Characterization of Serum Platelet-activating Factor (PAF) Acetylhydrolase

Correlation between Deficiency of Serum PAF Acetylhydrolase and Respiratory Symptoms in Asthmatic Children

Masao Miwa,* Takeshi Miyake,‡ Tatsuhiro Yamanaka,§ Junko Sugatani,‖ Yasuo Suzuki,* Shigeyo Sakata,¶ Yuji Araki,¶ and Makoto Matsumoto*

**Department of Biochemistry, School of Pharmaceutical Science, University of Shizuoka, Oshika 2-2-1, Shizuoka 422, Japan; ‡Division of Allergy, Clinical Immunology and Infectious Diseases, Shizuoka Children's Hospital, Shizuoka 420, Japan; §Department of Pediatrics, Faculty of Medicine, The University of Tokyo, Tokyo 113, Japan; ‖Department of Medical Chemistry, Kansai Medical School, Osaka 570, Japan; and ¶Shizuoka Red Cross Blood Center, Shizuoka 420, Japan*

Abstract

Platelet-activating factor (PAF) acetylhydrolase has been recognized as an enzyme that inactivates PAF. We developed a convenient and reproducible method for determining human serum PAF acetylhydrolase activity. The assay was based on measurement of [^{14}C]acetate produced from 1-*O*-alkyl-2-[^{14}C]-acetyl-*sn*-glycero-3-phosphocholine upon precipitation of the complex of radioactive substrate and albumin with TCA. The apparent K_m value of PAF acetylhydrolase (near the physiological concentration of serum protein) was 1.5×10^{-4} M PAF. 32 subjects with serum PAF acetylhydrolase deficiency were found among 816 healthy Japanese adults. The low PAF acetylhydrolase activity in the deficient serum might not be due to the presence of enzyme inhibitor. Both the sensitivity to PAF and the metabolism of PAF in platelets from PAF acetylhydrolase-deficient subjects were almost the same as those of normal subjects. Deficiency in serum PAF acetylhydrolase appeared to be transmitted by autosomal recessive heredity among five Japanese families. Among healthy adults, healthy children, and asthmatic children, who were grouped into five classes on the basis of respiratory symptoms (remission, wheezy, mild, moderate, and severe groups), the probability of PAF acetylhydrolase deficiency was significantly higher in groups with severe symptoms (moderate and severe) ($P < 0.01$). These results suggest that deficiency of serum PAF acetylhydrolase might be one of the factors leading to severe respiratory symptoms in asthmatic children.

Introduction

Platelet-activating factor ([PAF][1]; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine [1–4]) was originally discovered as a fluid-phase mediator released from antigen-stimulated IgE-sensitized basophils, but is now known to be formed in various cells including neutrophils, eosinophils, macrophages, mast cells, and vascular endothelial cells upon chemical or immune stimulation (5). Sensitized lungs also release PAF when challenged with antigen (6). This ether phospholipid causes platelet aggregation and degranulation (7–11), and is suggested to play an important role in the pathogenesis of anaphylaxis and inflammation (5). PAF has a wide spectrum of biological activity, not only stimulating platelets (7–11), neutrophils (12), and eosinophils (13, 14), but also enhancing vascular permeability (15–17) and inducing hypotension (18, 19), platelet-dependent bronchoconstriction (20), smooth muscle contraction (21, 22), and other alterations (e.g., acute inflammation and edema) in pulmonary dynamics (5). Because these biological effects are produced at very low concentrations of PAF, in the region of 10^{-10}–10^{-11} M, it seems that accumulation of PAF in body fluids and tissues, as in the case of anaphylaxis and endotoxic shock (23), is highly toxic. It has been reported by Halone et al. that a dose of PAF as low as 1 μg/kg is lethal in rabbits (24). Hence, it is considered that a system to regulate the concentration of PAF (e.g., PAF metabolism into an inactive product) is essential. Before the structure of PAF was identified, it was reported that human and rabbit serum contained an acid-labile factor that inactivated PAF (25, 26). Later, it was clarified that this PAF-inactivating factor in serum was acetylhydrolase, which hydrolyzes the *sn*-2-acetyl moiety of PAF to inactive lyso PAF (27, 28). This enzyme has since been found in the cytosolic fraction of several rat tissues (29, 30) and human blood cells (31). The current study was focused on acetylhydrolase in human serum and two major findings emerged. First, some Japanese families with serum PAF acetylhydrolase deficiency were found; second, such deficiency or low activity of serum PAF acetylhydrolase was suggested to be correlated with the severity of bronchial asthma in wheezy children.

Methods

1-*O*-Hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine ($C_{16:0}$-PAF) was obtained from BACHEM Feinchemikalien AG (Bunbendorf, Switzerland). [^{14}C]Acetyl-PAF (1-*O*-alkyl-2-[^{14}C]acetyl-*sn*-glycero-3-phosphocholine) was synthesized by acetylation of 1-*O*-alkyl-*sn*-glycero-3-phosphocholine (lyso PAF) with [^{14}C]acetic anhydride (Amersham Corp. Japan, Tokyo, Japan) according to the method of Blank et al. (29). 1-*O*-Alkyl-*sn*-glycero-3-phosphocholine was prepared by hydrogenation over platinum oxide and alkaline hydrolysis of choline glycerophospholipid from beef heart; the components were 1-*O*-hexadecyl (78%) and 1-*O*-octadecyl (22%)-*sn*-glycero-3-phosphocholine (1). [^{14}C]Acetyl-PAF was purified using a silicic acid column and preparative TLC (29). [^{14}C]Acetyl-PAF showed a single radioactive spot, which migrated between lysophosphatidylcholine and sphingomyelin in both neutral solvent (chloroform/methanol/water, 65:35:7, vol/vol/vol) and basic solvent (chloroform/methanol/28% ammonium

Address reprint requests to Dr. Masao Miwa, Department of Biochemistry, School of Pharmaceutical Science, University of Shizuoka, Oshika 2-2-1, Shizuoka 422, Japan.

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1. *Abbreviations used in this paper:* PAF, platelet-activating factor.

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hydroxide, 70:30:3, vol/vol/vol) on HPTLC plates (Merck Sharp & Dohme, West Point, PA). The specific radioactivity of [^{14}C]acetyl-PAF used for the measurement of PAF acetylhydrolase was 45 μCi/mmol. 1-*O*-[^{3}H]Hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (45 Ci/mmol) was purchased from New England Nuclear Japan (Tokyo).

