, and the results were compared with [^3H]PAF and cold PAF. The solvents used in HPLC were a mixture of 30% H_2O and 70% methanol initially for 3 minutes, followed by addition of increasing concentration of methanol up to 100% within 5 minutes, maintenance for 5 minutes, and then return to the original concentration of 30% methanol. Twenty micrograms in 20 μl of either labeled or cold PAF or 20 μl containing 5 μg each of cold PAF, lyso-PAF (Sigma), sphingomyelin (Sigma), and phosphatidylcholine (Sigma), or 20 μl of 100-fold concentrated extracted samples were added and eluted at a flow rate of 1 ml/min with increasing concentration of methanol up to 100%. Eighteen tubes (1 ml per tube) were collected and read at the wavelength of 215 nm in case of cold PAF, mixed phospholipids, and extracted tested samples, or they were counted on a beta counter in case of labeled PAF.

Specificity of PAF RIA kits

The specificity of PAF RIA kits from DuPont was checked. Two and a half nanograms each of PAF, lyso-PAF, sphingomyelin, and phosphatidylcholine in 0.1 ml, and 0.1

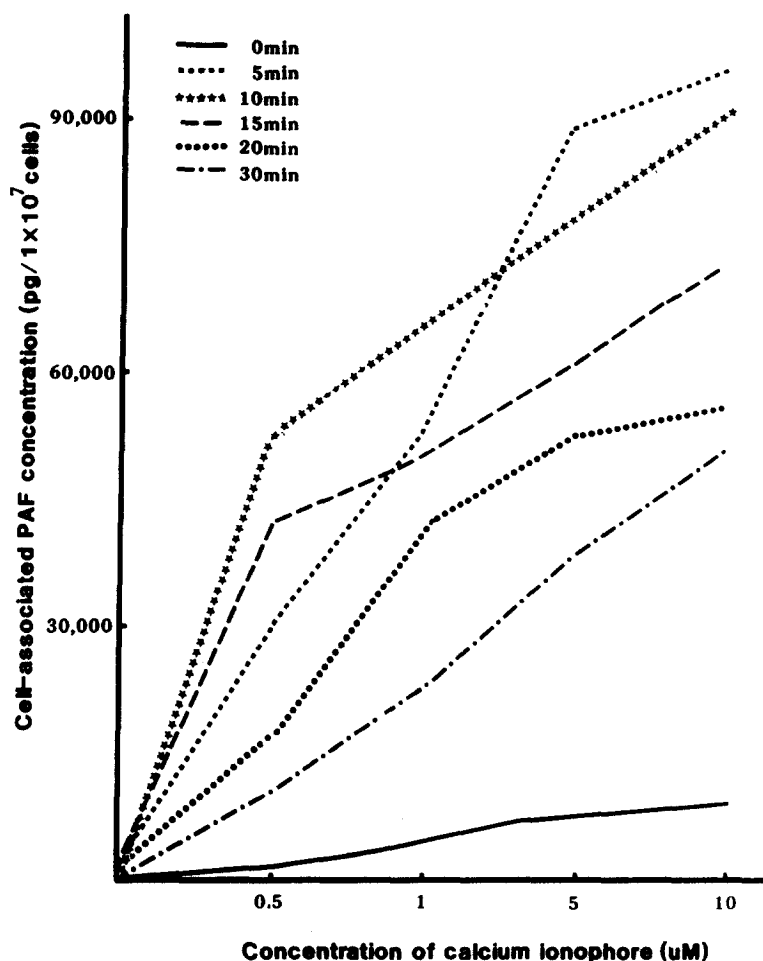


FIG. 1. Dose-response and time course of PAF production stimulated with calcium ionophore A23187. Granulocytes (5×10^6 cells/ml) incubated alone or stimulated with calcium ionophore at concentrations ranging from 0 to 10 $\mu\text{mol/L}$ and cultured for 0 to 30 minutes. Cell-associated and supernatant PAF determined by RIA kits after extraction. Optimal condition was 5 $\mu\text{mol/L}$ ionophore and incubation period of 10 minutes.

ml, of buffer were measured simultaneously for PAF concentration with use of PAF RIA kit.

