

The evidence of platelet activation in bronchial asthma

Hideaki Yamamoto, MD, Makoto Nagata, MD, Kazuaki Tabe, MD,
Isao Kimura, MD, Hidenori Kiuchi, MD, Yoshio Sakamoto, MD,
Keiichiro Yamamoto, MD, and Yutaka Dohi, MD Saitama, Japan

The possible involvement of platelets in bronchial asthma was investigated under three different conditions: (1) chronic asthma, (2) bronchial provocation inhaling house dust mite (HDM), and (3) status asthmaticus. Plasma levels of β -thromboglobulin (β -TG), platelet factor 4 (PF4), and in part, platelet-activating factor (PAF) were measured. Approximately one third of the subjects with symptomatic or asymptomatic chronic asthma showed an increased level of β -TG or PF4. Statistically significant differences occurred in β -TG and PF4 levels only between healthy controls and symptomatic subjects. Five out of six subjects showed no elevation of β -TG and PF4 during immediate asthmatic response. In two out of nine subjects with status asthmaticus, β -TG or PF4 was elevated, and statistically significant correlations occurred between the initial level of PAF and that of β -TG or PF4. Those results suggest that the platelet activation in the circulation is sometimes provoked in asthma, but plasma level of α -granule-derived proteins does not reflect the intensity or severity of asthma, and that PAF is likely to be a mediator responsible for the platelet activation. (J ALLERGY CLIN IMMUNOL 1993;91:79-87.)

Key words: Bronchial asthma, platelet-activating factor, β -thromboglobulin, platelet factor 4

Among various bone marrow-derived cells, mast cells, basophils, and eosinophils have been considered to play key roles in the pathophysiologic characteristics of bronchial asthma. Other kinds of cells such as macrophages have also been implicated to be involved in the cellular events in asthma.¹

It has recently been suggested that platelets, which are primarily essential for hemostasis, participate in bronchial asthma. In sensitized animals the administration of an antigen provokes bronchospasm accompanied by intravascular aggregation of platelets, the accumulation of platelets in the lung, and the release of platelet-specific granule proteins.²⁻⁴ In humans the evidence for platelet abnormalities has been reported in asthmatic patients by in vitro tests of platelet function, in which abnormal aggregation of platelets was demonstrated with use of various stimuli.⁵

The presence of intravascular platelet aggregation within bronchial biopsy specimens in asthmatic patients was also reported.⁶ Those findings suggest the possible involvement of platelets in the pathophysiology of asthma. In fact, the increase in plasma β -thromboglobulin (β -TG) and platelet factor 4 (PF4), which are platelet-specific proteins stored in alpha-granules and are useful markers of platelet activation in vivo, has been described during antigen-induced bronchoconstriction.^{7, 8}

Chemical mediators derived from inflammatory cells are considered to play important roles in the pathophysiologic mechanisms that underly the bronchoconstriction induced by allergens in bronchial asthma. Among these mediators, platelet-activating factor (PAF), a phospholipid known to be 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, also could play an important role in bronchial asthma not only through an augmentation of airway responsiveness but also through its effects on inflammatory cells such as eosinophils, neutrophils, or macrophages.^{9, 10} PAF is also a potent stimulator for platelets.

In the present study we attempted to confirm whether platelets are activated during an asthmatic attack, and whether the changes in PAF levels are parallel to those in the levels of β -TG or PF4 or both

From the Second Department of Internal Medicine, Saitama Medical School, Saitama, Japan.

Received for publication Dec. 30, 1991.

Revised Aug. 27, 1992.

Accepted for publication Aug. 27, 1992.

Reprint requests: Hideaki Yamamoto, MD, Second Department of Internal Medicine, Saitama Medical School, 38 Morohongo, Moroyama-cho, Iruma-gun, Saitama 350-04, Japan.

1/1/42082

TABLE I. Characteristics of subjects with chronic stable asthma

	No. of subjects	Age (yr) Mean $\pm$ SD (range)	Atopic status		Severity*		
			+	-	Mild	Moderate	Severe
Asymptomatic	66 (M40, F26)	44.0 $\pm$ 17.2 (17~75)	47	19	16	27	23
Symptomatic	21 (M14, F7)	47.0 $\pm$ 16.6 (18~74)	16	5	0	2	19
Healthy controls	30 (M21, F9)	42.3 $\pm$ 13.8 (20~68)					

M, Male; F, female; T, sustained release theophylline; Inh, inhalation; PO, per os; BDI, beclomethasone dipropionate; PSL, prednisolone (≤ 10 mg); DSCG, disodium cromoglycate.

*Severity determined according to the classification by Japanese Society of Allergology.

