Stimulation of neutrophil adherence to vascular endothelial cells by histamine and thrombin and its inhibition by PAF antagonists and dexamethasone

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1 In order to clarify the roles of platelet-activating factor (PAF) in histamine- and thrombin-induced neutrophil adhesion to vascular endothelial cells, the effects of several PAF antagonists were examined. The effects of the glucocorticoid dexamethasone were also examined in order to gain further insight into the anti-inflammatory actions of glucocorticoids.

2 In culture, histamine and thrombin stimulated the adherence of rat peritoneal neutrophils to human endothelial cells from the umbilical vein. They did not stimulate neutrophil adherence in the absence of endothelial cells, suggesting that the target cells for the histamine- and thrombin-induced adherence of neutrophils were endothelial cells, not neutrophils.

3 Several PAF antagonists, such as CV-3988, L-652,731 and Y-24,180 inhibited the histamine- and thrombin-induced neutrophil adherence in a concentration-dependent manner. Indomethacin failed to inhibit it.

4 Dexamethasone, a steroidal anti-inflammatory drug, did not inhibit the histamine- and thrombin-induced adherence of neutrophils to endothelial cells when the drug was present only during the 20 min incubation period for the adherence assay. When the endothelial cells were preincubated for 3 h with dexamethasone, the adherence of neutrophils to endothelial cells induced by histamine or thrombin was not inhibited.

5 When the neutrophils were preincubated for 3 h with dexamethasone, the histamine- and thrombin-induced adherence of neutrophils to endothelial cells was inhibited.

6 Our studies indicate that: (a) adherence of neutrophils to endothelial cells induced by histamine and thrombin is mediated by PAF production since PAF antagonists inhibited the adherence of neutrophils; and (b) neutrophils, not endothelial cells, are the target cells through which dexamethasone acts to inhibit adherence.

Introduction

The adhesion of circulating neutrophils to vascular endothelial cells may play a crucial role in the acute inflammatory responses. The mechanism of the adhesion process has not yet been clarified. Vascular endothelial cells produce various mediators which may regulate neutrophil adhesion, such as endothelial-leukocyte adhesion molecule-1 (ELAM-1) (Pober *et al.*, 1986; Bevilacqua *et al.*, 1989) and intercellular adhesion molecule-1 (ICAM-1) (Dustin *et al.*, 1986; Rothlein *et al.*, 1986). The other strong candidate for neutrophil adhesion is platelet-activating factor (PAF). PAF is reported to be synthesized after stimulation by thrombin (Prescott *et al.*, 1984), histamine, bradykinin, adenosine triphosphate (McIntyre *et al.*, 1985), leukotrienes C₄ and D₄ (McIntyre *et al.*, 1986) and interleukin-1 (Bussolino *et al.*, 1986). Among them, thrombin (Zimmerman *et al.*, 1985a) and leukotrienes C₄ and D₄ (McIntyre *et al.*, 1986) make endothelial cells adherent to neutrophils in a way which is compatible with the time course and dose-dependency of PAF production. Consequently, it is strongly suggested that PAF produced in the endothelial cells by such stimulators plays a significant role in neutrophil adhesion to endothelial cells.

In the present investigations, we found that histamine, like thrombin, also stimulated neutrophil adherence to endothelial cells. In order to clarify further the significance of the role of PAF in neutrophil adherence to endothelial cells, the effects of several PAF antagonists such as CV-3988, L-652,731 and

Y-24,180 on histamine- and thrombin-induced neutrophil adherence were examined.

Steroidal anti-inflammatory drugs inhibit neutrophil infiltration into the inflammatory locus (Ohuchi *et al.*, 1982; 1984). Although several reports have suggested that glucocorticoids inhibit neutrophil accumulation at inflammatory sites by inhibiting production of chemoattractants (Kurihara *et al.*, 1984; Tsurufuji *et al.*, 1984; Schleimer *et al.*, 1989), the precise mechanism for the inhibition of neutrophil infiltration by steroidal anti-inflammatory drugs has not yet been clarified. We studied whether histamine- and thrombin-induced neutrophil adherence to endothelial cells is inhibited by the steroidal anti-inflammatory drug dexamethasone, in order to gain further insight into the mechanism of anti-inflammatory activity of dexamethasone.

