Characterization of Serum Platelet-activating Factor (PAF) Acetylhydrolase Correlation between Deficiency of Serum PAF Acetylhydrolase and Respiratory Symptoms in Asthmatic Children

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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-[4$^{14}$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 $1.5 \times 10^{-4}$ 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 $\mu$g/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 (C16:0-PAF) was obtained from BACHEM Feinchemikalien AG (Bubendorf, 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 (Amer- sham 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 glyceroiphospholipid from beef heart; the components were 1-O-hexade cyl (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

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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 [14C]acetyle-PAF used for the measurement of PAF acetylhydrolase was 45 μCi/mmol. 1-O-[3H]Hexadecyl-2-acetyl-sn-glycerol-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 [14C]PAF. For the routine assay, [14C]acetyle-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 [14C]acetyle-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 acetate 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-[3H]Hexadecyl-2-acetyl-sn-glycerol-3-phosphocholine ([3H]alky-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 Cmax PAF (2 × 10^-5-2 × 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 Ca2+-free Tyrode-gelatin buffer (pH 7.2) (40 μl, 5 × 10^6 cells) were transferred to aggregomer (dual-channel hemoracetr; Niko Bioscience, Tokyo) cuvettes and made up to 200 μl with Tyrode-gelatin buffer (pH 7.2) containing 1 mM CaCl2.

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 Cmax PAF (1 × 10^-10-1 × 10^-5 M) was added to the platelets. Changes in light transmission 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. Upplands Västerås, 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 radioallergosorbence 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 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 experiencing 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. [14C]Acetyle-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 [14C]acetyle-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-[3H]alkyl-PAF, the acid-soluble fraction after incubation for 10 min with serum at 37°C contained only 0.12% of the radioactivity. When [14C]acetyle-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-[3H]alkyl-PAF with serum were mainly 1-O-[3H]alkyl-2-hydroxy-sn-glycerol-3-phosphocholine (99%). 1-O-[3H]alkyl-sn-glycerol-3-phosphocholine was not detected. The values of serum acetylhydrolase activity measured by our devised method (TCA precipitation method) were compatible
with those for deacylated 1-O-[3H]alkyl-PAF (1-O-[3H]-
al-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 [14C]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 acetyl-
hydrolase 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 (proen-
yzme); 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 ac-
tivities obtained from
radioactivities of supern-
atant upon TCA pre-
cipitation (released ace-
tate) and deacylated
products (lyso PAF) of
1-O-[3H]alkyl-PAF. (A)
50 µl of 1-O-alkyl-2-
[14C]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 centrifu-
gation, the radioactivity in 100 µl of the supernatant was measured by
liquid scintillation. (B) 50 µl of 1-O-[3H]alkyl-PAF (50 nmol), sus-
pended 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 radioactiv-
ities 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. o, normal serum. e, PAF acetyl-
hydrolase-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 (o) and 5 PAF ace-
tyhydrolase-deficient serum (e) samples.
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 sera 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 x 10^{-2}-5 x 10^{-4} 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 x 10^{-9}-5 x 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 x 10^{-9}-2 x 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 [3H]alkyl-PAF after incubation with platelets from normal and deficient subjects were 1-[3H]alkyl-2-lyso-sn-glycero-3-phosphocholine (31.4%) and 1-[3H]alkyl-2-lyso-sn-glycero-3-phosphocholine (31.4%).

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

<table>
<thead>
<tr>
<th>PAF·AH(+) Serum</th>
<th>PAF·AH(--) Serum</th>
<th>PAF acetylhydrolase activity</th>
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<tbody>
<tr>
<td>µl</td>
<td>µl</td>
<td>I</td>
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<tr>
<td>50</td>
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<tr>
<td>25</td>
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</table>

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.

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

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

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-[³H]-alkyl-2-acetyl-sn-glycero-3-phosphocholine in platelets from serum PAF acetylhydrolase-deficient and -normal subjects. 1-O-[³H]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 [³H]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-sensitive TV camera system (ARGUS-100) as described in Methods. PC, 1-O-alkyl-2-acetyl-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.