Abbreviations used

- PAF: Platelet-activating factor
- β -TG: Beta-thromboglobulin
- PF4: Platelet factor 4
- BPT: Bronchial provocation test
- IAR: Immediate asthmatic response
- LAR: Late asthmatic response
- HDM: House dust mite
- FEV₁: Forced expiratory volume in one second

released from activated platelets. For this purpose we measured β -TG, PF4, and in part, PAF levels in plasma in three different conditions: (1) at chronic stage with or without symptoms, (2) during immediate asthmatic response (IAR) induced by allergen inhalation, and (3) during status asthmaticus, which required intensive care in the hospital.

MATERIAL AND METHODS

Subjects

Eighty-seven subjects with stable atopic or nonatopic asthma were studied randomly irrespective of their asthmatic conditions (Table I). All subjects have been followed up in the outpatient clinic of Saitama Medical School and were asked to record a daily asthma diary. The judgment of the presence of a symptom was based on the subject's complaint, asthma diary, and the presence of wheeze on physical examination. Atopic status was defined by positive IgE antibody and/or immediate skin reaction against common inhalant allergen(s). The subjects have received variable combinations of antiasthma drugs. Thirty healthy volunteers with matched age and similar gender distribution were studied as controls.

Six subjects from 18 to 54 years of age with stable atopic

asthma were studied by bronchial provocation test (BPT) (Table II). All subjects had strongly positive IgE antibody against house dust mite (HDM). Nine subjects were studied during status asthmaticus (Table III). They were admitted to Saitama Medical School Hospital on an emergency basis and were treated intensively. The therapy included intravenous administration of corticosteroids and aminophylline, inhalation of salbutamol or subcutaneous injection of epinephrine or both, and oxygen administration. Consecutive measurement of forced expiratory volume in 1 second (FEV₁) was performed with a portable autospirometer (Microspiro HI-298, Chest Co., Tokyo, Japan). All subjects had no renal disease or thrombotic disorder or a history of taking drugs that affect platelet function.

Bronchial provocation test

BPT was carried out with informed consent, and the subjects were considered to be in an acceptable stable state at the time of the study. FEV₁ was greater than 70% of predicted value in all subjects. Before the study antiasthma drugs were discontinued for at least 12 hours for theophylline and β -adrenergic agonists and for 24 hours for corticosteroids and sodium cromoglycate.

BPT with HD extract containing a certain amount of HDM allergen was performed by use of the standardized method of the Japanese Society of Allergology: in brief, HD extract was diluted from 1/10 (wt/vol) to 1/10,000 serially by saline solution. Aerosol was delivered by a DeVilbiss model 646 nebulizer (De Vilbiss, Somerset, Pa.) at 5 L/min with compressed air and inhaled by tidal breathing while a nose clip was set. Spirometry was performed with use of an autospirometer (PK-Morgan, Kent, England). After the baseline FEV₁ was measured, saline was inhaled to check the absence of nonspecific bronchoconstriction. Thereafter, 1/10,000 HD extract was inhaled for 2 minutes, and FEV₁ was measured 10 minutes later. Unless the fall of FEV₁ was more than 20% of the baseline value, the 10-

Medications					
T	β -agonist		BDI	PSL	DSCG
	Inh	PO			
61 (92.4%)	41 (62.1%)	54 (81.8%)	35 (53.0%)	23 (34.8%)	10 (15.2%)
18 (85.7%)	14 (66.7%)	16 (76.2%)	8 (38.1%)	8 (38.1%)	4 (19.0%)

TABLE II. Characteristics of subjects in BPT

Subject no.	Age (yr)	Sex	Asthma duration (yr)	Baseline pulmonary function		PD ₂₀ (unit)*
				FEV ₁ (ml)	%FEV ₁	
1	18	M	16	3270	78.0%	8.7
2	44	F	21	1960	85.2%	5.9
3	47	M	1	3150	96.6%	16.9
4	54	F	5	2140	103.9%	99.9
5	22	M	15	4000	101.3%	51.4
6	51	F	18	2520	104.6%	19.8

*Provocative dose causing a 20% fall in FEV₁, (1 unit = 1 minute inhalation of 1 µg/ml of HD extract).

TABLE III. Characteristics of subjects with status asthmaticus

Subject no.	Age (yr)	Sex	Atopic status	Asthma duration (yr)	Baseline FEV ₁		Baseline Pao ₂ (Torr)
					(ml)	(% pred)	
7	20	F	—	7	540	18.3%	59.1
8	64	F	—	8	290	17.6%	59.1
9	54	M	—	6		ND	43.4
10	46	M	+	40	750	25.1%	66.4
11	32	F	—	1		ND	68.6
12	41	F	+	28	970	41.2%	59.0
13	67	M	+	22	370	20.1%	50.4
14	76	M	+	8	830	45.6%	87.4
15	45	M	+	11	2130	58.6%	75.3

ND, Not determined.

fold concentrated solution was inhaled. This procedure was repeated until FEV₁ decreased more than 20% of the baseline FEV₁. At the end of the test, 20 mg or 30 mg of oral prednisolone was administered to prevent a possible late asthmatic response (LAR). Every BPT was started between 9 and 11 AM.