Measurement of serum PAF acetylhydrolase activity

(a) Determination of acetate released from [^{14}C]PAF. For the routine assay, [^{14}C]acetyl-PAF was suspended in Tyrode buffer (pH 7.2) containing 2.5 mg/ml BSA so that its concentration was 1 mM. Serum (50 μl) was incubated with 50 μl of [^{14}C]acetyl-PAF (50 nmol) in a micro glass test tube at 37°C for 10 min unless otherwise indicated. The reaction was stopped by addition of 100 μl of 14% TCA and the mixture was left to stand for 10 min at 0°C. To separate the denatured protein, the reaction mixture was centrifuged for 5 min at 2,500 rpm (3,800 *g*). 100 μl of the supernatant was mixed with 5 ml of scintillation cocktail (Scintisol EX-H; Dojin Chemical Institute, Kumamoto, Japan) and the radioactivity determined in a liquid scintillation counter (LS602; Aloka Inc., Tokyo, Japan). The control values of released acetic acid were obtained for both free serum and serum heated for 10 min in boiling water. One unit of enzyme activity was defined as that which produced 1 nmol of acetate per minute at 37°C. 1-*O*-[^{3}H]Hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine ([^{3}H]alkyl-PAF) was used to determine the reaction product. 50 μl of PAF (50 nmol), suspended in 0.1% BSA/50 mM Tris-HCl buffer, pH 7.2/saline, was incubated with serum (50 μl) for the desired periods of time at 37°C. After terminating the reaction by adding 125 μl of chloroform and 250 μl of methanol, lipid was extracted by the method of Bligh and Dyer (32). Lipid samples were applied to a precoated Silica gel G TLC plate and run in a solvent system of chloroform/methanol/water (65:35:7, vol/vol/vol). Spots were identified by comigration with authentic standards after exposure with iodine vapor. After spraying with spray reagent (Enhance; New England Nuclear Japan, Tokyo), the radioactivity of each of the spots on the Silica gel G plate was determined with an ultra-high sensitivity TV camera system (ARGUS-100; Hamamatsu Photonics K.K., Hamamatsu, Japan) (33). Subsequently, individual spots were scraped into vials, extracted in 0.2 ml of chloroform/methanol/water (1:2:0.8, vol/vol/vol), and then mixed with 5 ml of scintillation cocktail. The radioactivity was determined in a liquid scintillation counter. Results shown in the tables and figures are the means of duplicate determinations unless otherwise stated. The variations were < 3%.

(b) Bioassay of serum PAF acetylhydrolase activity. 50 μl of $C_{16:0}$-PAF (2×10^{-9}–2×10^{-8} M) suspended in Tyrode buffer (pH 7.2) containing 2.5 mg/ml BSA was incubated with 50 μl of serum for 5 min at 37°C. 20-μl aliquots were removed and assayed immediately for platelet-aggregating ability. Rabbit platelets were isolated as previously described (34). The washed platelets suspended in Ca^{2+}-free Tyrode-gelatin buffer (pH 7.2) (40 μl, 5×10^{7} cells) were transferred to aggregometer (dual-channel hematracer; Niko Bioscience, Tokyo) cuvettes and made up to 200 μl with Tyrode-gelatin buffer (pH 7.2) containing 1 mM $CaCl_2$.

Measurement of PAF sensitivity of human platelets from normal and acetylhydrolase-deficient subjects. 5-ml samples of blood were collected in 0.8 ml of 3.8% sodium citrate. Platelet-rich plasma (PRP) was obtained by centrifuging citrated blood at 150 *g* for 10 min, and 200 μl of PRP was incubated in cuvettes for 5 min at 37°C. Then, 10 μl of authentic $C_{16:0}$-PAF (1×10^{-10}–1×10^{-6} M) was added to the platelets. Changes in light transmittance were monitored by aggregometer.

Patients. 175 elementary school children (6–12 yr old; 97 boys and 78 girls) with a history of wheezing, selected using the modified American Thoracic Society Division of Lung Disease children's questionnaire and subsequently by a second questionnaire to investigate the severity of respiratory symptoms over the last 2 yr, were classified into five groups (remission, 71 subjects; wheezy, 29; mild, 33; moderate, 24; and severe, 18), according to the grade of bronchial asthma (Japanese Society of Pediatric Allergy and Clinical Immunology) (35). In the remission group were 35 subjects who had experienced neither wheezing nor dyspnea attacks over the previous 2 yr. In the wheezy group were subjects who had experienced wheezing attacks without dyspnea less than four times per year. In the mild group were subjects who had experienced wheezing attacks without dyspnea between five and nine times, or dyspnea attacks without orthopnea less than four times per year. Moderate subjects had experienced wheezing attacks without dyspnea > 10 times, dyspnea attacks without orthopnea between five and nine times, or orthopnea attacks less than four times per year. Severe subjects had experienced dyspnea attacks > 10 times, or orthopnea attacks more than five times per year. All patients in the combined groups of mild, moderate, and severe had displayed the typical clinical characteristics of bronchial asthma. The diagnosis was confirmed hematologically by the total IgE level and by the presence of specific serum IgE antibodies to *Dermatophagoides pteronyssinus* (radioallergosorbence test [RAST]; Pharmacia Fine Chemicals Co. Uppsala, Sweden) using mite as the allergen (35). Of 175 patients, 90 had a serum total IgE value of 300 IU/ml or over, as measured by a paper radioimmunosorbence test, and 85 subjects had levels < 300 IU/ml. Serum total IgE levels in groups with severe symptoms (moderate and severe) were significantly higher than in those with slight symptoms (remission and wheezy). Upon IgE-RAST for measuring the level of specific serum IgE antibodies to *Dermatophagoides pteronyssinus,* 127 subjects had a RAST score of 2 or over, and 48 subjects scored less than 1. The percentages of subjects exhibiting a RAST score of 2 or over in each of the groups (remission, wheezy, mild, moderate, and severe) were 51, 79, 88, 92, and 94, respectively. These results are compatible with those of previous reports on outpatients attending pediatric allergy clinics in Japan. Blood from the patients was collected when asthmatic attacks were absent, unless otherwise stated. In addition, we selected 36 children (6–12 yr old; 18 boys and 18 girls) without any history of wheezing as a control group. The healthy control children had no history of allergic disease. The RAST score was 0, and the total IgE value was < 300 IU/ml. The serum PAF acetylhydrolase activity of 816 healthy adults (20–45 yr old; 408 men and 408 women), who were selected at random from among 1,600 volunteers and had normal values of glutamic oxalic-acetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, and blood pressure, was also assayed. Serum samples were separated from venous blood by centrifugation after being left to stand for 30 min at room temperature, and kept at −80°C until use.

Results and Discussion

A simple and reproducible method for measuring serum PAF acetylhydrolase activity was developed as follows. [^{14}C]Acetyl-PAF was incubated with human serum, and then TCA was added to stop the reaction. The TCA-soluble materials were separated from the denatured protein by centrifugation. The acid-soluble fraction after 0-, 5-, and 10-min incubation of [^{14}C]acetyl-PAF at 0°C with the HCl-treated serum, whose PAF acetylhydrolase had been inactivated by 30-min incubation at pH 1.0 and 37°C and then neutralized, contained 0.98, 1.8, and 2.3% of the radioactivity, respectively. In the case of 1-*O*-[^{3}H]alkyl-PAF, the acid-soluble fraction after incubation for 10 min with serum at 37°C contained only 0.12% of the radioactivity. When [^{14}C]acetyl-PAF was hydrolysed with 0.5 N NaOH at 50°C for 30 min, neutralized with HCl, and then incubated with serum for the assay, 98–99% of the radioactivity was recovered in the supernatant. The products of reaction of 1-*O*-[^{3}H]alkyl-PAF with serum were mainly 1-*O*-[^{3}H]alkyl-2-lyso-*sn*-glycero-3-phosphocholine (99%). 1-*O*-[^{3}H]Alkyl-2-acyl-*sn*-glycero-3-phosphocholine was not detected. The values of serum acetylhydrolase activity measured by our devised method (TCA precipitation method) were compatible

with those for deacetylated 1-*O*-[^{3}H]alkyl-PAF (1-*O*-[^{3}H]-alkyl-2-lyso-*sn*-glycero-3-phosphocholine) obtained by the conventional TLC method (Fig. 1). The correlation coefficient between the results obtained by the TCA precipitation method and the TLC method was 0.993, indicating that it would be possible to determine PAF acetylhydrolase activity from the radioactivity recovered in the supernatant. Fig. 2 shows the kinetics of the time-, serum protein concentration-, and PAF concentration-dependent metabolism. Linearity for up to 15 min of incubation and 3.1 mg of serum protein (~ 50 μl serum) was observed. As the apparent K_m value of human serum PAF acetylhydrolase was 1.5×10^{-4} M PAF, the routine enzyme assay used 5×10^{-4} M [^{14}C]acetyl-PAF and 50 μl of human serum, unless otherwise stated. The enzyme activity was not affected by EDTA (1–10 mM). The PAF antagonist CV3988 (8 mM) completely inhibited the serum PAF acetylhydrolase activity, indicating that the antagonist competed with PAF for PAF acetylhydrolase. These observations showed that this enzyme activity was not due to phospholipase A_2, but mainly to specific PAF acetylhydrolase.