Methods

Human endothelial cell culture

Human endothelial cells were isolated from the umbilical vein with 0.25% trypsin (Flow Laboratories, McLean, VA, U.S.A.)–0.01% EDTA in phosphate-buffered saline (PBS) solution, and cultured in 75-cm² tissue culture flasks (Iwaki Glass, Tokyo, Japan) according to a slight modification of the method described by Jaffe *et al.* (1973b) and Gimbrone (1976). Primary cultures of endothelial cells were grown in medium 199 (M199) (Nissui Seiyaku Co., Tokyo, Japan) containing 20% foetal bovine serum (FBS) (Flow Laboratories) supplemented with amphotericin B solution (1%) (Flow

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Laboratories), endothelial mitogen ($20 \mu\text{g ml}^{-1}$) (Biomedical Technologies, Inc., Stoughton, MA, U.S.A.), porcine intestinal heparin ($90 \mu\text{g ml}^{-1}$) (Novo Industries A/S, Denmark), penicillin G potassium ($18 \mu\text{g ml}^{-1}$) and streptomycin sulphate ($50 \mu\text{g ml}^{-1}$) (Meiji Seika Co., Tokyo, Japan). Culture flasks were incubated at 37°C in an atmosphere of 5% carbon dioxide (CO_2) in air. The culture media were changed every 3 days. When primary cultures were almost confluent, endothelial cells were harvested from flasks by treatment with 0.25% trypsin, and plated into well-plates with a 16-mm diameter (Tissue Culture Cluster 24, Costar, Cambridge, MA, U.S.A.). Subcultures were grown in M199 containing 10% FBS supplemented with the reagents used for primary cultures. The cells were used at the first passage in each experiment. When monolayers of endothelial cells were tightly confluent, they were used for assay of endothelial cell-neutrophil adhesion. Cultures were characterized as endothelial cells based on morphological criteria (Jaffe *et al.*, 1973b) and by indirect immunofluorescence using a specific antiserum to human factor VIII antigen (ICN Biomedicals Inc., Costa Mesa, CA, U.S.A.), a usual marker of endothelial cells (Jaffe *et al.*, 1973a).

Preparation of rat neutrophils

Male rats (350–500 g, Sprague-Dawley strain, specific pathogen-free, Charles River Japan Inc., Kanagawa, Japan) were injected i.p. with Ca^{2+} -free Krebs-Ringer bicarbonate buffer (pH 7.4, $12 \text{ ml } 100 \text{ g}^{-1}$ body weight) containing 1% casein (from milk, vitamin-free, Wako Pure Chemical Ind., Tokyo, Japan) under light diethyl ether anaesthesia. After 15 h, rats were killed under diethyl ether anaesthesia by cutting the carotid artery, and peritoneal fluid was collected. The collected fluid was centrifuged at 250 g for 3 min at 4°C , and the cell pellet was washed twice with PBS and once with M199. Cells were finally suspended in M199 containing 0.1% (w/v) essentially fatty acid-free bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO, U.S.A.), at a concentration of 6×10^6 cells ml^{-1} . Under microscopic observation after staining with haematoxylin and eosin, more than 95% of the cells were identified as neutrophils.

Adhesion of neutrophils to gelatin-coated dishes

Neutrophils (3×10^6 cells) in 1.0 ml of the M199-BSA solution containing various concentrations of histamine or thrombin were incubated in each well of gelatin-coated plates with a 16-mm well diameter (Iwaki Glass) at 37°C for 20 min in an atmosphere of 5% CO_2 in air. After the incubation, the wells were washed 3 times with 0.5 ml of M199 to remove non-adherent neutrophils, then 0.4 ml of 0.25% trypsin-EDTA in PBS solution was added, and the samples were incubated for 10 min at 37°C to detach the adherent neutrophils from the wells. After an addition of 0.1 ml of M199 containing 10% calf serum (Flow Laboratories Inc.), the neutrophils were suspended by use of siliconized Pasteur pipettes, and were counted in a haemocytometer.