Blood samples

Blood from the antecubital vein was collected as slowly as possible without venous occlusion to avoid mechanical agitation of platelets. A part of the blood was mixed with 1.1 volumes of saline containing theophylline, adenosine, dipyridamole, citric acid, and sodium citrate, then gently

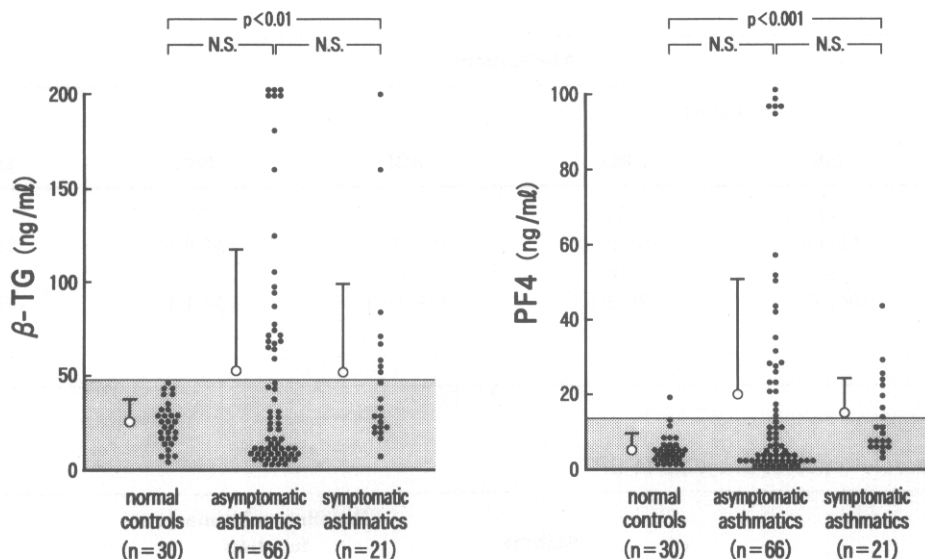


FIG. 1. Plasma levels of β -TG and PF4 in chronic asthma. Shaded area represents normal range.

TABLE IV. Effects of antiasthma drugs on plasma β -TG and PF4 levels in chronic stable asthma

	β -agonist							
	T		Inh		PO		BDI	
	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
β -TG	55.7	25.0	54.9	49.2	58.2	31.0	50.1	55.6
(ng/ml)	± 58.1	± 20.3	± 54.3	± 60.1	± 60.5	± 25.4	± 56.0	± 57.1
PF4	19.1	7.4	18.2	17.7	20.3	8.6	17.8	18.3
(ng/ml)	± 26.5	± 8.1	± 23.4	± 29.4	± 27.7	± 9.0	± 25.3	± 26.1

Data presented as mean $\pm$ SD.

inverted, submerged in crushed ice between 30 and 120 minutes, and centrifuged at 3000 g for 30 minutes at 4° C.

The rest of the blood was mixed with ethylenediamine tetraacetic acid. Plasma was immediately separated by centrifugation at 670 g for 10 minutes at 4° C, and lipid fraction was extracted for the measurement of PAF. Blood samples obtained from healthy volunteers were used to determine the normal range.

Lipid extraction and thin-layer chromatography

Plasma was mixed with eight volumes of saline, then the total lipid fraction was extracted by the method of Bligh and Dyer.¹¹ The chloroform layer was removed and evaporated under nitrogen gas. The residue was dissolved with 100 μ l of chloroform:methanol (4:1 [vol/vol]) and was subjected to thin-layer chromatography.

Each sample was spotted on a silica gel G thin-layer chromatography plate (Merck, Darmstadt, Germany). Authentic PAF (Bachem, Bubendorf, Switzerland), sphingomyelin, and lysophosphatidylcholine (Sigma Chemical Co., St. Louis, Mo.) were applied simultaneously as standards. The plates were developed with use of the solvent system of chloroform:methanol:water (65:35:6 [vol/vol]). The area corresponding to PAF was identified from the position between lysophosphatidylcholine and sphingomyelin detected by their fluorescence under ultraviolet light after spraying with 0.1% primlin. It was scraped and extracted by the aforementioned method.

