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The Existence of a Platelet-Activating Factor (Paf-Acether)-Like Substance in Blister Fluid Derived from Patients with Bullous Pemphigoid as Demonstrated by Human Platelet Aggregation Techniques

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Summary: We investigated the presence of a platelet-activating factor-like substance in blister fluid obtained from patients with bullous pemphigoid. Human platelet aggregation activity was present in blister fluids obtained from 4 out of 6 patients with bullous pemphigoid and in blister fluids obtained from 3 cases with contact dermatitis. Platelet aggregation activity of pemphigoid blister fluids was inhibited by pretreatment with lyso-PAF, which is a precursor metabolite of platelet activating factor. The activity of blister fluids obtained from patients suffering from contact dermatitis was not inhibited by precursor. Blister fluids obtained from burn lesions and blisters of normal skin induced in the suction revealed no platelet aggregation activity. These results suggest that platelet-activating factor or a similar substance is present in the blister fluid obtained from suffered bullous pemphigoid.

Key words: Paf-acether—blister fluid—bullous pemphigoid—human platelet—eosinophil

Introduction

Platelet-activating factor (Paf-acether) is a metabolite of long chain fatty acids which originates made from the lipid cell membrane (Albert et al. 1984). It is released after various stimuli from several cell types including macrophages, neutrophils, eosinophils and endothelial cells after various stimuli (Clark et al. 1980; Jouvin-Marche et al. 1984). It may have an important role as an immune mediator on the cell to cell interaction including platelet aggregation and secretion, neutrophil aggregation and degranulation, and monocyte and eosinophil chemotaxis (McManus et al. 1981; Czarnetzki, 1983; Pinckard, 1983; Lee et al. 1984; Dulioust et al. 1988).

Bullous pemphigoid (BP) which is a subepidermal blistering dermatosis, is characterized by the presence of circulating IgG autoantibodies which react with antigens located in the lamina lucida region of the basement membrane zone (BMZ) and a variety of inflammatory cells. In particular, many eosinophils are found in the blister cavity and in the dermal papillae.

It is suggested that many factors and enzymes released from the infiltrating cells cause tissue injury leading to blister

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formation in experimental bullous pemphigoid (Naito et al. 1984). However, the presence of Paf-acether in BP blister fluid was not demonstrated as yet. Since the biological activities of Paf-acether are linked with inflammatory cells, we expected that Paf-acether may exist a role in the blister formation, and we proceeded to attempt to demonstrate Paf-acether-like activity in BP blister fluid, using platelet aggregation assay for this purpose.

Materials and Methods

Subjects and controls

Blister fluids (BFs) was obtained from 6 patients suffering from BP, 3 patients with burns (2 males and one female, 20-72 years old), 3 patients with contact dermatitis (one male and 2 females, 45-78 years old) and a suction blister obtained from a normal volunteer (one male, 35 years old). BP was diagnosed on the basis of clinical and immunological findings. All of BFs were aspirated by needle syringe from a freshly formed blister within 24hr of its formation. BFs were immediately centrifuged at 1650G for 10 min at 4℃ and were filtered through a Millex-GV filter (pore size; 0.22μm, MILLIPORE, Tokyo, Japan). Steriled BFs were stocked in the deep freezer at -80℃ and thawed out before use. Immunological findings which were demonstrated as attachment of immunoglobulins and complements to the basement membrane of epidermis and existence of circulating autoantibodies to BMZ in serum from BP patients were studied by direct and indirect immunofluorescence staining method using rabbit anti-human immunoglobulin and anti-human complement antibodies. These results were shown in Table 1.

Chemicals and buffers

Paf-acether (1-o-octadecyl-2-acetyl-sn-glycero-3-phosphocholine) and lyso-PAF (1-o-alkyl-sn-glycero-3-phosphocholine) were purchased from Bachem (Marina Del Rey, CA). Buffers and modified Tyrode solution were prepared as described elsewhere (Farr et al. 1980). BFs which showed strong activity of platelet aggregation were diluted to minimum concentration which exhibit platelet aggregation activity in Tyrode solution or saline containing 5mg/ml bovine serum albumin before measurement of activity on the aggregation response of human platelets (Mallet and Cunningham, 1985).