Production of PAF in various patient groups

Blood samplings and measurements of PAF were done by different technicians, and the matchings of the results of PAF with the clinical response to immunotherapy for each individual were done after the completion of the whole study. Thus the classification of response to immunotherapy was made independently of the PAF data.

Statistics

Student's *t* test was used for statistical analysis. A *p* value of <0.05 was considered significant.

RESULTS

The extracted substances from plasma, culture supernatants, and cell pellets were eluted from the HPLC column at a retention time of 5.6 minutes, which was

similar to [^3H]PAF, but the HPLC system was unable to resolve [^3H]PAF from other phospholipids such as lyso-PAF, phosphatidylcholine, and sphingomyelin (data not shown). Because PAF RIA kits (DuPont) used in this study detected specifically only PAF but not other phospholipids (Table I), and the extracted sample could aggregate rabbit platelets in a bioassay,³⁴ with use of an aggregometer (data not shown), the data presented in this paper are actually about PAF that was coeluted with other phospholipids.

Fig. 1 shows the dose-response and time course of production of PAF by granulocytes stimulated with calcium ionophore A23187. The production of PAF peaked at 5 to 10 minutes after incubation and then decreased. The production of PAF increased with the dose of ionophore, but no more difference was found between 5 $\mu\text{mol/L}$ and 10 $\mu\text{mol/L}$. Thus, a concentration of ionophore of 5 $\mu\text{mol/L}$ and an incubation period of 10 minutes at 37° C were used thereafter

TABLE II. Plasma PAF concentrations in healthy controls, newly diagnosed and hyposensitized asthmatic children, and patients with acute attacks

Subjects	No. cases	Plasma PAF concentration (pg/10 ml whole blood)
Healthy controls	18	4090.1 ± 2909.1*‡
Newly diagnosed	25	8747.0 ± 3378.9†‡§
Good responders	29	3877.4 ± 1958.3†
Poor responders	18	9586.5 ± 3748.2
Acute attacks	15	75432.6 ± 14653.7‡§

PAF in plasma obtained from 10 ml whole blood was measured (mean ± SD), * $p < 0.005$, † $p < 0.005$, ‡ $p < 0.005$, § $p < 0.005$.

TABLE III. PAF concentrations in culture supernatants of healthy controls, newly diagnosed and hyposensitized asthmatic children, and patients with acute attacks

Subjects	No. cases	PAF concentration (pg/1 × 10 ⁷ cells)		
		Buffer	A23187	Mite
Healthy controls	18	467.8 ± 386.1* ^{ac}	1960.1 ± 1592.9 ^d	463.4 ± 169.1 ^f
Newly diagnosed	25	1108.7 ± 599.1 ^{ab}	3771.0 ± 2815.4 ^{de}	1857.3 ± 1647.2 ^{gh}
Good responders	29	388.4 ± 315.8 ^b	4374.2 ± 2453.5	360.2 ± 310.8 ^s
Poor responders	18	923.4 ± 542.3	3420.5 ± 1655.9	1928.5 ± 1344.6
Acute attacks	15	201.6 ± 135.4 ^c	33355.5 ± 29567.1 ^e	256.7 ± 120.4 ^h

Granulocytes (5×10^6 cells/ml) stimulated with A23187 (5 μ mol/L) or mite allergen (10 μ g/ml) at 37° C for 10 minutes. Cells and culture supernatants separated by centrifugation. PAF in 2 ml culture supernatants obtained from 1×10^7 cells was measured.