PAF bioassay

Plasma PAF activity was assayed by the aggregating activity of washed rabbit platelets with use of an aggregometer (Nikko Hematracer NKK-2; Nikko Biomedical Co., Tokyo, Japan).¹² In brief, 200 μ l of washed rabbit platelets (a final

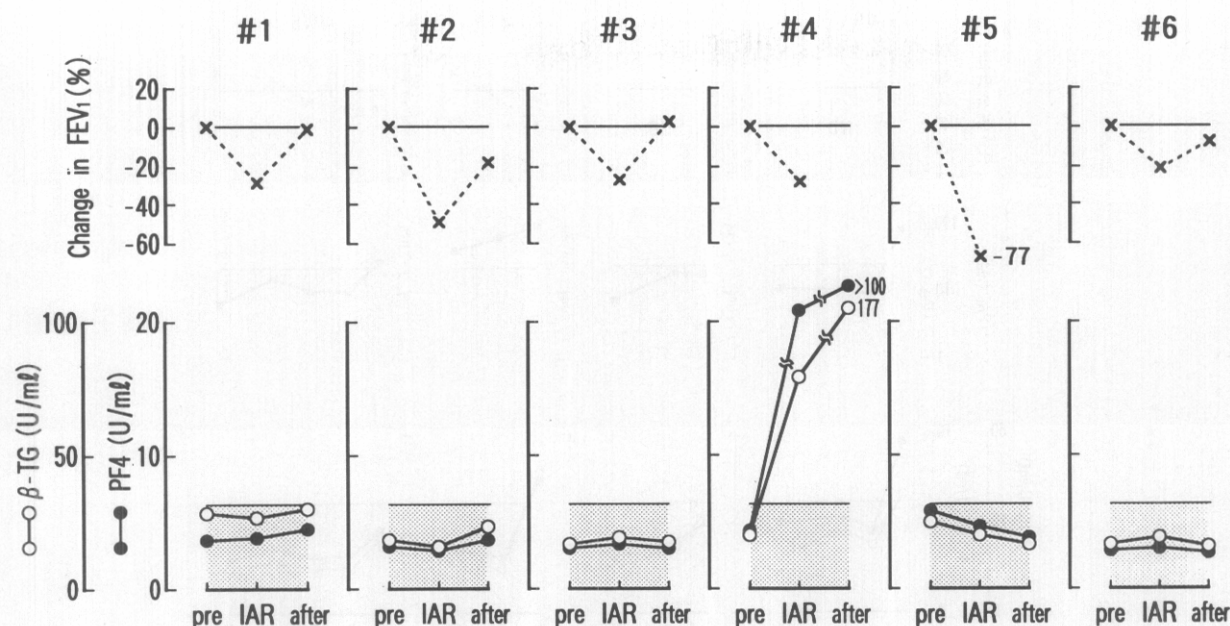


FIG. 2. Changes in FEV₁, β -TG, and PF₄ during BPT. Shaded area represents normal range.

PSL		DSCG	
(+)	(-)	(+)	(-)
53.8	52.4	54.8	52.5
± 60.3	± 54.5	± 52.2	± 57.4
18.5	17.8	16.8	18.2
± 25.3	± 26.0	± 15.0	± 27.2

correlation coefficient between ELISA and RIA was 0.96 for β -TG and 0.99 for PF₄ according to the manufacturer's description. Samples were assayed in duplicate. The coefficient of variation among assays on the same sample was less than 5%.

Statistical analysis

Data were analyzed by Wilcoxon's rank-sum test. Correlation coefficients were calculated from linear regression by the least-square method. *P* values less than 0.05 were considered statistically significant.

RESULTS

Chronic asthma

A statistically high correlation occurred ($r = 0.95$) between β -TG and PF₄ when all subjects were included.

The subjects with chronic asthma were divided into the symptomatic group and the asymptomatic group when each subject's blood was drawn at the outpatient clinic. The presence of wheeze or asthmatic complaints or both was regarded as symptomatic. Twenty-one subjects were symptomatic, and 66 were asymptomatic. Plasma β -TG and PF₄ levels in the healthy volunteers (the control group) were 25.1 ± 11.0 (mean $\pm$ SD) and 5.1 ± 3.7 ng/ml, respectively. In the symptomatic group plasma β -TG and PF₄ levels were 52.2 ± 47.3 and 13.4 ± 10.1 ng/ml, exceeding the normal range (mean $\pm$ 2 SD) in eight subjects

concentration of 2 to 4×10^8 /ml) suspended in Tyrode's solution containing 1.8 mmol/L Ca^{2+} , 0.5 mmol/L Mg^{2+} , pH 7.35 was added to an aggregometer cuvette. The sample residue was dissolved with 0.9% saline solution containing 0.25% bovine serum albumin, then $20 \mu\text{l}$ was added to the cuvette, and the change in light transmission was measured. Standard curve was prepared simultaneously with authentic PAF. The identification of PAF in each sample was confirmed by the absence of aggregation after the preincubation of platelets with a specific antagonist, CV-3988 (10^{-5} mol/L) (Takeda, Osaka, Japan).