The mean serum PAF acetylhydrolase activities in healthy Japanese adults and healthy children were 1.83±0.75 nmol/min per 50 μl serum for 816 subjects (20–45 yr old) and 1.25±0.49 nmol/min per 50 μl serum for 211 subjects (6–12 yr old; 30 subjects of each age), respectively. Although the mean enzyme activity in healthy adults was higher than that in healthy children, there were no significant differences in the enzyme activity among children of each of the various ages (6–12 yr old). Serum PAF acetylhydrolase activities of healthy subjects showed no change over the course of 1 d, 1 wk, and also over several months (Fig. 3). There was no statistical difference in enzyme activity between men and women (data not shown).

We found 32 and 8 subjects with PAF acetylhydrolase activity–negative serum among 816 healthy adults ($P = 0.039$) and 211 ($P = 0.038$) healthy children, respectively. The low or undetectable level of serum PAF acetylhydrolase activity might have been due to one of the following: (*a*) the presence of PAF acetylhydrolase inhibitor; (*b*) a deficiency of enzyme(s) necessary to activate the inactive PAF acetylhydrolase (proenzyme); or (*c*) a defect of the PAF acetylhydrolase itself. As shown in Table I, the influence of negative serum on PAF acetylhydrolase activity was investigated; PAF acetylhydrolase

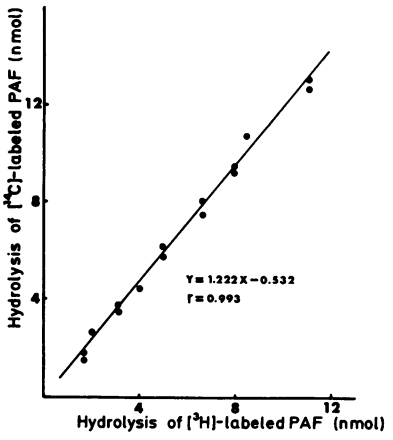

Figure 1. Correlation of PAF acetylhydrolase activities obtained from radioactivities of supernatant upon TCA precipitation (released acetate) and deacetylated products (lyso PAF) of 1-*O*-[^{3}H]alkyl-PAF. (*A*) 50 μl of 1-*O*-alkyl-2-[^{14}C]acetyl-*sn*-glycero-3-phosphocholine (50 nmol), suspended in Tyrode buffer, pH 7.2, containing 1 mg/ml BSA, was incubated with 50 μl serum for 10 min at 37°C. The reaction was stopped by addition of 14% TCA (100 μl). After precipitation of the denatured protein by centrifugation, the radioactivity in 100 μl of the supernatant was measured by liquid scintillation. (*B*) 50 μl of 1-*O*-[^{3}H]alkyl-PAF (50 nmol), suspended in 0.1% BSA/50 mM Tris-HCl buffer, pH 7.2/saline, was incubated with serum (50 μl) for 10 min at 37°C. After terminating the reaction by adding 125 μl of chloroform and 250 μl of methanol, lipid was extracted by the method of Bligh and Dyer. The radioactivities of reaction products were determined as described in Methods.

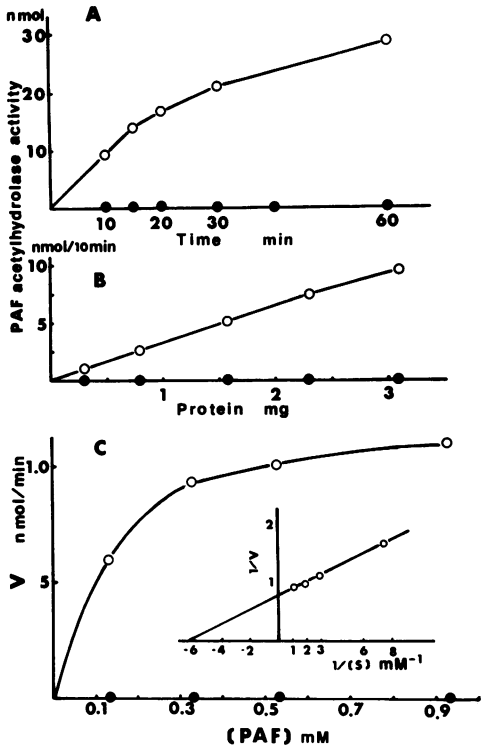

Figure 2. Effect of incubation time and the concentrations of serum protein and substrate on PAF acetylhydrolase activity. Serum PAF acetylhydrolase activity was measured by our TCA precipitation method, as described in Methods. ○, normal serum. ●, PAF acetylhydrolase-deficient serum.

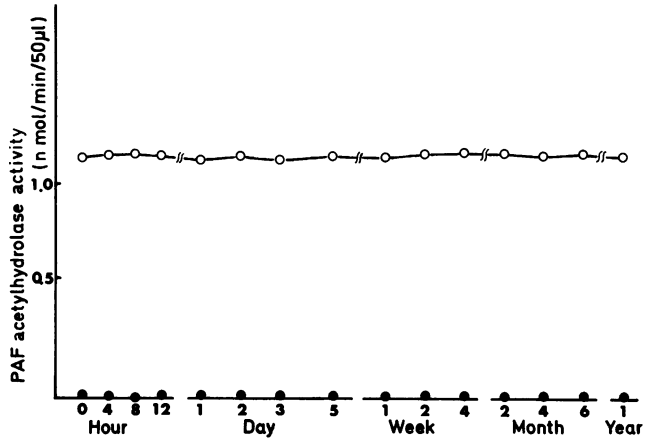

Figure 3. Variability of serum PAF acetylhydrolase activity in the same subject at different times. PAF acetylhydrolase activity from 10 normal subjects and 5 PAF acetylhydrolase-deficient subjects (healthy adults) was measured at the indicated times. Typical data are expressed in the figure from 10 normal serum (○) and 5 PAF acetylhydrolase-deficient serum (●) samples.

Table I. Influence of PAF Acetylhydrolase-deficient Serum on PAF Acetylhydrolase Activity

PAF·AH(+) Serum		PAF·AH(−) Serum		PAF acetylhydrolase activity
A	B	I	II	
	μl			*nmol/min*
50	0	0	0	1.20±0.02
25	25	0	0	1.45±0.04
0	50	0	0	1.81±0.06
0	30	0	0	1.08±0.05
0	20	0	0	0.73±0.03
0	30	20	0	1.04±0.02
0	20	30	0	0.71±0.02
0	10	40	0	0.36±0.01
0	0	50	0	NEG
0	10	0	0	0.36±0.01
0	10	0	10	0.35±0.01
0	10	0	20	0.36±0.01
0	10	0	30	0.31±0.01
0	10	0	40	0.32±0.01
0	0	0	50	NEG

A, B, I, and II are different sera, which were selected randomly among 816 healthy adults. Each combination was done to final volume as 50 μl of serum. Determination of PAF acetylhydrolase (PAF AH) activity was shown in detail in the text. Results are the means±SD for three separate experiments. NEG, negligible.