To study the genetic linkage of serum PAF acetylhydrolase deficiency, we selected 10 families in which either parent had PAF acetylhydrolase activity-normal 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 = 2P) 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 nor 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-

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

<table>
<thead>
<tr>
<th>PAF acetylhydrolase activity</th>
<th>Deacetylation activity (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subject</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.71±0.13</td>
</tr>
<tr>
<td>B</td>
<td>2.66±0.01</td>
</tr>
<tr>
<td>C</td>
<td>2.85±0.07</td>
</tr>
<tr>
<td>Deficient subject</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2.16±0.05</td>
</tr>
<tr>
<td>II</td>
<td>2.05±0.09</td>
</tr>
<tr>
<td>III</td>
<td>2.21±0.08</td>
</tr>
</tbody>
</table>

PAF acetylhydrolase and deacetylation activities are determined as described in Fig. 5 and expressed as nanomoles of [¹⁴C]acetate released from 1-O-hexadeyl-2-[¹⁴C]acetetyl-sn-glycero-3-phosphocholine and and 1-O-[³H]-alkyl-2-acetyl-sn-glycero-3-phosphocholine and 1-O-[³H]-alkyl-2-acetyl-sn-glycero-3-phosphocholine* and 1-O-[³H]-alkyl-sn-glycero-3-phosphocholine (lyso PAF) produced from 1-[³H]-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 mean±half range of duplicate determinations.

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 [¹⁴C]acetate released per 50 μl of serum per min. Results from one of five groups divided randomly are shown (184 subjects).
The leukemic and other three families were heterozygous, as expected. These observations suggest that the asthmatic patient with deficient and/or low activity of serum PAF acetylhydrolase activity 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 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).

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 unrelated, independent families. a, b, completely deficient subject; c, d, heterozygous subject, for whom PAF acetylhydrolase activity was 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).

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.
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 Km 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 con-
centration of serum, because serum albumin easily binds PAF
and this would affect the interaction between PAF and acetyl-
hydrolase (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-den-
sity and high-density lipoprotein) and is hypothesized to inac-
tivate PAF in order to maintain circulating PAF below harm-
ful levels (26–28, 39, 40). This paper is the first to describe a
deficiency of serum PAF acetylhydrolase, which was indepen-
dent of the level of LDL and total cholesterol.

To date, there has been no information regarding serum
PAF acetylhydrolase production in body organs. PAF acetyl-
hydrolase 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 acetyl-
hydrolase, 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 intra-
cellular acetylhydrolase might be different proteins with dif-
f erent functions produced by different genes. Studies are cur-
rently 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

Figure 9. Serum PAF
acetylhydrolase activity
of a (A) subject with in-
tolerance to aspirin dur-
ing sublingual challenge
and of (B) subjects with
atopic asthma during
the course of asthmatic
attack. (A) A 13-year-
old girl with intolerance to
aspirin, who had nei-
ther nasal polyps nor
sinusitis on physical
and radiological exami-
nations, was sublin-
gually 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 (dys-
pnea) are shown.

the blood from the organ of origin or an abnormal distribution
or association with lipoprotein of the enzyme.

Serum PAF acetylhydrolase deficiency is apparently trans-
mitted autosomal (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 accumula-
tion is not known at present.

It would be interesting to explain the correlation of the deficiency of serum PAF acetylhydrolase with some syn-
dromes potentially attributable to excess PAF in view of the
hypothesis that PAF may be a humoral mediator in such syn-
dromes. We found that deficiency of serum PAF acetylhydro-
ase 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 asth-
matic 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 hypersen-
sitivity 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 ba-
sophils is released, but many inflammatory cells such as
human lung mast cells, endothelial cells, and neutrophils re-
tain 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 sub-
jects 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 chil-
dren. Further studies will be needed in order to confirm these
possibilities.

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References

Platelet-activating factor: evidence for 1-O-alkyl-2-acetyl-sn-glyceryl-
ether glyceryl gation of rabbit platelets