Measurement of β -TG and PF₄

Both β -TG and PF₄ were measured by either ELISA kit (Boehringer-Mannheim-Yamanouchi Co., Tokyo, Japan) or RIA kit (β -TG; Amersham, Buckinghamshire, England; PF₄; Dainabot, Tokyo, Japan). The thresholds of detection for β -TG and PF₄ were 5 and 1 U/ml, respectively, for ELISA and 7 and 3 ng/ml, respectively, for RIA. The

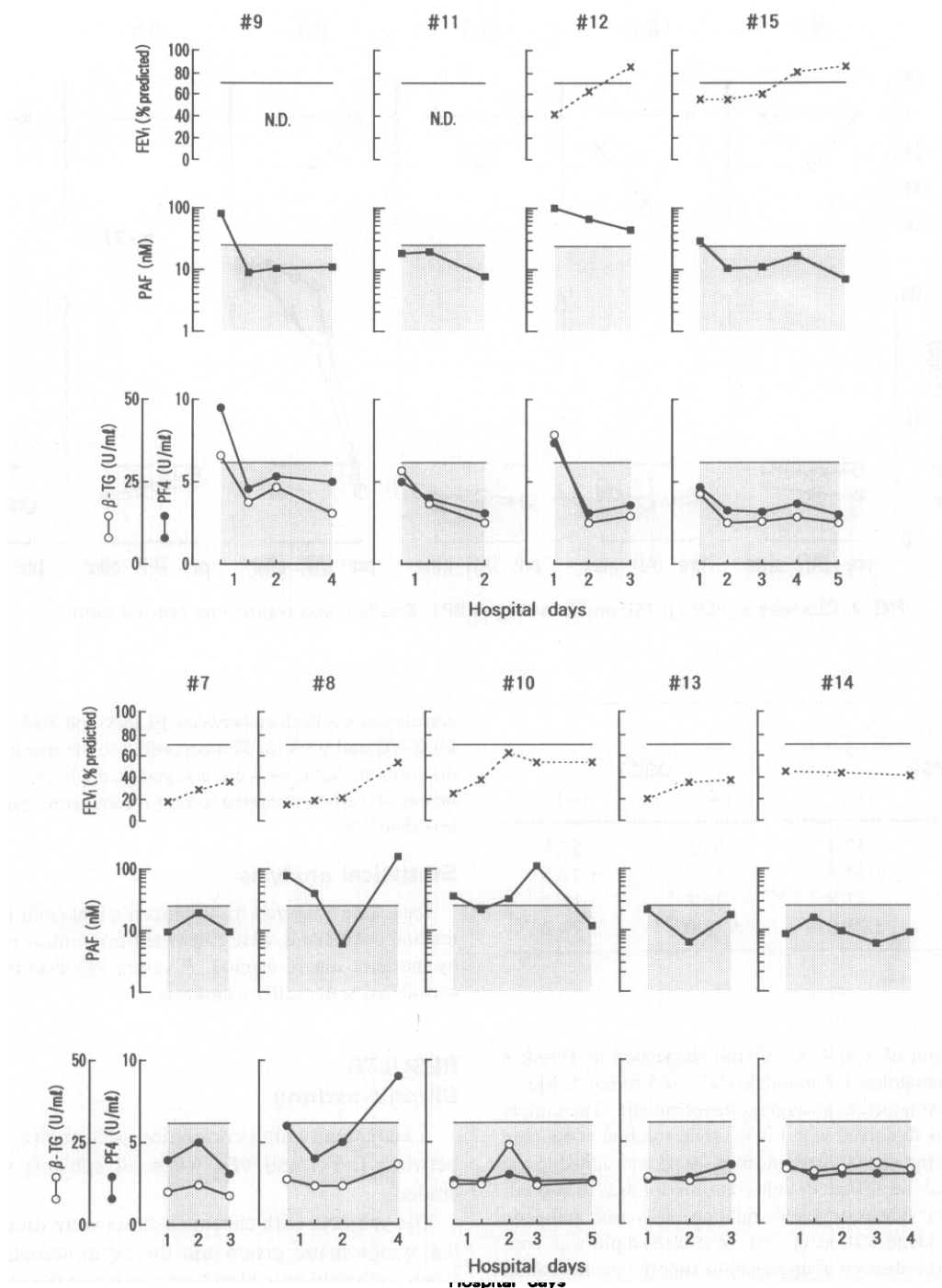


FIG. 3. Changes in FEV₁, PAF, β-TG, and PF4 in status asthmaticus. Shaded area represents normal range. ND, Not determined.