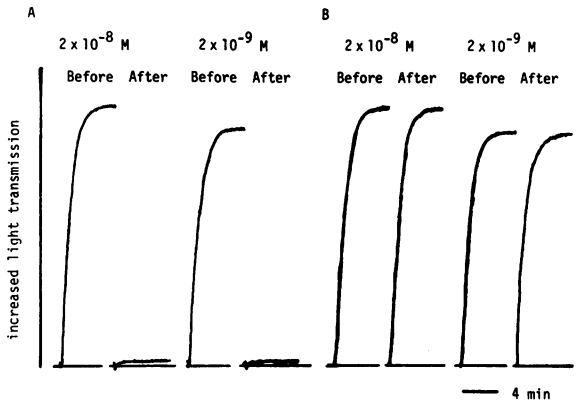

Figure 4. Platelet aggregation activities of $C_{16:0}$-PAF before and after incubation with normal (PAF acetylhydrolase-positive) or deficient (PAF acetylhydrolase-negative) serum. 50-μl aliquots of $C_{16:0}$-PAF suspension at the concentrations indicated in the figure were each incubated with 50 μl of (*A*) PAF acetylhydrolase activity-positive or (*B*) PAF acetylhydrolase activity-negative serum at 37°C for 5 min. After incubation, the mixtures were cooled rapidly in an ice bath. Platelet aggregation activity was measured by adding the incubation mixture (20 μl) to 200 μl of washed rabbit platelets suspended in Tyrode gelatin buffer, pH 7.2, containing 1 mM $CaCl_2$. Changes in light transmittance were monitored using an aggregometer. The results from one of six separate experiments using six deficient sera are shown in the figure.

activities of the mixture of PAF acetylhydrolase-positive sera A and B were additive. On the other hand, PAF acetylhydrolase activities of the mixture of PAF acetylhydrolase-positive and negative serum were not different from the respective activities in the control experiments (Table I), even though the mixtures were preincubated for 5–20 min at 37°C (data not shown). Next, to confirm whether PAF acetylhydrolase activity–negative serum was unable to inactivate PAF even at a lower concentration (close to the physiological concentration), 1×10^{-9}–5×10^{-8} M $C_{16:0}$-PAF was incubated with negative serum at 37°C for 5 min. The original platelet aggregation was induced by the $C_{16:0}$-PAF solution incubated with negative serum, but not upon incubation with normal serum (Fig. 4). These observations indicated that the acetylhydrolase activity–negative serum had no effect on 1×10^{-9}–5×10^{-4} M PAF. PAF-acetylhydrolase activity was not observed in the deficient serum at the reaction times, serum concentrations and PAF concentrations investigated, as shown in Fig. 2. Deficiency of PAF acetylhydrolase activity was also observed over the course of 1 d, 1 wk, and several months (Fig. 3). These results suggested that the undetectable or very low level of PAF acetylhydrolase activity was not due to the presence of its inhibitor and/or a deficiency of enzyme(s) necessary for activating the inactive PAF acetylhydrolase (proenzyme), and that it was the result of a defect of the PAF acetylhydrolase itself.

As it has been reported that serum PAF acetylhydrolase is associated with LDL (36), the levels of LDL, cholesterol, and PAF acetylhydrolase activity in normal and PAF acetylhydrolase-deficient sera were determined. Table II indicates that serum PAF acetylhydrolase activity, LDL cholesterol, and triacylglycerol levels of deficient subjects were not significantly different from those of the normal subjects. The deficiency of PAF acetylhydrolase was not correlated with the levels of LDL or total cholesterol.

Whether such PAF target cells as platelets in PAF acetylhydrolase-deficient blood might have reduced the responsiveness to PAF and whether intracellular acetylhydrolase might also have been lacking in persons showing a deficiency of the serum enzyme are indeed pertinent questions. Platelets from normal and deficient subjects were aggregated to almost the same extent by 2×10^{-9}–2×10^{-7} M PAF (data not shown). This indicated that PAF acetylhydrolase deficiency was not correlated with the decreased responsiveness of platelets to PAF. As for the latter question, we succeeded in measuring the intracellular PAF acetylhydrolase activity of platelets. Fig. 5 shows that the reaction products from [^{3}H]alkyl-PAF after incubation with platelets from normal and deficient subjects were 1-[^{3}H]alkyl-2-lyso-*sn*-glycero-3-phosphocholine (31.4%)

Table II. Content of LDL, Free and Total Cholesterol and Triacylglycerol in Serum from Normal and Deficient Subjects

	Normal subjects (20)	Deficient subjects (20)
PAF acetylhydrolase (*U*)	1.97±0.61	0
LDL (*mg/dl*)	374±55	345±51
Free cholesterol (*mg/dl*)	39±4	39±7
Total cholesterol (*mg/dl*)	148±13	145±15
Triacylglycerol (*mg/dl*)	77±29	69±22

Normal subjects (20) and PAF acetylhydrolase deficient subjects (20) were selected randomly among 816 healthy adults. Blood from 12–15-h fasted subject was collected for determination of lipid levels.

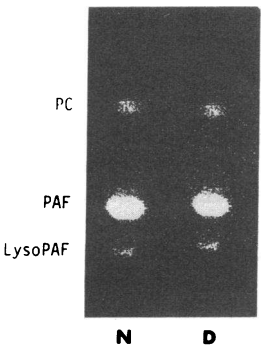

Figure 5. Metabolites of 1-*O*-[^{3}H]-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine in platelets from serum PAF acetylhydrolase-positive and -negative subjects. 1-*O*-[^{3}H]Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (50 μl) suspended in 50 mM Tris-HCl buffer, pH 7.2, containing 20 mM EDTA, was incubated with the platelet lysate (50 μl, protein 0.125–0.150 mg), which was prepared by freezing and thawing followed by 5-min sonication. After terminating the reaction by adding 125 μl of chloroform and 250 μl of methanol, lipid was extracted by the method of Bligh and Dyer. Metabolites of [^{3}H]PAF were separated on Silica gel G plates using a chloroform/methanol/water (65:35:7, vol/vol/vol) solvent system. The radioactivity was determined using an ultra-high sensitivity TV camera system (ARGUS-100) as described in Methods. PC, 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine; PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; LysoPAF, 1-*O*-alkyl-*sn*-glycero-3-phosphocholine. N, normal subject; D, serum PAF acetylhydrolase-deficient subject.

and 1-[^{3}H]alkyl-2-acyl-*sn*-glycero-3-phosphocholine (68.6%). The different procedure we adopted involved the addition of BSA (final concentration, 7 mg/ml) to the incubation mixture to bind [^{14}C]acetyl-PAF after the reaction. PAF acetylhydrolase activities in platelets from normal and deficient subjects determined by the TCA precipitation and TLC method are compared in Table III. The amount of free [^{3}H]acetate in the supernatant obtained by TCA precipitation reflected the extent of deacetylation of PAF (~ 87%). PAF acetylhydrolase activity in platelets from a person with the deficiency was similar to that in platelets from a normal person, indicating that intracellular acetylhydrolase was active in persons with a deficiency of the serum enzyme.