Endothelial cell-neutrophil adhesion assay

Monolayers of endothelial cells which were tightly confluent were washed twice with 0.5 ml of M199 at 37°C . Then 0.5 ml of neutrophil suspension (3×10^6 cells 0.5 ml^{-1}) was layered over the endothelial cells, 0.5 ml of the medium containing various concentrations of reagents was added, and the samples were incubated at 37°C for 20 min in an atmosphere of 5% CO_2 in air. After the incubation, the wells were washed 3 times with 0.5 ml of M199 to remove non-adherent neutrophils, then 0.4 ml of 0.25% trypsin–0.01% EDTA in PBS solution was added and the samples were incubated for 10 min at 37°C to detach the endothelial cells from the wells and the neutrophils from the endothelial cells. After addition of 0.1 ml of M199 containing 10% calf serum (Flow Laboratories, Inc.), endothelial cells and neutrophils were suspended by use of sili-

conized Pasteur pipettes. The neutrophils and endothelial cells were counted in a haemocytometer. Neutrophil adhesion is expressed as the number of neutrophils that adhered to 100 endothelial cells.

Chemicals

Human plasma thrombin (Sigma Chemical Co.) and histamine (free base, Wako Pure Chemical Ind.) were dissolved in the M199-BSA solution. PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a mixture of C_{16} and C_{18} forms, Avanti Polar Lipids Inc., Birmingham, AL, U.S.A.) was dissolved in ethanol and an aliquot of the ethanol solution was added to the M199-BSA solution. Synthetic PAF antagonists, CV-3988 ((*RS*)-2-methoxy-3-(octadecylcarbamoyloxy) propyl-2-(3-thiazolo) ethyl phosphate) (Terashita *et al.*, 1983), L-652,731 (*trans*-2,5-bis-(3,4,5-trimethoxyphenyl) tetrahydrofuran) (Hwang *et al.*, 1985) and Y-24,180 (($\pm$)-4-(2-chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (Terasawa *et al.*, 1990) were dissolved in ethanol or dimethylsulphoxide (DMSO), and an aliquot of the solution was added to the M199-BSA solution. Dexamethasone (Sigma Chemical Co.) and indomethacin (Sigma Chemical Co.) were dissolved in ethanol, and an aliquot of the solution was added to the M199-BSA solution. The final concentration of ethanol or DMSO in the M199-BSA solution was adjusted to 0.1% (v/v). Control medium contained the same amount of the vehicle.

Statistical analysis

Results were analyzed for statistical significance by Student's *t* test for paired observations.

Results

Effects of thrombin, histamine and PAF on neutrophil adherence to vascular endothelial cells

Figure 1 shows the effects of thrombin, histamine and PAF on the adherence of rat peritoneal neutrophils to human endothelial cells from the umbilical vein. Thrombin (1 unit ml^{-1}) and histamine (10^{-5} M) increased the adherence about two fold. PAF at 10^{-7} M was not as active as thrombin and histamine. These drugs increased the adherence in a concentration-

Figure 1 Effects of thrombin, histamine and PAF on neutrophil adhesion to endothelial cells. Neutrophils (3×10^6 cells) in 1 ml of M199 supplemented with 0.1% (w/v) BSA (M199-BSA solution) which contained thrombin (1 unit ml^{-1}), histamine (10^{-5} M) or PAF (10^{-7} M) were incubated with the monolayer of endothelial cells (2×10^5 cells) at 37°C for 20 min. Neutrophil adhesion is expressed as the number of neutrophils that adhered to 100 endothelial cells (EC). Values are the means from 5 to 6 samples with s.e.mean shown by vertical bars. Statistical significance: * $P < 0.01$; ** $P < 0.001$ vs. 'None'. Comparable results were obtained in two additional experiments.

dependent manner. Significant increases in the adherence were observed at concentrations of more than $0.1 \text{ units ml}^{-1}$ for thrombin, 10^{-6} M for histamine and 10^{-8} M for PAF (data not shown). Leukotriene B_4 failed to stimulate adherence even at a concentration of $3 \times 10^{-6} \text{ M}$ (data not shown).

Effects of thrombin, histamine and PAF on neutrophil adherence to gelatin-coated dishes

To identify the target cells for thrombin, histamine and PAF, neutrophils plus the medium containing one of each of the stimulants were incubated at 37°C for 20 min in gelatin-coated dishes in the absence of endothelial cell monolayers. As shown in Figure 2, thrombin at concentrations of 0.01 to 1 unit ml^{-1} did not affect adherence of neutrophils to gelatin-coated dishes. Histamine at concentrations of 10^{-7} to 10^{-5} M significantly decreased the adherence in a concentration-dependent manner. In contrast, PAF at 10^{-7} M significantly stimulated the adherence. A significant increase in neutrophil adherence was also observed at 10^{-8} M PAF (data not shown).