(38%), respectively (Fig. 1). In the asymptomatic group plasma β-TG and PF4 levels were 53.1 ± 59.2 and 19.5 ± 28.7 ng/ml, exceeding the normal range in 23 (35%) and 24 subjects (36%), respectively. Statistically significant differences occurred in β-TG and

PF4 levels between the control group and the symptomatic group ($p < 0.01$ and 0.001). No significant difference occurred between the symptomatic group and the asymptomatic group. When asymptomatic subjects were divided into subgroups according to the

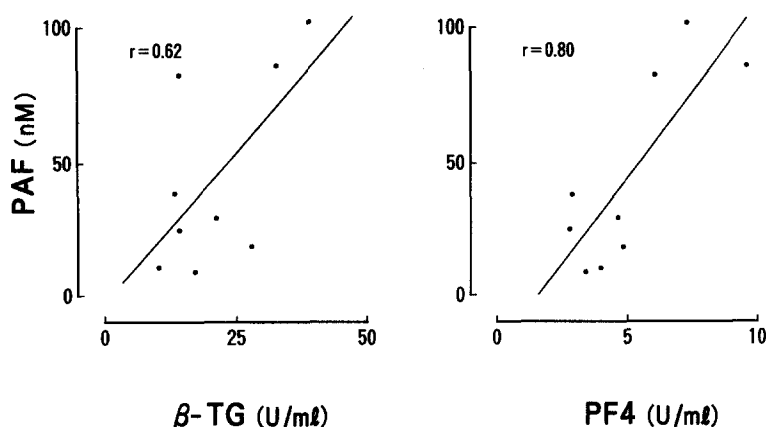


FIG. 4. Correlations between β -TG and PAF (left), and between PF4 and PAF (right) in status asthmaticus at time of admission.

atopic status or severity of asthma, no significant difference occurred in plasma β -TG and PF4 levels among those subgroups (data not shown).

The effect of each antiasthma drug on β -TG or PF4 levels was evaluated (Table IV). No statistically significant difference occurred between subjects with medication and those without medication in each drug. Even when subjects were divided into the group showing higher levels exceeding the normal range and that showing normal levels, then analyzed by χ^2 -test, no significant difference occurred in each drug.

The changes during IAR after BPT

Inhalation of HD extract provoked the fall of FEV₁ more than 20% in every subject. The mean values of FEV₁ before, during, and after IAR were 2.84 ± 0.32 , 1.70 ± 0.26 , and 2.60 ± 0.38 L, respectively (mean $\pm$ SE). Plasma β -TG and PF4 levels were within normal range before BPT. Those levels did not change during IAR except one (no. 4) in whom β -TG and PF4 levels increased during IAR and even after the inhalation of β -agonist (Fig. 2). In this subject plasma PAF level did not change and remained within the normal range (data not shown). Overall, no statistically significant change occurred in β -TG, PF4, or PAF levels throughout BPT (data not shown).

The changes during status asthmaticus

Blood was collected before and after starting the emergency therapy. The measurement of FEV₁ was not performed in two out of nine subjects (no. 9 and 11) because consent was not obtained. In only two subjects (no. 9 and 12) plasma β -TG and PF4 levels were high at the time of admission (Fig. 3). Elevated β -TG and PF4 decreased within normal range during

1 to 2 hospital days and kept constant levels thereafter. In another two subjects (no. 11 and 15) β -TG and PF4 levels were within normal range at the time of admission; however, those levels decreased further within a day. Among the above four subjects the value of FEV₁ increased gradually over 70% of the predicted value in two subjects (no. 12 and 15). In another two subjects (no. 9 and 11) FEV₁ was not measured, but their clinical signs and symptoms improved. In a subject (no. 8) β -TG and PF4 levels were within normal range at the time of admission. PF4 levels decreased within a day, but again increased and exceeded the normal range at the fourth hospital day. In the other four subjects (no. 7, 10, 13, and 14) β -TG and PF4 levels were low at the time of admission and changed little throughout the hospital days. The values of FEV₁ increased in four subjects (no. 7, 8, 10, and 13) or did not change in one (no. 14). However, FEV₁ has been less than 70% of the predicted value in all of the above five subjects. No significant correlation occurred between the values of PaO₂ and the levels of β -TG ($r = -0.22$) or PF4 ($r = -0.52$) at the time of admission.

The plasma PAF level in healthy volunteers was 9.3 ± 7.6 (mean $\pm$ 1 SD) nmol/L, and the upper limit of normal range was set at 24.5 nmol/L. Plasma PAF levels were considered high at the time of admission in three subjects (no. 8, 9, and 12; 82.6, 85.8, 102.1 nmol/L, respectively), among whom two subjects also showed high levels of β -TG and PF4 (no. 9 and 12); in another subject the PF4 level was near the upper limit of normal range, but β -TG was not (Fig. 3). Relatively high correlations occurred between PAF and β -TG ($r = 0.62$), or PAF and PF4 ($r = 0.80$), which were values at the time of admis-

sion (Fig. 4). The change in PAF level seemed to be generally parallel to the change in β -TG and/or PF4 level in each subject except two (no. 10 and 14).