Table III. PAF Acetylhydrolase Activities in Platelet from Normal and Deficient Subjects

	PAF acetylhydrolase activity	Deacetylation activity
	nmol/mg protein	
Normal subject		
A	2.71±0.13	3.20±0.11 (2.15*, 1.05‡)
B	2.66±0.01	3.14±0.13 (2.19*, 0.95‡)
C	2.85±0.07	3.42±0.09 (2.35*, 1.07‡)
Deficient subject		
I	2.16±0.05	2.42±0.16 (1.68*, 0.74‡)
II	2.05±0.09	2.28±0.13 (1.57*, 0.71‡)
III	2.21±0.08	2.50±0.17 (1.74*, 0.76‡)

PAF acetylhydrolase and deacetylation activities are determined as described in Fig. 5 and expressed as nanomoles of [^{14}C]acetate released from 1-*O*-hexadecyl-2-[^{14}C]acetyl-*sn*-glycero-3-phosphocholine and of 1-*O*-[^{3}H]-alkyl-2-acyl-*sn*-glycero-3-phosphocholine* and 1-*O*-[^{3}H]-alkyl-*sn*-glycero-3-phosphocholine(lyso PAF)‡ produced from 1-[^{3}H]-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine per milligram protein of platelet, respectively. The subjects were selected randomly among 816 healthy adults. Results are presented as the means±half range of duplicate determinations.

To study the genetic linkage of serum PAF acetylhydrolase deficiency, we selected 10 families in which either parent had PAF acetylhydrolase activity-positive serum but the filial relationships consisted of more than one person with PAF acetylhydrolase deficiency. Among a total of 25 children in 10 families, 12 subjects with PAF acetylhydrolase deficiency were observed. On analysis according to the a priori method (37), the expected number of deficient subjects was 12.20±1.39, which thus agreed with the observed number. This observation strongly suggested that PAF acetylhydrolase deficiency is transmitted as a recessive trait, assuming that the deficiency is genetically controlled. Fig. 6 shows the distributions of serum PAF acetylhydrolase activity in healthy adults and healthy children. Three groups with different levels of serum PAF acetylhydrolase activity (normal, 124/211; low, 79/211, $P = 0.374$; deficient, 8/211, $P = 0.038$) were recognized in healthy children. Assuming that PAF acetylhydrolase deficiency is inherited as a recessive trait as described above, the probability of a subject having heterozygosity ($q = 2\sqrt{P}$) is 0.39, since 32 and 8 subjects with PAF acetylhydrolase deficiency (homozygous, $P = 0.038$) were found among 816 healthy adults and 211 healthy children, respectively. The theoretically calculated probability (q) of heterozygosity in children, agreed with the observed probability of subjects with low enzyme activity (low group) in Fig. 6 (79/211, $P = 0.394$). This shows that the subjects, for whom serum PAF acetylhydrolase activity among children was < 1.2 nmol/min per 50 μl serum (not deficient), may be heterozygous. On the other hand, for adult enzyme activities, neither a bimodal or normal distribution was observed, indicating that the enzyme activity may be genetically controlled, whereas the adult enzyme activity could be complicated by other factors. Fig. 7 shows PAF acetylhydrolase activity in serum from five families, each of which had more than one person with PAF acetylhydrolase deficiency. The subjects with the deficiency of serum PAF acetylhydrolase were healthy in families A (second generation), B (third generation), C (first and second generations), D (first generation), and E (first, second, and third generations), and the second and third generations in family D suffered from acute myelo-

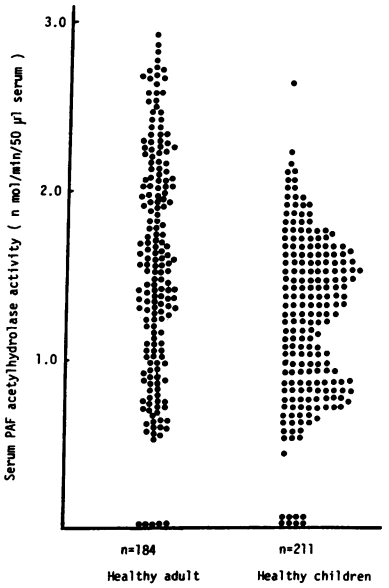

Figure 6. The distribution of serum PAF acetylhydrolase activity in healthy Japanese adults and children. PAF acetylhydrolase activity was measured by the TCA precipitation method as described in Methods. The activity is expressed as nanomoles of [^{14}C]acetic acid released per 50 μl of serum per min. Results from one of five groups divided randomly are shown (184 subjects).

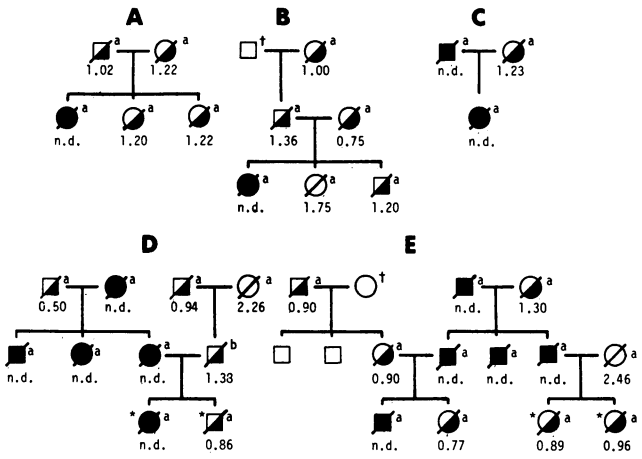

Figure 7. Pedigree of serum PAF acetylhydrolase activities. Figures and those marked with an asterisk indicate the PAF acetylhydrolase activity (nmol/min per 50 μl of serum) among adults and children, respectively. A, B, C, D, and E are nonrelated, independent families. ■, ●, completely deficient subject; ◪,◐, heterozygous subject, for whom PAF acetylhydrolase activity was lower than 1.45 nmol/min per 50 μl of serum in adults (> 18 yr old) and 1.2 nmol/min per 50 μl of serum in children (6–12 yr old); n.d.: not detected. [a], healthy subject; [b], acute myelocytic leukemia; [c], bronchial asthma (moderate).

cytic leukemia and bronchial asthma (moderate), respectively. The probability of PAF acetylhydrolase deficiency appearing in these families was significantly higher than that in healthy adults (32/816) and healthy children (8/211) ($P = 0.038$). These observations show that the appearance of PAF acetylhydrolase deficiency may be genetically controlled. We hypothesized that PAF acetylhydrolase deficiency is transmitted as an inherited autosomal dominant trait from the pedigrees of family C, family D (second generation) and family E (second and third generations). However, when the subjects for whom serum PAF acetylhydrolase activity was < 1.45 nmol/min per 50 μl serum among adults and 1.2 nmol/min per 50 μl serum among children are grouped as heterozygotes with a probability ($q = 2\sqrt{P}$) of 0.39, we were able to explain the trait of PAF acetylhydrolase inheritance as follows. In family A (first generation) and family B (second generation), the enzyme activities of the parents (heterozygotes) were not deficient, but below 1.45 nmol/min per 50 μl of serum. One of the three daughters in family A had a deficiency of PAF acetylhydrolase and the others appeared to be heterozygotes. The third generation of family B probably consisted of one homozygous (dominant), one heterozygous (codominant), and one deficient subject. In the other three families (C, D, and E), we found one PAF acetylhydrolase activity-negative person in each generation (Fig. 7). Hence, it seemed reasonable to assume that PAF acetylhydrolase deficiency is transmitted as an inherited autosomal recessive trait.