*Mean ± SD, ^a $p < 0.05$, ^b $p < 0.05$, ^c $p < 0.05$, ^d $p < 0.05$, ^e $p < 0.05$, ^f $p < 0.005$, ^g $p < 0.005$, ^h $p < 0.005$.

for calcium ionophore study. A similar study had been done for the house dust mite allergen, and the optimal condition was found to be 10 μ g/ml of mite protein, an incubation period of 10 minutes, and an incubation temperature of 37° C (data not shown).

The plasma PAF concentrations are shown in Table II. The PAF level was higher in new patients than in healthy patients ($p < 0.005$), and it decreased in good responders ($p < 0.005$) but not in poor responders. It is important to note that the PAF level in patients with acute asthmatic attacks was nearly 20 times higher than that of healthy patients.

Table III shows the secretion of PAF by granulocytes under various conditions. The spontaneous secretion of new patients was the greatest among all studied groups. After immunotherapy the spontaneous secretion was decreased in good ($p < 0.05$) but not in poor responders. The granulocytes of new patients also secreted the greatest amount of PAF when stimulated with specific allergen ($p < 0.0001$) and after immunotherapy; the secretory capacity was decreased to a level comparable to healthy patients' in good ($p < 0.005$) but not in poor responders. When stimulated with calcium ionophore A23187, all patient groups secreted a greater amount of PAF than did

healthy patients, and the amount secreted was highest in patients with acute attacks.

Table IV shows the cell-associated PAF in five studied groups under various conditions. All the patients synthesized spontaneously a greater amount of PAF than did healthy control subjects. But the difference was significant only between healthy control subjects and new patients. The new patients produced the greatest amount of PAF when stimulated with mite allergen, and the amount was decreased after immunotherapy, particularly in good responders (new patients vs good responders, $p < 0.0001$; new patients vs poor responders, $p < 0.05$). When stimulated with calcium ionophore, all patient groups, particularly the patients with acute attacks, tended to produce a greater amount of PAF than did healthy controls, but the difference did not reach a significant degree. It is interesting to note that although a major portion of synthesized PAF induced by allergen stimulation was secreted, less than 10% of synthesized PAF was secreted when stimulated with calcium ionophore.

Table V shows the total PAF production (sum of supernatants and cells) by granulocytes under various condition. The spontaneous and mite-stimulated total

TABLE IV. Cell-associated PAF concentration in healthy, newly diagnosed and hyposensitized asthmatic children and patients with acute attacks

Subjects	No. cases	PAF concentration (pg/1 × 10 ⁷ cells)		
		Buffer	A23187	Mite
Healthy controls	18	167.6 ± 180.9 ^a	47664.6 ± 39331.7 ^d	256.3 ± 140.6 ^e
Newly diagnosed	25	419.3 ± 187.7 ^{ab}	66730.2 ± 47330.1 ^c	514.3 ± 254.6 ^{efgh}
Good responders	29	305.1 ± 279.9	82197.7 ± 53505.0	182.2 ± 139.7 ^f
Poor responders	18	310.2 ± 249.8	85753.0 ± 40438.0	359.7 ± 283.6 ^g
Acute attacks	15	171.5 ± 136.5 ^b	100444.4 ± 26934.9 ^{cd}	251.6 ± 201.9 ^h

PAF in 1 × 10⁷ cells was measured.*Mean ± SD, ^a*p* < 0.005, ^b*p* < 0.05, ^c*p* < 0.05, ^d*p* < 0.05, ^e*p* < 0.005, ^f*p* < 0.0001, ^g*p* < 0.05, ^h*p* < 0.05.**TABLE V.** Total PAF concentrations (supernatants and cells) of healthy controls, newly diagnosed and hyposensitized asthmatic children, and patients with acute attacks