DISCUSSION

The present study demonstrated clinically that platelets are activated in bronchial asthma. The presence of platelet activation in asthma was suggested by the fact that plasma levels of β -TG and PF4 were elevated in a certain proportion among subjects with chronic asthma. Elevated levels were found not only in the subjects with obvious asthma symptoms but also in those without any symptoms. This result might be explained by speculating that the *in vivo* release of platelet-activating mediator(s) triggered by allergic or nonallergic mechanisms occurs frequently in chronic asthma. For example, the occurrence of nocturnal asthma is a usual phenomenon frequently observed in patients with chronic asthma. During such an episode the platelet activation might be provoked, meanwhile the appearance of symptoms could be suppressed by the prophylactic use of appropriate bronchodilators. In concert with it, there has been a report that atropine increased the peak expiratory flow rate without affecting the circadian elevation of plasma β -TG in the early morning.¹³

The plasma β -TG and PF4 levels did not seem to reflect the intensity of asthma because approximately 60% of symptomatic subjects with chronic asthma showed normal levels of β -TG and PF4. Furthermore, seven out of nine subjects with status asthmaticus showed normal levels. Two possible explanations exist for such results: one is that platelet activation is not always provoked in acute asthmatic attack, and the other is that the desensitization of platelets is caused by continuous stimulation by an agonist(s).

Among various agonists such as PAF, thrombin, and thromboxane A_2 , we measured PAF as a possible candidate. PAF is also an important mediator possibly involved in the airway inflammation of asthma by attracting eosinophils and by increasing vascular permeability and mucus secretion.^{14, 15} PAF binds to a specific receptor and leads to the desensitization of platelets against subsequent stimulation by PAF.¹⁶ The results suggested, however, that the platelet desensitization by PAF is unlikely to occur, since plasma PAF levels were well correlated with plasma PF4 or β -TG levels in subjects with status asthmaticus. Thus we might be able to conclude that platelet activation in the circulation is not always provoked in asthma attack, although we have not measured PAF in subjects with chronic asthma, and also we cannot exclude the possibility of platelet desensitization by thromboxane A_2 , which is also an important mediator in asthma.

Whether or not platelet activation occurs during IAR has been controversial. Some investigators have reported the elevation of plasma β -TG and PF4 were associated with bronchoconstriction induced by allergen inhalation^{7, 8}; on the other hand, others have failed to confirm it.¹⁷ In the present study all subjects except one showed no increase of plasma β -TG and PF4 during IAR. In only one subject plasma β -TG and PF4 increased prominently during and after IAR, therefore it is not conclusive but quite possible that the platelet activation seldom occurs during the IAR. Several reports suggest that the platelet activation by PAF would be an event occurring in LAR but not in IAR.¹⁸⁻²¹

We have discussed the platelet activation in the circulation. Since bronchial asthma is an allergic inflammation essentially occurring in bronchial mucosa, even if platelets are activated *in situ*, the levels of platelet-derived substances in the circulation might not reflect the intensity of platelet activation in the airways.

The mean level of PAF in plasma of healthy controls was 9.3 nmol/L in this study. This value was more than 10 times higher than that reported by Diez et al.²² Although we could not identify what caused such a difference, it might be, in part, due to the difference of bioassay used.

In conclusion, our data suggest that the platelet activation in circulation is sometimes provoked in asthma, but plasma level of α -granule-derived proteins does not reflect the intensity or severity of asthma, and data also suggest that PAF is likely to be a mediator responsible for the platelet activation.

The authors thank Mr. N. Arita for his technical assistance.

REFERENCES

1. MacDermot J, Fuller RW. Macrophages. In: Barnes PJ, Rodger IW, Thomson NC, eds. *Asthma: basic mechanisms and clinical management*. London: Academic Press, 1988:97-113.
2. Pinckard RN, Halonen M, Palmer JD, Butler C, Shaw JO, Henson PM. Intravascular aggregation and pulmonary sequestration of platelets during IgE-induced systemic anaphylaxis in the rabbit: abrogation of lethal anaphylactic shock by platelet depletion. *J Immunol* 1977;119:2185-93.
3. Page CP, Paul W, Morley J. Platelets and bronchospasm. *Int Arch Allergy Appl Immunol* 1984;74:347-50.
4. McManus LM, Morley CA, Levine SP, Pinckard RN. Platelet activating factor (PAF) induced release of platelet factor 4 (PF₄) *in vitro* and during IgE anaphylaxis in the rabbit. *J Immunol* 1979;123:2835-41.
5. Maccia CA, Gallagher JS, Ataman G, Glueck HI, Brooks SM, Bernstein IL. Platelet thrombopathy in asthmatic patients with elevated immunoglobulin E. *J ALLERGY CLIN IMMUNOL* 1977;59:101-8.
6. Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma: an ultrastructural, quantitative