As several reports have suggested that PAF might play an important role in bronchial asthma as a chemical mediator (38), the correlation between serum PAF acetylhydrolase activity of asthmatic children and their symptoms was investigated. Asthmatic children (175) were grouped into five classes (remission [71], wheezy [29], mild [33], moderate [24], and severe [18]) on the basis of their respiratory symptoms over the previous 2 yr. The lack of variation of serum PAF acetylhydrolase activity at different times, which was shown for healthy adults in Fig. 3, was also observed among the same patients without asthmatic attacks (data not shown). These patients had not ingested medicine(s) for at least 1 wk before donation except for theophylline and aspirin, which were administered to some of the patients. Administration of theophylline and aspirin to asthmatic children did not affect the enzyme activity (data not shown). PAF acetylhydrolase activity in patients without asthmatic attacks was reduced in the severe group in comparison with the groups showing slight symptoms (remission, wheezy, and mild, $P < 0.05$, $P < 0.1$, $P < 0.01$, respectively) (Fig. 8). There were no significant differences between the enzyme activities of the patients with and without asthmatic attacks (data not shown). There were five deficient subjects in the most severe groups (42 subjects in the moderate and severe groups). The probability of occurrence in the most severe group (5/42) was significantly higher ($P < 0.01$) than those in healthy adults (32/816), healthy children of a similar age (1/36) and in the groups showing slight symptoms (remission, wheezy, and mild groups, 2/133). Next, to explain the effect of asthmatic attacks with airway obstruction on PAF acetylhydrolase activity, a 13-yr-old girl with intolerance to aspirin was investigated. A sublingual challenge with aspirin induced symptoms such as urticaria, angioedema, wheezing, and dyspnea with a decline in peak expiratory flow rate (Fig. 9). Venous blood samples, which were collected during the challenge, showed a rise in plasma histamine and a decrease in platelet count at the onset of these symptoms (data not shown). However, the serum PAF acetylhydrolase activity did not change (Fig. 9). Furthermore, the enzyme activities of patients with atopic asthma were not affected by severe attacks (dyspnea) lasting 12 h or more (Fig. 9).

These observations suggest that the asthmatic patient with deficient and/or low activity of serum PAF acetylhydrolase might tend to have the severe symptoms.

Concluding remarks. The method we developed to measure the radioactivity of the supernatant after precipitation of the complex of radioactive substrate and albumin with TCA was reliable and useful for the routine assay of PAF acetylhydrolase activity (Figs. 1 and 5, and Table III). Intracellular

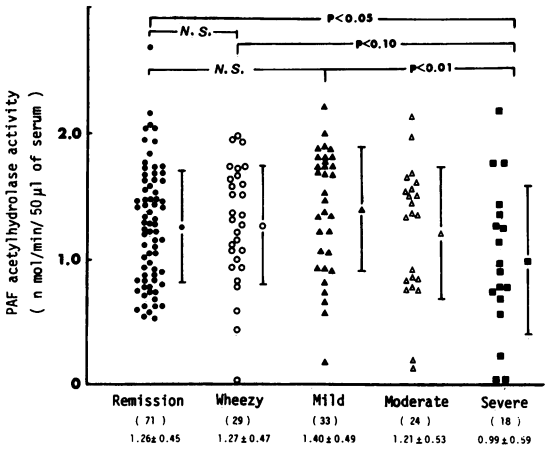

Figure 8. Serum PAF acetylhydrolase activity of asthmatic children grouped on the basis of wheezing symptoms. Figures in parentheses are the numbers of subjects in each group and other figures are the levels of PAF acetylhydrolase activity expressed as mean±SD. NS, no significant difference.

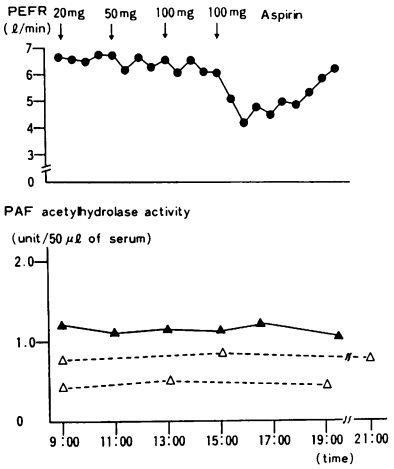

Figure 9. Serum PAF acetylhydrolase activity of a (*A*) subject with intolerance to aspirin during sublingual challenge and of (*B*) subjects with atopic asthma during the course of asthmatic attack. (*A*) A 13-yr-old girl with intolerance to aspirin, who had neither nasal polyps nor sinusitis on physical and radiological examinations, was sublingually challenged with aspirin (20, 50, and 100 mg) at the indicated times and symptoms were induced. Peak expiratory flow rate (PEFR) was determined by autospirometer (AS-500; Minato Inc., Japan) for measurement of airway obstruction. (*B*) Measurements were made in five severely asthmatic children at the indicated times, and typical data (– – – △ – – –) obtained during the course of severe attacks (dyspnea) are shown.

metabolites of PAF such as those in platelets were 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine and 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine, but almost all metabolites in serum were 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine. The serum PAF acetylhydrolase activity obtained by the TCA precipitation method was similar to that obtained by the TLC method (Fig. 1). The different K_m value (1.5×10^{-4} M) from the data (1.37×10^{-5} M) reported by Stafforini et al. (39) might have been caused by the use of a 250-fold higher concentration of serum, because serum albumin easily binds PAF and this would affect the interaction between PAF and acetylhydrolase (40). To determine the enzyme activity under assay conditions close to the physiological concentration of serum protein, we used a higher concentration of serum in this study, at which the PAF acetylhydrolase activity showed linearity with both time and protein concentration (Fig. 2). Serum PAF acetylhydrolase is associated with lipoprotein (both low-density and high-density lipoprotein) and is hypothesized to inactivate PAF in order to maintain circulating PAF below harmful levels (26–28, 39, 40). This paper is the first to describe a deficiency of serum PAF acetylhydrolase, which was independent of the level of LDL and total cholesterol.

To date, there has been no information regarding serum PAF acetylhydrolase production in body organs. PAF acetylhydrolase production is known to occur in tissues (29, 30) and blood cells (31) such as neutrophils, eosinophils, and platelets. Intracellular PAF acetylhydrolase plays an important role in metabolizing PAF within the cell (41–45). Some differences in enzyme properties between serum and intracellular acetylhydrolase, e.g., substrate specificity, have been reported (29, 46). The intracellular PAF acetylhydrolase activity even in platelets from subjects with enzyme deficiency was similar to that from normal subjects. These results indicate that serum and intracellular acetylhydrolase might be different proteins with different functions produced by different genes. Studies are currently in progress to explain whether the deficiency of serum PAF acetylhydrolase activity is due to lack of production or degradation of the enzyme, the prevention of its secretion into the blood from the organ of origin or an abnormal distribution or association with lipoprotein of the enzyme.

Serum PAF acetylhydrolase deficiency is apparently transmitted autosomally (Fig. 7), although a more detailed family study will be needed to clarify the inheritance pattern of this deficiency. It was found that not all subjects with serum PAF acetylhydrolase deficiency had clinically apparent symptoms, for example, asthma, thrombosis and hypertension. The mechanism that protects these persons from PAF accumulation is not known at present.