Effects of PAF antagonists on neutrophil adherence to vascular endothelial cells

The effects of the specific PAF antagonists, CV-3988, L-652,731 and Y-24,180, on thrombin-induced (1 unit ml^{-1}) and histamine-induced (10^{-5} M) neutrophil adherence to endothelial cells are shown in Figures 3, 4, and 5, respectively. These PAF antagonists inhibited both thrombin- and histamine-induced neutrophil adherence in a concentration-dependent manner. Among the three antagonists, Y-24,180 was the most active. Higher concentrations of PAF antagonists tended to inhibit the spontaneous adhesion of neutrophils. The PAF antagonists showed no cytotoxicity to either type of cell as revealed by the dye exclusion test.

Effects of dexamethasone on thrombin- and histamine-induced adherence of neutrophils to vascular endothelial cells

When endothelial cell monolayers were incubated at 37°C for 20 min with neutrophils in the medium containing thrombin (1 unit ml^{-1}) or histamine (10^{-5} M) and various concentrations of dexamethasone (3×10^{-8} to $3 \times 10^{-6} \text{ M}$), neutrophil adherence induced by thrombin or histamine was not affected by the dexamethasone (data not shown). Furthermore, no inhibition of neutrophil adherence was seen when endothelial cell

Figure 2 Effects of thrombin, histamine and PAF on neutrophil adherence to gelatin-coated dishes. Neutrophils (3×10^6 cells) in 1 ml of the M199-BSA solution containing thrombin (1 unit ml^{-1}), histamine (10^{-5} M) or PAF (10^{-7} M) were incubated at 37°C for 20 min in gelatin-coated dishes with a 16 mm diameter, and the number of neutrophils adhering to the dishes was counted. Values are the means from 5 to 6 samples with s.e.mean shown by vertical bars. Statistical significance: ** $P < 0.001$ vs. control. Comparable results were obtained in two additional experiments.

Figure 3 Effects of CV-3988 on thrombin- and histamine-induced adhesion of neutrophils to endothelial cells. Neutrophils (3×10^6 cells) in 1 ml of the M199-BSA solution containing 1 unit ml^{-1} of thrombin (a) or 10^{-5} M of histamine (b) and indicated concentrations of CV-3988 were incubated with a monolayer of endothelial cells (2×10^5 cells) at 37°C for 20 min. Open columns represent the medium in the absence of stimulant. Hatched columns represent the medium which contained thrombin (a) and histamine (b). Values are the means from 5 to 6 samples with s.e.mean shown by vertical bars. Statistical significance: * $P < 0.01$; ** $P < 0.001$ vs. corresponding control. Comparable results were obtained in two additional experiments.

monolayers were preincubated for 3 h in medium containing various concentrations of dexamethasone (3×10^{-8} to $3 \times 10^{-6} \text{ M}$), washed with fresh dexamethasone-free medium, and incubated with neutrophils at 37°C for 20 min in dexamethasone-free medium containing thrombin (1 unit ml^{-1}) or histamine (10^{-5} M) (Figure 6). Neutrophil adherence was not inhibited when the above experiment was repeated with exposure to dexamethasone throughout and with incubation in the conditioned medium derived from the initial incubation with endothelial cells. However, as shown in Figure 7, when neutrophils were preincubated for 3 h in the presence of various concentrations of dexamethasone (3×10^{-8} to $3 \times 10^{-6} \text{ M}$), washed with fresh dexamethasone-free medium, and incubated with endothelial cell monolayers at 37°C for 20 min in the presence of thrombin (1 unit ml^{-1}) or histamine (10^{-5} M) but the absence of dexamethasone, neutrophil adherence to endothelial cells induced by thrombin and histamine was significantly inhibited.