Subjects	No. cases	Total PAF concentration (picograms)		
		Buffer	A23187	Mite
Healthy controls	18	635.4 ± 568.2 ^{ac}	49624.7 ± 40924.7 ^c	719.7 ± 309.1 ^e
Newly diagnosed	25	1528.0 ± 788.5 ^{abd}	70501.2 ± 50145.5 ^f	2371.6 ± 1901.8 ^{ghi}
Good responders	29	693.5 ± 594.6 ^b	86571.9 ± 55958.5	542.4 ± 450.5 ^h
Poor responders	18	1233.6 ± 792.3	89173.5 ± 42094.2	2288.2 ± 1628.2
Acute attacks	18	373.1 ± 272.0 ^{cd}	133799.9 ± 56502.0 ^{ef}	508.3 ± 322.3 ⁱ

Total PAF represents the sum of PAF in supernatants and that in cells.

*Mean ± SD, ^a*p* < 0.0001, ^b*p* < 0.0001, ^c*p* < 0.05, ^d*p* < 0.0001, ^e*p* < 0.0001, ^f*p* = 0.0002, ^g*p* = 0.0004, ^h*p* < 0.0001, ⁱ*p* = 0.0001.

PAF production were highest in new patients, and the production after immunotherapy was decreased significantly only in good responders (*p* < 0.0001) but not in poor responders.

DISCUSSION

PAF is a potent inflammatory autacoid produced by a number of cell types and involved in a wide variety of diseases, including allergic and inflammatory acute reactions.^{1, 2, 35} Several lines of evidence from recent studies in both animals and human beings strongly suggest that PAF may be an important mediator in the pathogenesis of bronchial asthma.³⁻¹⁵ This postulation is further supported by the reports that a specific PAF receptor antagonist, BN 52021, was able to suppress the allergen-induced immediate bronchoconstrictive response in a certain portion of asthmatic patients via both aerosol and oral route.¹⁶⁻¹⁷ Inhaled BN52021 inhibited the PAF-induced bronchoconstriction more efficiently than the allergen-induced one.¹⁶ To further clarify the role of PAF in the pathogenesis of bronchial asthma on the one hand and delineate the working mechanisms of immunotherapy on the other hand, we measured in this study the plasma levels of PAF in children with acute asthmatic attacks and the *in vitro*

and *in vivo* production of PAF in asthmatic children before and after immunotherapy.

Several types of cell have been found to produce PAF, including basophils, mast cells, neutrophils, eosinophils, monocyte-macrophages, platelets, and endothelial cells.^{2-4, 36} The capability of PAF production by human basophils has been a matter of debate since the synthesis of PAF was first described in rabbit basophils.¹ Although Betz et al.³⁷ and Sanchez-Crespo et al.³⁸ reported the failure of human basophils to produce PAF, Camussi et al.³⁹ reported a contradictory result. Most recently Triggiani et al.⁴⁰ described a novel observation that human inflammatory cells can be divided into two groups according to the predominant type of 1-acyl-2-acetyl-GPC (glycero-3-phosphocholine [1-acyl-2-acetyl]) they produce on stimulation: those that produce predominantly 1-acyl-2-acetyl-GPC (mast cells, basophils, and endothelial cells) and those that produce almost exclusively PAF, that is, 1-alkyl-2-acetyl-GPC, (neutrophils, eosinophils, and macrophages). Furthermore, their data indicate that human basophils synthesize predominantly 1-acyl-2-acetyl-GPC in response to IgE-dependent stimuli and predominantly PAF in response to A23187. Because 1-acyl-2-acetyl-GPC can not be de-

tected by bioassay for PAF, this observation may explain partly the confusion in the previous studies. Therefore the very small number of basophils and eosinophils contaminated in the granulocyte suspension would contribute, to a certain degree, to the total PAF measured in this study, particularly if 1-acyl-2-acetyl-GPC (PAF analogue) can be detected by the PAF RIA kit used in this report.