It would be interesting to explain the correlation of the deficiency of serum PAF acetylhydrolase with some syndromes potentially attributable to excess PAF in view of the hypothesis that PAF may be a humoral mediator in such syndromes. We found that deficiency of serum PAF acetylhydrolase was correlated with the severity of respiratory symptoms of bronchial asthma in elementary school children (Fig. 8). Although the capacity to degrade PAF was slightly elevated in atherosclerotic patients (47), the enzyme activity showed no significant change during asthmatic attacks, which caused a decrease in peak expiratory flow rate (Fig. 9). Furthermore, the enzyme activities of five severely asthmatic children showed no change during the course of severe attacks (dyspnea) lasting 12 h or more (Fig. 9). This shows that PAF acetylhydrolase was not released from inflammatory tissues or cells during asthmatic attacks. Even if acetylhydrolase was released from these cells, it is presumed that the amount of released enzyme would be small and have no direct effects upon the acetylhydrolase level in circulating blood. PAF is a potent lipid mediator that induces pulmonary recruitment of eosinophils (13, 14) and causes platelet-dependent bronchoconstriction and hypersensitivity of the respiratory tract (38, 48). Acute IgE anaphylaxis in the rabbit has been speculated to occur secondary to the release of PAF (49). PAF synthesized by stimulated rabbit basophils is released, but many inflammatory cells such as human lung mast cells, endothelial cells, and neutrophils retain almost all of the synthesized PAF (50, 51). Furthermore, in this study, both the platelet responsiveness to PAF and the intracellular metabolism of PAF were normal even in the subjects who showed deficiency of serum PAF acetylhydrolase (Fig. 5 and Table III).

From these observations, we suggest that (*a*) PAF may be released into the blood only in specific syndromes such as asthma, (*b*) the action of PAF in blood may be controlled by serum PAF acetylhydrolase or endogenous PAF inhibitor(s) (52), and (*c*) an abnormality in the blood PAF level due to deficiency or low activity of serum PAF acetylhydrolase might cause more severe respiratory symptoms in asthmatic children. Further studies will be needed in order to confirm these possibilities.

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References

1. Demopoulos, C. A., R. N. Pinckard, and D. J. Hanahan. 1979. Platelet-activating factor: evidence for 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-

3-phosphocholine as the active components. *J. Biol. Chem.* 254:9355–9358.

2. Benveniste, J., M. Tence, P. Varenne, J. Bidault, C. Boullet, and J. Polonsky. 1979. Semi-synthèse et structure proposée du facteur activant les plaquettes (P.A.F.): PAF-acetherm un alkyl ether analogue de la lysophosphatidylcholine. *C.R. Hebd. Seances Acad. Sci. Paris. (ser. D).* 289:1037–1040.

3. Polonsky, J., M. Tence, P. Varenne, B. C. Das, B. J. Lunel, and J. Benveniste. 1980. Release of 1-*O*-alkylglyceryl-3-phosphorylcholine, *O*-deacetyl platelet-activating factor, from leukocytes: chemical ionization mass spectrometry of phospholipids. *Proc. Natl. Acad. Sci. USA.* 77:7019–7023.

4. Hanahan, D. J., C. A. Demopoulos, J. Liehr, and R. N. Pinckard. 1980. Identification of platelet activating factor isolated from rabbit basophils as acetyl glyceryl ether phosphorylcholine. *J. Biol. Chem.* 255:5514–5516.

5. McManus, L. M. 1986. Pathobiology of platelet-activating factors. *Pathol. Immunopathol. Res.* 5:104–117.

6. Cammussi, G., I. Pawlowski, F. Bussolino, P. R. B. Caldwell, J. Brentjens, and G. Andres. 1983. Release of platelet activating factor in rabbits with antibody-mediated injury of the lung: the role of leukocytes and of pulmonary endothelial cells. *J. Immunol.* 131:1802–1807.

7. Lynch, J. M., G. Z. Lotner, S. J. Betz, and P. M. Henson. 1979. The release of a platelet-activating factor by stimulated rabbit neutrophils. *J. Immunol.* 123:1219–1226.

8. Cazenave, J. P., J. Benveniste, and J. F. Mustard. 1979. Aggregation of rabbit platelets by platelet-activating factor is independent of the release the reaction and arachidonate pathway and inhibited by membrane-active drugs. *Lab. Invest.* 41:275–285.

9. McManus, L. M., D. J. Hanahan, and R. N. Pinckard. 1981. Human platelet stimulation by acetyl glyceryl ether phosphorylcholine. *J. Clin. Invest.* 67:903–906.

10. Marcus, A. J., L. B. Safier, H. L. Ullman, K. T. H. Wong, M. J. Broekman, B. B. Weksler, and K. L. Kaplan. 1981. Effects of acetyl glyceryl ether phosphoryl choline on human platelet function in vitro. *Blood.* 58:1027–1031.

11. Lapetina, E. G. 1982. Platelet-activating factor stimulates the phosphatidylinositol cycle. *J. Biol. Chem.* 257:7314–7317.

12. O'Flaherty, J. T., R. L. Wykle, C. H. Miller, J. C. Lewis, M. Waite, D. A. Bass, C. E. McCall, and L. R. DeChatelet. 1981. The 1-*O*-alkyl-*sn*-glyceryl-3-phosphorylcholines. A novel class of neutrophil stimulants. *Am. J. Pathol.* 103:70–78.

13. Wardlaw, A. J., R. Moqbel, O. Cromwell, and A. B. Kay. 1986. Platelet-activating factor. A potent chemotactic and chemokinetic factor for human eosinophils. *J. Clin. Invest.* 78:1701–1706.

14. Tamura, N., D. K. Agrawal, F. A. Suliaman, and R. G. Townley. 1987. Effects of platelet activating factor on the chemotaxis of normodense eosinophils from normal subjects. *Biochem. Biophys. Res. Commun.* 142:638–644.

15. Humphrey, D. M., L. M. McManus, K. Satouchi, D. J. Hanahan, and R. N. Pinckard. 1982. Vasoactive properties of acetyl glyceryl ether phosphorylcholine and analogues. *Lab. Invest.* 46:422–427.

16. Humphrey, D. M., D. J. Hanahan, and R. N. Pinckard. 1982. Induction of leukocytic infiltrates in rabbit skin by acetyl glyceryl ether phosphorylcholine. *Lab. Invest.* 47:227–234.

17. Humphrey, D. M., L. M. McManus, D. J. Hanahan, and R. N. Pinckard. 1984. Morphologic basis of increased vascular permeability induced by acetyl glyceryl ether phosphorylcholine. *Lab. Invest.* 50:16–25.

18. Muairhead, E. E., J. A. Stirman, and F. Jones. 1960. Renal autoexplantation and protection against renoprival hypertensive cardiovascular disease and hemolysis. *J. Clin. Invest.* 39:266–281.

19. Blank, M. L., F. Snyder, L. W. Byers, B. Brooks, and E. E. Muirhead. 1979. Antihypertensive activity of an alkyl ether analog of phosphatidyl choline. *Biochem. Biophys. Res. Commun.* 90:1194–1200.

20. Heffner, J. E., S. A. Shoemaker, E. M. Canham, M. Patel, I. F. McMurty, H. G. Morris, and J. E. Repine. 1983. Acetyl glyceryl ether phosphorylcholine-stimulated human platelets cause pulmonary hypertension and edema in isolated rabbit lungs. Role of thromboxane A_2. *J. Clin. Invest.* 71:351–357.

21. Stimler, N. P., C. M. Bloor, T. E. Hugli, R. L. Wykle, C. E. McCall, and J. T. O'Flaherty. 1981. Anaphylactic actions of platelet-activating factor. *Am. J. Pathol.* 105:64–69.

22. Findlay, S. R., L. M. Lichtenstein, D. J. Hanahan, and R. N. Pinckard. 1981. Contraction of guinea pig ileal smooth muscle by acetyl glyceryl ether phosphorylcholine. *Am. J. Physiol.* 241:C130–C133.