Platelet isolation

Venous blood from healthy volunteers was collected into 1ml of 130mM-trisodium

TABLE 1

Immunological findings of six patients with bullous pemphigoid stained by immunofluorescence method

Case	Patients		Immunoglobulins and complements detected on BMZ	Titer of anti-BMZ antibody in serum
	Age	sex		
1	46	F	IgG, IgM, C_3	×640
2	84	M	negative	×80
3	79	F	IgG, C_3	×320
4	66	F	IgG, C_3	×320
5	73	F	IgG, C_3	×64
6	70	M	IgG, C_3	×1280

citrate. The donors did not take any medicine for 7 days prior to blood collection. Platelet-rich plasma (PRP) was prepared as previously described (Cox et al. 1984), and platelets were resuspended at 1.25×10^8/ml in Tyrode solution.

Measurement of the platelet response

0.18ml of PRP was added to siliconized cuvette containing a stir bar revolving at 500 rpm at 37℃ in an aggregometer (Platelet Aggregation Tracer, NKK, Tokyo, Japan). After incubation for 60sec, 0.02 ml of samples or reagents were added to PRP in the cuvette. To examine the existence of Paf-acether in BF, 0.02ml of lyso-PAF was added to an aliquot of platelets for one minute in siliconized cuvettes before Paf-acether or BFs were added.

The aggregation response was monitored by means of an aggregometer for four to seven min after the addition of samples, and changes of transmittance were measured. Activities of Paf-acether and BFs were recorded in terms of % aggregation response of human platelets (Mallet and Cunningham, 1985).

Results

Response of human platelets to Paf-acether and lyso-PAF

The human platelet aggregation response to Paf-acether and lyso-PAF are shown in Figs 1 and 2. The aggregations of human platelets induced by Paf-acether were observed at the concentrations of Paf-acether between 10^{-6}M and 10^{-12}M (Fig. 1). Paf-acether could not induce human platelet aggregation at the concentration lower than 10^{-13}M (data not shown). On the other hand, lyso-PAF, which is an inactive precursor metabolite of Paf-acether, and saline did not exhibit platelet aggregation activity at the concentrations of lyso-PAF between 10^{-4}M and 10^{-8}M (Fig. 2).

Since it was indicated lyso-PAF itself

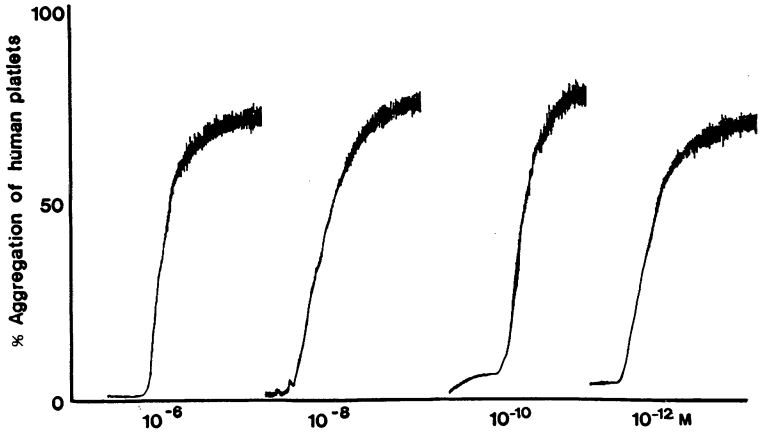

Fig. 1. Response of human platelets to Paf-acether as evaluated by aggregation behavior. This assay was conducted using the change in transmittance of a platelet suspension. The molar concentrations of Paf-acether are indicated in this figure. Aggregation of human platelets was observed at concentrations of higher than 10^{-12}M Paf-acether in cuvette.