In this study we found that PAF was present in the blood of healthy control subjects (Table II). This finding was consistent with previous reports;^{35, 41-43} moreover, the concentration of approximately 6×10^{-11} mol/L was also comparable to that reported by Carameo et al.⁴² and Lazanas et al.⁴³ During acute asthmatic attacks, the plasma concentration of PAF increased to nearly 20 times that of healthy control subjects, and it is reasonable to speculate that the local concentration of PAF in the airways must be much higher than that of blood because in addition to mast cells and basophils, alveolar macrophages, neutrophils, and other cell types can also be activated to produce PAF. Recently increased PAF in cerebrospinal fluid was reported in patients with bacterial meningitis, suggesting that neutrophil is a major cell type producing PAF.⁴⁴ The increased production of PAF during acute asthmatic attacks may account partly for the pathologic changes found in bronchial asthma such as bronchoconstriction, mucosal edema, increased mucus secretion, and infiltration of airways with inflammatory cells, particularly eosinophils.^{5, 11} Lazanas et al.⁴³ also reported markedly increased circulating PAF during acute allergic drug reaction in patients receiving nonsteroid antiinflammatory drugs (NSAIDs). Thus the present study and the study of Lazanas et al.⁴³ support the postulation that PAF may be involved in allergic reaction in humans.

In spite of increased circulating PAF in patients with acute asthmatic attacks, both the spontaneous and allergen-stimulated *in vitro* synthesis of PAF (secreted, and cell-associated forms) by granulocytes of those patients were not increased (Tables III to V). Moreover, among the five studied groups, granulocytes of those patients with acute attacks still produced the greatest amount of PAF when stimulated with calcium ionophore. These findings, in conjunction with the fact that a major portion of allergen-induced PAF, but less than 10% of that induced by ionophore was secreted, strongly suggest that the pathway by which allergen stimulates production of PAF is different from that of calcium ionophore.

To the authors' knowledge, the present report was the first attempt in the literature to study the effect of immunotherapy on the *in vivo* and *in vitro* production of PAF. Our study showed that both the plasma con-

centration (Table II) and the *in vitro* production of PAF (Tables III to V) were significantly higher in new patients than in healthy control subjects, and after immunotherapy both the *in vivo* (plasma) and *in vitro* production of PAF (spontaneous and allergen-stimulated) were decreased in good but not in poor responders. These results suggest that immunotherapy may affect the production, metabolic pathway, or secretion of inflammatory mediators including PAF; however, the mechanism and the reason why only a certain portion of treated patients are affected beneficially need further study.

REFERENCES

1. Benveniste J, Henson PM, Cochrane CG. Leukocyte-dependent histamine release from rabbit platelet. The role of IgE, basophils, and a platelet-activating factor. *J Exp Med* 1972;136:1365-76.
2. Braquet P, Touqui L, Shen TY, Vargaftig BB. Prospectives in platelet activating factor research. *Pharmacol Rev* 1987;39:97-145.
3. Vargaftig BB, Lefort J, Chignard M, Benveniste J. Platelet-activating factor induces a platelet-dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. *Eur J Pharmacol* 1980;65:185-92.
4. Pinckard RN, McManus LM, Halonen M, Hanahan D. Immunopharmacology of acetyl glyceryl ether phosphorylcholine (AGEPC). In: Newball HH, ed. *Immunopharmacology of the lung*. New York: Marcel Dekker, 1984:73-103.
5. Fabbri L. Airway inflammation and asthma. Importance of arachidonate metabolites for airway hyperresponsiveness. *Prog Biochem Pharmacol* 1985;20:18-25.
6. Mazzoni L, Morley J, Page CP, Sanjar S. Induction of airway hyper-reactivity by platelet-activating factor in the guinea-pig. *J Physiol* 1983;365:107.
7. Aoki S, Boubekeur K, Burrows L, Morley J, Sanjar S. Recruitment of eosinophils by platelet-activating factor (PAF) in the guinea pig lung. *J Physiol* 1987;394:130.
8. Stevenson JS, Tallent M, Blinder B, Abraham WM. Modification of antigen-induced late responses with an antagonist of platelet-activating factor (WEB 2086). *Fed Proc* 1987;46:1454A.
9. Coyle AJ, Sjoerdsma K, Page CP, Brown L, Metzger WJ. Modification of the late-phase asthmatic response and bronchial hyperactivity by BN52021, a platelet-activating factor antagonist. *Clin Res* 1987;35:254A.
10. Knauer KA, Lichtenstein LM, Franklin Adkinson N Jr, Fish JE. Platelet activation during antigen-induced airway reactions in asthmatic subjects. *N Engl J Med* 1981;304:1404-7.
11. Metzger WJ, Hunninghake GW, Richerson HB. Late asthmatic responses: inquiry into mechanisms and significance. *Clin Rev Allergy* 1985;3:145-65.
12. Grandel KE, Wardlow ML, Fare RS. Platelet activating factor in sputum of patients with asthma and COPD. *Fed Proc* 1985;44:184.
13. Gateau O, Arnoux B, Deriaz H, Viars P, Benveniste J. Acute effects of intratracheal administration of PAF-acether (platelet-activating factor) in humans. *Am Rev Respir Dis* 1984;129:A3.
14. Cuss FM, Dixon CMS, Barnes PJ. Effect of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. *Lancet* 1986;2:189-92.