Effects of indomethacin on thrombin- and histamine-induced adherence of neutrophils to vascular endothelial cells

Thrombin-stimulated (1 unit ml^{-1}) neutrophil adherence was not inhibited when endothelial cell monolayers were incubated at 37°C for 20 min with neutrophils in the medium containing various concentrations of indomethacin (3×10^{-8} to

Figure 4 Effects of L-652,731 on thrombin- and histamine-induced adhesion of neutrophils to endothelial cells. Neutrophils (3×10^6 cells) in 1 ml of the M199-BSA solution containing 1 unit ml^{-1} of thrombin (a) or 10^{-5} M of histamine (b) and indicated concentrations of L-652,731 were incubated with a monolayer of endothelial cells (2×10^5 cells) at 37°C for 20 min. Open columns represent the stimulant-free medium. Hatched columns represent the medium which contained thrombin (a) and histamine (b). Values are the means from 5 to 6 samples with s.e.mean shown by vertical bars. Statistical significance: * $P < 0.01$; ** $P < 0.001$ vs. corresponding control. Comparable results were obtained in two additional experiments.

Figure 5 Effects of Y-24,180 on thrombin- and histamine-induced adhesion of neutrophils to endothelial cells. Neutrophils (3×10^6 cells) in 1 ml of the M199-BSA solution containing 1 unit ml^{-1} of thrombin (a) or 10^{-5} M of histamine (b) and indicated concentrations of Y-24,180 were incubated with a monolayer of endothelial cells (2×10^5 cells) at 37°C for 20 min. Open columns represent the stimulant-free medium. Hatched columns represent the medium which contained thrombin (a) and histamine (b). Values are the means from 5 to 6 samples with s.e.mean shown by vertical bars. Statistical significance: * $P < 0.01$; ** $P < 0.001$ vs. corresponding control. Comparable results were obtained in two additional experiments.

3×10^{-6} M) (data not shown). Furthermore, when endothelial cell monolayers or neutrophils were preincubated with indomethacin at the same range of concentrations for 1 h, thrombin-induced (1 unit ml^{-1}) or histamine-induced (10^{-5} M) neutrophil adherence was not inhibited by indomethacin at concentrations up to 3×10^{-6} M (data not shown).

Discussion

Preliminary experiments revealed that the adherence rate of peripheral neutrophils from healthy volunteers to vascular endothelial cells derived from one donor differed greatly from volunteer to volunteer. Therefore, in the present investigations, in order to minimize individual differences of neutrophil adherence, rat peritoneal neutrophils were used instead of human peripheral neutrophils. Furthermore, in order to minimize changes in characteristics of vascular endothelial cells caused by a long incubation period with many passages, primary cultured endothelial cells from different human donors were used in each experiment. Consequently, depending upon the differences in the donated umbilical veins, the adherence rate of neutrophils to vascular endothelial cells changed from experiment to experiment. But the inter-experimental difference in the neutrophil adherence rate was much less than that obtained by use of human neutrophils and human vascular endothelial cells.

In the present investigations, the effects of several drugs on adherence of rat peritoneal neutrophils to human endothelial cells from the umbilical vein were examined in order to gain further insight into the mechanism of neutrophil adherence and the anti-inflammatory activity of the glucocorticoid, dexamethasone. In this study, we showed that both histamine and thrombin stimulated adherence of rat peritoneal neutrophils to vascular endothelial cells from human umbilical veins. However, when rat peritoneal neutrophils were incubated in gelatin-coated plastic dishes which did not contain vascular endothelial cells, the adherence was not stimulated by thrombin treatment (Figure 2). These results indicate that thrombin-stimulated neutrophil adherence to vascular endothelial cells is dependent on the endothelial cells and does not result from direct activation of the neutrophils by thrombin, as suggested by Zimmerman *et al.* (1985a). In contrast, incubation of neutrophils in the medium containing histamine resulted in a concentration-dependent inhibition of adherence to gelatin-coated plastic dishes (Figure 2). Since histamine is reported to inhibit neutrophil adherence to glass beads by a histamine H_2 receptor-dependent mechanism (Robinson, 1982), the concentration-dependent inhibition to gelatin-coated dishes might be induced by the same mechanism. However, when neutrophils were incubated on a monolayer of endothelial cells in the presence of histamine, neutrophil adhesion to endothelial cells was significantly stimulated (Figure 1). This result indicates that endothelial cells might produce some potent stimulator for adherence which counteracts the H_2