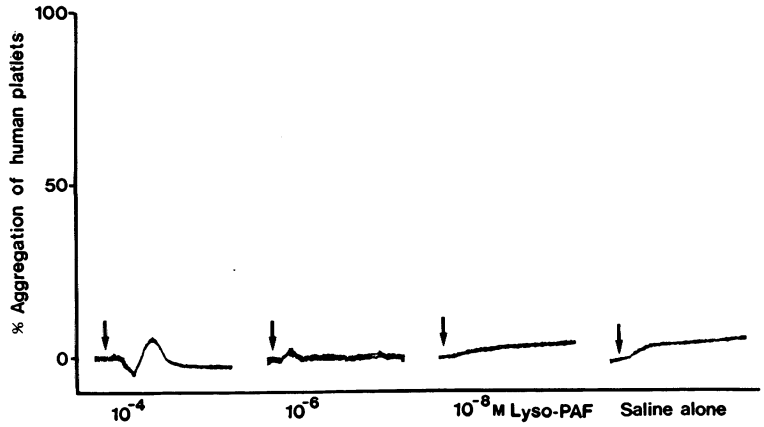

Fig. 2. Response of human platelets to lyso-PAF and saline as evaluated by aggregation behavior. Aggregation of human platelets was not observed at concentration of Lyso-PAF ranging from 10^{-4}M to 10^{-8}M.

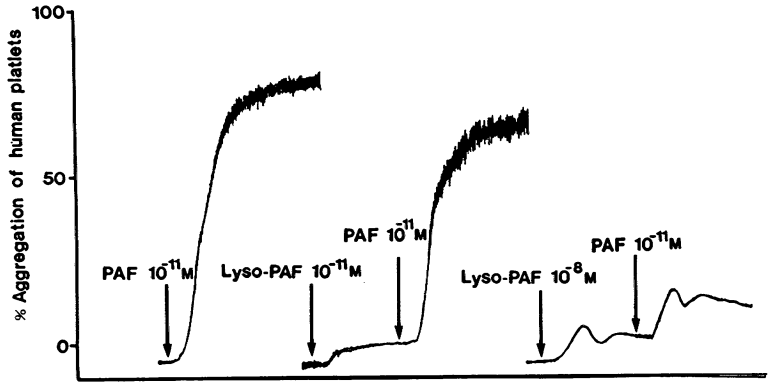

Fig. 3. Effect of lyso-PAF on the aggregation response of human platelets by Paf-acether. Aggregation of human platelets by addition of 10^{-11}M Paf-acether was inhibited by pretreatment with lyso-PAF at concentration higher than 10^{-8}M.

did not aggregate human platelet, the effect of pretreatment with lyso-PAF was studied in Paf-acether induced platelet aggregation. 10^{-11}M of Paf-acether exhibited platelet aggregation, and pretreatment with an equal concentration of lyso-PAF sohwed no inhibition of Paf-acether induced platelet aggregation (Fig. 3). However, 10^{-8}M of lyso-PAF inhibited the aggregation induced with 10^{-11}M of PAF-acether (Fig. 3).

Detection of platelet aggregation activity in blister fluid and inhibition test by pretreatment with lyso-PAF

Platelet aggregation activities induced

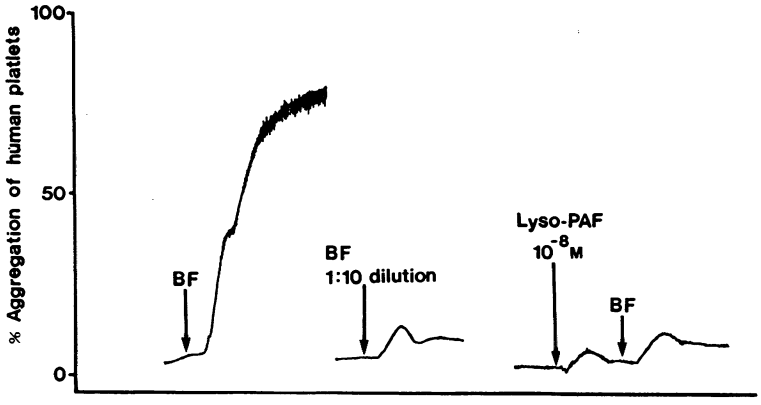

Fig. 4. The activity of blister fluid obtained from patient with bullous pemphigoid (Case 2) and the inhibition by lyso-PAF of the aggregation response of human platelets. Aggregation of human platelets was induced by addition of BF obtained from BP patient and the aggregation was inhibited by 10^{-8}M lyso-PAF.

TABLE 2

Results of Platelet aggregation activity in BFs and inhibition test by lyso-PAF.

case	BF obtained from	Activity of platelets aggregation in BF	Inhibition of platelets aggregation by lyso-PAF
1	BP	+	+
2	BP	+	+
3	BP	−	N. D.
4	BP	−	N. D.
5	BP	+	+
6	BP	+	+
7	burn	−	N. D.
8	burn	−	N. D.
9	burn	−	N. D.
10	contact dermatitis	+	−
11	contact dermatitis	+	−
12	contact dermatitis	+	−
13	healthy volunteer (suction blister)	−	N. D.