15. Rubin HE, Smith LJ, Patterson R. The bronchoconstrictor properties of platelet-activating factor in humans. *Am Rev Respir Dis* 1987;136:1145-51.
16. Hsieh KH. Effects of PAF antagonist, BN52021, on the PAF-, methacholine- and allergen-induced bronchoconstriction in asthmatic children. *Chest* 1991;99:877-82.
17. Guinot P, Brambiilla C, Duchieer J, Braquet P, Bonvoisin B, Cournot A. Effect of BN52063, a specific PAF-acether antagonist, on bronchial provocation to allergens in asthmatic patients. A preliminary study. *Prostaglandins* 1987;34:723-31.
18. Muino JC. Asthma and a specific inhibitor of platelet activating factor. *Allergy Clin Immunol News* 1990;2:75-7.
19. Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911;1:1572-3.
20. Lockey RF, Bukantz SC. Diagnostic tests and hyposensitization therapy in asthma. In: Weiss EB, Segal MS, eds, *Bronchial asthma: mechanisms and therapeutics*. Boston: Little Brown and Company, 1976:613-37.
21. Patterson R, Liebman P, Irons JS, et al. Immunotherapy. In: Middleton E Jr, Read CE, Ellis EF, eds, *Allergy: principles and practices*. St. Louis: CV Mosby, 1983:119-42.
22. Evans R, Pence H, Kaplan GH, Rocklin RE. The effect of immunotherapy on humoral and cellular response in ragweed hay fever. *J Clin Invest* 1976;57:1378-83.
23. Rocklin RE, Sheffer AL, Greineder DK, Melmon KL. Generation of antigen-specific suppressor cells during allergy desensitization. *N Engl J Med* 1980;302:1213-8.
24. Hsieh KH. Change of lymphoproliferative responses of T-cell subsets to allergen and mitogen after hyposensitization in asthmatic children. *J ALLERGY CLIN IMMUNOL* 1984;74:34-7.
25. Hsieh KH. Altered interleukin-2 (IL-2) production and responsiveness after hyposensitization to house dust. *J ALLERGY CLIN IMMUNOL* 1985;76:188-94.
26. Tamir R, Castracan JM, Rocklin RE. Generation of suppressor cells in atopic patients during immunotherapy that moderate IgE synthesis. *J ALLERGY CLIN IMMUNOL* 1987;79:591-8.
27. Hsieh KH. Decreased production of interleukin-2 receptors after immunotherapy to house dust. *J CLIN IMMUNOL* 1988;8:171-7.
28. Liao TN, Hsieh KH. Altered production of histamine-releasing factor (HRF) activity and responsiveness to HRF after immunotherapy in asthmatic children. *J ALLERGY CLIN IMMUNOL* 1991;86:894-901.
29. Pruzansky JJ, Patterson R. Histamine release from leukocytes of hypersensitive patients, II. Reduced sensitivity of leukocytes after injection therapy. *J Allergy* 1967;39:44-56.
30. Borowska SC, Kuczevska B, Rogalewska A, Hofman J. Immunologic histamine release from isolated human basophils during specific immunotherapy. *Agents Actions* 1986;18:176-7.
31. Wang JY, Hsieh KH. The effect of immunotherapy on the in vitro productions of histamine, prostaglandin E₂ and leukotriene C₄ in asthmatic children. *Asian Pacific J Allergy Immunol* 1989;7:119-24.