Figure 6 Effects of dexamethasone treatment of endothelial cells on thrombin- and histamine-induced adhesion of neutrophils to endothelial cells. Endothelial cell monolayers (2×10^5 cells) were incubated at 37°C for 3 h in 1 ml of the M199-BSA solution containing indicated concentrations of dexamethasone, washed and further incubated in the medium containing neutrophils (3×10^6 cells) and 1 unit ml^{-1} of thrombin (a) or 10^{-5} M of histamine (b). Open columns represent the stimulant-free medium. Hatched columns represent the medium which contained thrombin (a) and histamine (b). Values are the means from 5 to 6 samples with s.e.mean shown by vertical bars. Comparable results were obtained in three additional experiments.

receptor-induced inhibition of adherence. In contrast, LTB_4 failed to induce neutrophil adherence to endothelial cells (data not shown). The lack of effect of LTB_4 could be because rat neutrophils are weak responders to this lipid mediator (Kreisle *et al.*, 1985). It is reported that histamine (McIntyre *et al.*, 1985) as well as thrombin (Prescott *et al.*, 1984; Zimmerman *et al.*, 1987) produces PAF in endothelial cells, and exogenous PAF stimulates adhesion of neutrophils to vascular endothelial cells in hamster cheek pouch microcirculation (Dillon *et al.*, 1988). Furthermore, Zimmerman *et al.* (1987) demonstrated that in thrombin-stimulated human endothelium, PAF synthesis and neutrophil adhesion to the endothelial cells were tightly coupled events. Consequently, it is conceivable that histamine as well as thrombin stimulates PAF generation in endothelial cells and promotes neutrophil adherence. In order to investigate this idea, effects of several PAF antagonists or histamine- and thrombin-induced neutrophil adherence to endothelial cells were examined.

Since thrombin- and histamine-induced adherence of neutrophils to vascular endothelial cells was inhibited by several PAF antagonists in a concentration-dependent manner (Figures 3, 4 and 5), PAF produced by vascular endothelial cells or by neutrophils might be responsible for the neutrophil adherence to vascular endothelial cells. However, in human polymorphonuclear leukocytes (PMN), thrombin at 10 units ml^{-1} or histamine at $50 \mu\text{M}$ failed to induce PAF production (Sisson *et al.*, 1987). Consequently, it is possible that vascular endothelial cells produce PAF, which in turn makes

Figure 7 Effects of dexamethasone treatment of neutrophils on thrombin- and histamine-induced neutrophil adhesion to endothelial cells. Neutrophils (3×10^6 cells) were incubated at 37°C for 3 h in 1 ml of the M199-BSA solution containing indicated concentrations of dexamethasone, washed and further incubated with endothelial cell monolayer (2×10^5 cells) in 1 ml of the M199-BSA solution containing 1 unit ml^{-1} of thrombin (a) or 10^{-5} M histamine (b). Open columns represent the stimulant-free medium. Hatched columns represent the medium which contained thrombin (a) and histamine (b). Values are the means from 5 to 6 samples with s.e.mean shown by vertical bars. Statistical significance: * $P < 0.01$; ** $P < 0.001$ vs. corresponding control. Comparable results were obtained in three additional experiments.

neutrophils adhere to the endothelial cells. This idea is also supported by Zimmerman *et al.* (1990) demonstrating that L-652,731 inhibited the binding of human neutrophils to human endothelial cells activated by thrombin but not to endothelial cells activated by tumor necrosis factor α . Most of the PAF produced by endothelial cells remains cell-associated and it has been suggested that PAF is appropriately located on the endothelial cells to interact with neutrophils (Prescott *et al.*, 1984; McIntyre *et al.*, 1986; Zimmerman *et al.*, 1990). However, in interleukin-1-induced adhesion of PMN to cultured human endothelial cells, Breviaro *et al.* (1988) reported that PAF production by endothelial cells did not significantly contribute to PMN adhesion because CV-3988 acts as an inhibitor in paraformaldehyde-fixed endothelial cells. They considered the inhibitory effect of CV-3988 was directed at PAF produced by the PMN themselves in interleukin-1-stimulated adherence. We reported that in an allergic inflammation model of air pouch type in rats (Tsurufuji *et al.*, 1982), neutrophil infiltration into the pouch fluid was significantly inhibited by PAF antagonists (Sugidachi *et al.*, 1988), suggesting that PAF generated in the locus of allergic inflammation plays some significant role in neutrophil infiltration *in vivo*.

As to the effects of indomethacin, Zimmerman *et al.* (1985b) found that PAF production was enhanced by treatment of endothelial cells with indomethacin, indicating that endogenously generated prostacyclin might modulate PAF synthesis.