- study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989;140:1745-53.
7. Knauer KA, Lichtenstein LM, Adkinson NF Jr, Fish JE. Platelet activation during antigen-induced airway reactions in asthmatic subjects. *N Engl J Med* 1981;304:1404-7.
 8. Gresele P, Todisco T, Merante F, Nenci GG. Platelet activation and allergic asthma. *N Engl J Med* 1982;306:549.
 9. Cuss FM, Dixon CMS, Barnes PJ. Effects of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. *Lancet* 1986;2:189-92.
 10. Vargaftig BB, Pretolani M, Coëffier E, Chignard M. Platelet activating factor: biology, receptors and antagonists. In: Henson PM, Murphy RC, eds. *Mediators of the inflammatory process*. Amsterdam: Elsevier, 1989:113-46.
 11. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911-7.
 12. Satouchi K, Oda M, Saito K, Hanahan DJ. Metabolism of 1-*O*-alkyl-2-acetyl-*sn*-glycerol by washed rabbit platelets: formation of platelet activating factor. *Arch Biochem Biophys* 1984;234:318-21.
 13. Morrison JFJ, Pearson SB, Dean HG, Craig IR, Bramley PN. Platelet activation in nocturnal asthma. *Thorax* 1991;46:197-200.
 14. Wardlaw AJ, Moqbel R, Cromwell O, Kay AB. Platelet-activating factor: a potent chemotactic and chemokinetic factor for human eosinophils. *J Clin Invest* 1986;78:1701-6.
 15. Evans TW, Chung KF, Rogers DF, Barnes PJ. Effect of platelet-activating factor on airway vascular permeability: possible mechanisms. *J Appl Physiol* 1987;63:479-84.
 16. Klopogge E, Akkerman JWN. Binding kinetics of PAF-acether (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) to intact human platelets. *Biochem J* 1984;223:901-9.
 17. Durham SR, Dawes J, Kay AB. Platelets in asthma. *Lancet* 1985;2:36.
 18. Coyle AJ, Page CP, Atkinson L, Flanagan R, Metzger WJ. The requirement for platelets in allergen-induced late asthmatic airway obstruction: eosinophil infiltration and heightened airway responsiveness in allergic rabbits. *Am Rev Respir Dis* 1990;142:587-93.
 19. Averill FJ, Hubbard WC, Proud D, Gleich GJ, Liu MC. Platelet activation in the lung after antigen challenge in a model of allergic asthma. *Am Rev Respir Dis* 1992;145:571-6.
 20. Hemmendinger S, Pauli G, Tenabene A, et al. Platelet function: aggregation by PAF or sequestration in lung is not modified during immediate or late allergen-induced bronchospasm in man. *J ALLERGY CLIN IMMUNOL* 1989;83:990-6.
 21. Nakamura T, Morita Y, Kuriyama M, Ishihara K, Ito K, Miyamoto T. Platelet-activating factor in late asthmatic response. *Int Arch Allergy Appl Immunol* 1987;82:57-61.
 22. Diez FL, Nieto ML, Fernandez-Gallardo S, Gijon MA, Crespo MS. Occupancy of platelet receptors for platelet activating factor in patients with septicemia. *J Clin Invest* 1989;83:1733-40.

AVAILABLE NOW! The FIVE-YEAR (1986-1990) CUMULATIVE INDEX TO THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY can be purchased from the Publisher for \$48.00. This comprehensive, 188-page reference guide is a current presentation of all topics included in the JOURNAL from January 1986 to December 1990 (volumes 77-86)—the past 10 volumes. It incorporates complete references to over 1500 original articles, abstracts, case reports, letters, editorials, and CME articles. It features 1662 subject headings, under which there are 5863 entries. Each subject entry lists the complete article title, author(s), volume, page, and year of publication. It also includes 5801 author entries, listing contributors, along with their respective titles, author-to-author referral, volume, page, and publication date.

To purchase, call or write: Mosby-Year Book, Inc., 11830 Westline Industrial Dr., St. Louis, MO 63146-3318, or telephone FREE 1-800-325-4177, Subscription Services, ext. 4351, or 314-453-4351. PREPAYMENT REQUIRED. Make checks payable to Mosby-Year Book, Inc. (All payments must be in US funds drawn on a US bank.) Price: \$48.00 in the US, \$54.35 in Canada, and \$51.00 international (price includes mailing charges).