32. Hsieh KH. Immunological changes after hyposensitization in asthmatic children—a review. *J Asthma* 1987;24:19-27.
33. Nakada S, Ito K, Urata C, et al. Allergenicity and immunogenicity of house-dust mite (*Dermatophagoides farinae*) antigens treated with glutaraldehyde. *Ann Allergy* 1985;54:437-41.
34. Ardlic NG, Packham MA, Mustard JF. Adenosine diphosphate-induced platelet aggregation in suspensions of washed rabbit platelets. *Br J Haematol* 1970;19:7-17.
35. Pinckard RN. Physicochemical and functional identity of rabbit platelet-activating factor (PAF) released in vivo during IgE anaphylaxis with PAF released in vitro from IgE sensitized basophils. *J Immunol* 1979;123:1234-47.
36. Arnoux B, Simoes-Cacioro MH, Landes A, Mathieu M, Duroux P, Benveniste J. Alveolar macrophages from asthmatics release PAF-acether and lyso-PAF-acether when stimulated with specific antigen. *Am Rev Respir Dis* 1982;125:70.
37. Betz SJ, Lotner GZ, Henson PM. Generation and release of platelet-activating factor (PAF) from enriched preparations of rabbit basophils; failure of human basophil to release PAF. *J Immunol* 1980;125:2749-55.
38. Sanchez-Crespo M, Alonso F, Egido J. Platelet-activating factor in anaphylaxis and phagocytosis. I. Release from human peripheral polymorphonuclears and monocytes during the stimulation by ionophore A23187 and phagocytosis but not degranulating basophils. *Immunology* 1980;40:645-55.
39. Camussi G, Aglietta M, Coda R, Bussolino F, Piacibello W, Teta C. Release of platelet-activating factor (PAF) and histamine II. The cellular origin of human PAF: monocyte, polymorphonuclear neutrophils and basophils. *Immunology* 1981;42:191-9.
40. Triggiani M, Schleimer RP, Warner JA, Chilton FH. Differential synthesis of 1-acyl-2-acetyl-glycero-3-phosphocholine and platelet-activating factor by human inflammatory cells. *J Immunol* 1991;147:660-6.
41. Sanchez-Crespo M, Inarrea P, Aevarez V, Alonso F, Egido J, Hernando L. Presence in normal human urine of a hypotensive and platelet-activating phospholipid. *Ann J Physiol (Renal Fluid Electrolyte Physiol)* 1983;244:706-11.
42. Caramelo C, Fernandez-Gallardos S, Marin-Cao D, et al. Presence of platelet-activating factor in blood from humans and experimental animals. Its absence in anephric individuals. *Biochem Biophys Res Commun* 1984;120:789-96.
43. Lazanas M, Demopoulos CA, Tournis S, Koussissis S, Labrakis-Lazanas K, Tsaroukas X. PAF of biological fluids in disease: i.v. levels in blood in allergic reactions induced by drugs. *Arch Dermatol Res* 1988;280:124-6.
44. Odio CM, Faingezicht I, Paris M, et al. The beneficial effects of early dexamethasone administration in infants and children with bacterial meningitis. *N Engl J Med* 1991;324:1525-31.