23. Doebber, T. W., M. S. Wu, J. C. Robbins, B. M. Choy, M. N. Chang, and T. Y. Shen. 1985. Platelet activating factor (PAF) involvement in endotoxin-induced hypotension in rats. Studies with PAF-receptor antagonist kadsurenone. *Biochem. Biophys. Res. Commun.* 127:799–808.

24. Halonen, M., J. D. Palmer, I. C. Lohman, L. M. McManus, and R. N. Pinckard. 1980. Respiratory and circulatory alterations induced by acetyl glyceryl ether phosphorylcholine (AGEPC), a mediator of IgE anaphylaxis in the rabbit. *Am. Rev. Respir. Dis.* 122:915–924.

25. Pinckard, R. N., R. S. Farr, and D. J. Hanahan. 1979. 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.* 123:1847–1857.

26. Farr, R. S., C. P. Cox, M. L. Wardlow, and R. Jorgensen. 1980. Preliminary studies of an acid-labile factor (ALF) in human sera that inactivates platelet-activating factor (PAF). *Clin. Immunol. Immunopathol.* 15:318–330.

27. Cox, C. P., M. L. Wardlow, K. E. Meng, D. R. Creene, and R. S. Farr. 1983. Substrate specificity of the phosphatide 2-acylhydrolase that inactivates AGEPC. *J. Pharmacol. (Paris).* 14:37(Suppl. 1).

28. Blank, M. L., M. N. Hall, E. A. Cress, and F. Snyder. 1983. Inactivation of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by a plasma acetyl-hydrolase: higher activities in hypertensive rats. *Biochem. Biophys. Res. Commun.* 113:666–671.

29. Blank, M. L., T-C. Lee, V. Fitzgerald, and F. Snyder. 1981. A specific acetylhydrolase for 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid). *J. Biol. Chem.* 256:175–178.

30. Nijssen, J. G., C. F. P. Roosenboom, and H. van den Bosch. 1986. Identification of a calcium-independent phospholipase A_2 in rat lung cytosol and differentiation from acetylhydrolase for 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine(PAF-acether). *Biochim. Biophys. Acta.* 876:611–618.

31. Lee, T-C., B. Malone, S. I. Wasserman, V. Fitzgerald, and F. Snyder. 1982. Activities of enzymes that metabolize platelet-activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in neutrophils and eosinophils from humans and the effect of a calcium ionophore. *Biochem. Biophys. Res. Commun.* 105:1303–1308.

32. Bligh, E. G., and W. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911–918.

33. Miwa, M., M. Matsumoto, M. Tezuka, S. Okada, S. Ohsuka, and H. Fujiwake. 1986. Quantitative fluorographic detection of ^3H and ^{14}C on two-dimensional thin-layer chromatographic sheets by an ultra-high-sensitivity TV camera system. *Anal. Biochem.* 152:391–395.

34. Sugatani, J., M. Miwa, and D. J. Hanahan. 1987. Platelet-activating factor stimulation of rabbit platelets is blocked by serine protease inhibitor (chymotryptic protease inhibitor). *J. Biol. Chem.* 262:5740–5747.

35. Miyake, T., J. Kawamori, T. Yoshida, and T. Hirao. 1985. Analysis of wheezy children in elementary school: prevalence of wheeze, bronchial asthma, and correlation between serum IgE levels, RAST score to mite and severity of respiratory symptomes. *Jpn. J. Allergol.* 34:939–947.

36. Stafforini, D. M., T. M. McIntyre, M. E. Carter, and S. M. Prescott. 1987. Human plasma platelet-activating factor acetylhydrolase: association with lipoprotein particles and role in the degradation of platelet-activating factor. *J. Biol. Chem.* 262:4215–4222.

37. McKusick, V. A. 1969. Human Genetics. Foundations of Modern Genetics Series. Prentice-Hall, Inc., Englewood Cliffs, NJ. 125.

38. Morley, J., C. P. Page, L. Mazzoni, and S. Sanjar. 1986. Effects of ketotifen upon responses to platelet activating factor: a basis for asthma prophylaxis. *Ann. Allergy.* 56:335–340.

39. Stafforini, D. M., S. M. Prescott, and T. M. McIntyre. 1987. Human plasma platelet-activating factor acetylhydrolase. Purification and properties. *J. Biol. Chem.* 262:4223–4230.

40. Yamashita, M., H. Homma, K. Inoue, and S. Nojima. 1983. The metabolism of platelet activating factor in platelets and plasma of various animals. *J. Toxicol. Sci.* 8:177–188.

41. Malone, B., T-C. Lee, and F. Snyder. 1985. Inactivation of platelet activating factor by rabbit platelets: lyso-platelet activating factor as a key intermediate with phosphatidylcholine as the source of arachidonic acid in its conversion to tetraenoic acylated product. *J. Biol. Chem.* 260:1531–1534.

42. Alam, I., J. B. Smith, and M. J. Silver. 1983. Metabolism of platelet-activating factor by blood platelets and plasma. *Lipids.* 18:534–538.

43. Chilton, F. H., J. T. O'Flaherty, J. M. Ellis, C. L. Swendsen, and R. L. Wykle. 1983. Metabolic fate of platelet-activating factor in neutrophils. *J. Biol. Chem.* 258:6357–6361.

44. Chilton, F. H., J. T. O'Flaherty, J. M. Ellis, C. L. Swendsen, and R. L. Wykle. 1983. Selective acylation of lyso platelet activating factor by arachidonate in human neutrophils. *J. Biol. Chem.* 258:7268–7271.

45. Chilton, F. H., J. M. Ellis, S. C. Olson, and R. L. Wykle. 1984. 1-*O*-alkyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine: a common source of platelet-activating factor and arachidonate in human polymorphonuclear leukocytes. *J. Biol. Chem.* 259:12014–12019.

46. Yanoshita, R., I. Kudo, K. Ikizawa, H. W. Chang, S. Kobayashi, M. Ohno, S. Nojima, and K. Inoue. 1988. Hydrolysis of platelet-activating factor and its methylated analogs by acetylhydrolase. *J. Biochem.* 103:815–819.

47. Ostermann, G., K. Ruhling, R. Zabel-Langhennig, L. Winkler, B. Schlag, and U. Till. 1987. Plasma from atherosclerotic patients exerts an increased degradation of platelet-activating factor. *Thromb. Res.* 47:279–285.

48. Patterson, R., P. R. Bernstein, K. E. Harris, and R. D. Krell. 1984. Airway responses to sequential challenges with platelet-activating factor and leukotriene D_4 in rhesus monkeys. *J. Lab. Clin. Med.* 104:340–345.

49. Pinkard, R. N., R. S. Farr, and D. J. Hanahan. 1979. 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.* 123:1847–1857.

50. Henson, P. M. 1987. Extracellular and intracellular activities of PAF. *In* Platelet-activating Factor and Related Lipid Mediators. F. Snyder, editor. Plenum Press, New York. 255–272.

51. Oda, M., K. Satouchi, K. Yasunaga, and K. Saito. 1986. Polymorphonuclear leukocyte-platelet interactions: acetyl glyceryl ether phosphocholine-induced platelet activation under stimulation with chemotactic peptide. *J. Biochem.* 100:1117–1123.

52. Miwa, M., C. Hill, R. Kumar, J. Sugatani, M. S. Olson, and D. J. Hanahan. 1987. Occurrence of an endogeneous inhibitor of platelet-activating factor in rat liver. *J. Biol. Chem.* 262:527–530.