However, in the present investigations, indomethacin treatment did not affect neutrophil adherence to endothelial cells even at a concentration of 3×10^{-6} M (data not shown).

As to the action of steroidal anti-inflammatory drugs on neutrophil adhesion to vascular endothelial cells, Schleimer *et al.* (1989) observed that 24 h culture of human neutrophils with dexamethasone failed to inhibit the adherence induced by either leukocyte activators, such as formyl-methionyl-leucyl-phenylalanine and PAF, or endothelial activators, such as interleukin-1, lipopolysaccharide or 12-*O*-tetradecanoylphorbol-13-acetate. However, judging from the half-life of neutrophils, the 24 h of culture might be too long to examine the adherence ability of neutrophils. In contrast, the oral administration of prednisone in humans significantly inhibited the adherence of peripheral neutrophils to nylon-wool columns when examined 4 h after prednisone administration (Clark *et al.*, 1979). Furthermore, Rosenbaum *et al.* (1986) reported that spontaneous adherence of rabbit peripheral PMN to rabbit coronary microvascular endothelial cells was reduced when the leukocytes were pretreated with 10^{-7} M dexamethasone for 4 h. However, when the rabbit coronary microvascular endothelial cells were pretreated with dexamethasone, granulocyte adhesion was not inhibited. In the present investigations, we showed that when human endothelial cells were pretreated with dexamethasone for 3 h, neutrophil adhesion induced by thrombin or histamine was not inhibited (Figure 6), but when neutrophils were pretreated with dexamethasone for 3 h, thrombin- and histamine-induced neutrophil adhesion to vascular endothelial cells was significantly inhibited (Figure 7). These results strongly suggest that dexamethasone suppresses histamine- and thrombin-induced neutrophil adhesion to vascular endothelial cells and thus may inhibit neutrophil infiltration into the inflammatory locus. Probable target cells for glucocorticoids might be neutrophils, but not vascular endothelial cells. Our preliminary experiments revealed that the inhibitory effect of dexamethasone did not become apparent when endothelial cells were pretreated for 1 h with dexamethasone. The requirement of a time lag before manifesting the effect of dexamethasone might suggest that biosynthesis of certain proteins is necessary for the suppression of neutrophil adherence.

If phospholipase A_2 is exclusively essential for PAF synthesis in vascular endothelial cells, glucocorticoid treatment of

vascular endothelial cells might suppress PAF production by inhibiting phospholipase A_2 (Parente & Flower, 1985). However, dexamethasone treatment of vascular endothelial cells failed to inhibit neutrophil adherence (Figure 6), suggesting that dexamethasone could not reduce PAF production by inhibiting phospholipase A_2 . It is possible that the alternative physiological pathway involving choline phosphotransferase (Lee *et al.*, 1986) may subserve the generation of intracellular PAF in vascular endothelial cells.

It has been suggested that in thrombin-induced neutrophil adherence to vascular endothelial cells, CDw18 antigen expression on neutrophils plays a significant role in the neutrophil adherence (Bizios *et al.*, 1988). Since PAF is reported to express CDw18 antigen on the outer membrane of neutrophils (Zimmerman & McIntyre, 1988), it is possible that PAF, which is generated in endothelial cells by histamine treatment, might also induce CDw18 complex on neutrophils, which in turn makes neutrophils adherent to endothelial cells. One of the possible mechanisms of steroidal anti-inflammatory drugs on inhibition of neutrophil adherence to vascular endothelial cells might be an inhibition of the expression of CDw18 complex on neutrophils. However, this hypothesis requires further investigation.

In conclusion, histamine as well as thrombin stimulated neutrophil adherence to vascular endothelial cells. The histamine- and thrombin-induced neutrophil adherence was inhibited in a concentration-dependent manner by several PAF antagonists, suggesting that PAF probably produced by endothelial cells might be responsible for neutrophil adherence. The steroidal anti-inflammatory drug dexamethasone inhibited neutrophil adherence when neutrophils were pretreated for 3 h. However, when endothelial cells were pretreated with dexamethasone for 3 h, neutrophil adhesion was not inhibited. Consequently, neutrophils might be target cells for dexamethasone in inhibiting histamine- and thrombin-induced neutrophil adherence to vascular endothelial cells.

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