N. D. : Not done.

with BFs and inhibition test with lyso-PAF were tested in patients with BP, burns, contact dermatitis and in a healthy volunteer. The results are shown in Table 2. Human platelet aggregation activity was detected in BF obtained from 4 out of 6 patients exhibiting BP, and platelet aggregation induced with BF obtained from these four cases was inhibited with 10^{-8}M of lyso-PAF pretreatment. Platelet aggregation activity was not detected in BF obtained from three cases with burns and one healthy volunteer. Since BFs obtained from three cases with contact dermatitis

showed strong platelet aggregation activity, these BFs was diluted at minimum concentration exhibiting platelet aggregation. However platelet aggregation activity was not inhibited by addition of 10^{-8}M of Lyso-PAF.

Discussion

Platelet aggregation is known to occur not only with Paf-acether but also with ADP, serotonin, epinephrine, prostaglandin, collagen and thrombin (Davie and Ratnoff, 1965) in vitro. In this study, we showed that Paf-acether at the concentrations ranging 10^{-6}M and 10^{-12}M aggregated human platelets and lyso-PAF at the concentration lower than 10^{-4}M did not aggregate them. Aggregation of human platelets induced by 10^{-11}M Paf-acether was inhibited by pretreatment with lyso-PAF at concentration above 10^{-8}M. This result indicates that lyso-PAF can inhibit aggregation of human platelets induced with Paf-acether.

We found that four of BFs among six cases obtained from BP patients showed the human platelets aggregation activity, and that the activity of BF was inhibited by pretreatment with 10^{-8}M lyso-PAF. BFs from burns and via suction BF from a healthy volunteer revealed no platelets aggregation. Although BFs from contact dermatitis showed platelet aggregation activity, these activities were not inhibited with 10^{-8}M lyso-PAF. These results demonstrate that only BFs obtained from BP patients exhibit Paf-acether-like activity, while BFs obtained from patients suffering from contact dermatitis cause platelets aggregation due to via factors other than Paf-acether. However paf-acether like activity could not be detected in the cases of two patients suffering from BP. We suppose that it may be due to the existence of low amount of Paf-acether-like substance which we could not detect in the BF.

A variety of inflammatory cells have been identified in inflammatory lesions of the skin obtained from patients suffering from BP and they included polymorphonuclear leukocytes, eosinophils and mononuclear cells (Lever and Schaumburg-Lever, 1983). Immunological abnormalities of circulating complement activating autoantibody to the BP antigen, and IgG and C_3 attached on BMZ may play an important role in the tissue injury present in BP (Gammon et al. 1982).

Paf-acether is an inflammatory mediator with a wide range of biological activities, including chemotaxis and aggregation of neutrophils and eosinophils. Although a number of immune modulating factors including eosinophil chemotactic factor of anaphylaxis (ECF-A), leukotriene B4 (LTB4) and prostaglandin (PG) have been detected in BFs derived from BP, the presence of Paf-acether has not been previously demonstrated (Jordan et al. 1985).

It is known that activated eosinophils have high ability to synthesize Paf-acether as compared with resting eosinophils (Lee et al. 1984). Also, monocytes have a high ability to produce Paf-acether (Sanches-Crespo et al. 1980) and BP lesions contain significantly more macrophages compared to normal skin and the skin obtained from patients suffering from pemphigus vulgaris (Nestor et al. 1987).

In this study, we found that four out of six BFs from BP showed human platelets aggregation activity, and that the activity was inhibited by pretreatment with 10^{-8}M lyso-PAF. As it is not known whether lyso-PAF is able to inhibit selectively the aggregation activity of human platelets induced with Paf-acether in blister fluid, it is unclear whether this substance is identical with Paf-acether.

The role and origin of Paf-acether-like substance and the identification of Paf-acether in BF derived from patients suf-

fering from BP remain unresolved in this study. However, our group has already reported the existence of a substance which induced eosinophil activation in blister fluid derived from patients suffering from BP (Iryo et al. 1987), and we think Paf-acether might play a role in blister formation in these patients.

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