Arachidonic Acid Metabolism and the Adhesion of Human Polymorphonuclear Leukocytes to Cultured Vascular Endothelial Cells

By M. R. Buchanan, M. J. Vazquez, and M. A. Gimbrone, Jr.

Polymorphonuclear leukocytes (PMN) adhere to the vascular endothelial lining in vivo and to the surfaces of cultured endothelial cells in vitro, but the mechanisms of these cellular interactions remain unclear. Arachidonic acid metabolites, both cyclooxygenase- and lipoxygenase-derived, have been shown to influence PMN locomotion, secretion, and adhesion to artificial surfaces. To determine whether such mediators also are involved in regulating PMN–endothelial cell interactions, we have examined the effects of prostacyclin and various inhibitors of arachidonic acid metabolism on the adherence of radiolabeled PMN to cultured bovine aortic endothelial cells. Confluent endothelial monolayers were incubated with washed suspensions of radiolabeled human PMN (which contained less than 1% platelet contamination) at 37°C for 30 min, then subjected to a standardized wash procedure and the number of adherent leukocytes determined radiometrically. Under basal conditions, i.e., in the absence of exogenous activating stimuli, 4,163 ± 545 PMN adhered per square millimeter of endothelial surface (mean ± SEM, n = 12). This basal adhesion (which corresponds to approximately 4–5 leukocytes per endothelial cell) was unaffected when the leukocytes and endothelial monolayers were pretreated with cyclooxygenase inhibitors (100 µM aspirin or 1–5 µM indomethacin) or PGI2 (10−10−10 M). Thus, basal PMN–endothelial adhesion in this in vitro model system does not appear to be dependent on endogenous cyclooxygenase derivatives of arachidonate or to be sensitive to inhibition by exogenous prostacyclin. In contrast, leukocyte adhesion was significantly reduced by pretreatment with 5,8,11,14- or 4,7,10,13-eicosatetraenoic acid, 0.5–5 mM sodium salicylate, or 10–1,000 µM indomethacin, antiinflammatory agents that can interfere with the metabolism of arachidonic acid via non-cyclooxygenase-dependent mechanisms. These observations may be relevant to the interactions of circulating PMN with vascular endothelium under both physiologic and pathophysiologic conditions in vivo.

CIRCULATING polymorphonuclear leukocytes (PMN) interact with the vascular endothelium under both physiologic and pathologic conditions. Normally, approximately one-half of blood PMN are maintained in a “marginated pool,” reversibly associated with the vascular endothelial surface, and, in the presence of a systemic stimulus, such as endotoxia, this fraction can be markedly increased.1–3 At sites of tissue injury, PMN characteristically become concentrated in the microvasculature and their emigration across the endothelial lining is enhanced, presumably in response to locally generated chemotactic factors.1,4 Although the phenomena of PMN margination and transendothelial migration are well recognized, the cellular and molecular mechanisms involved and their regulation, especially under physiologic conditions, are not well understood.

During the last 5 yr, several laboratories have utilized cell culture systems to explore various aspects of leukocyte–endothelial interactions.5–11 For example, Hoover and coworkers have observed that human PMN appear to adhere preferentially to cultured human or bovine endothelial cells in comparison with other cell types.7 This interaction requires divalent cations8,9 and is enhanced by treatment with chemotactic agents, such as formyl-methionyl-leucyl-phenylalanine.8 Studies with such in vitro systems, in which selective pretreatment of either cell type is possible, have suggested that the endothelial cell may be playing an active role in modulating leukocyte–vessel-wall interactions.8,11

There is much evidence that derivatives of arachidonic acid, arising via cyclooxygenase and/or lipoxygenase pathways, or even nonenzymatic mechanisms, can influence various aspects of endothelial and leukocyte function, and thus might act as potent, short-range mediators at the vessel-wall–blood interface.14–18 Both cyclooxygenase and lipoxygenase products can influence leukocyte chemokinesis, chemotaxis, lysosomal enzyme release, aggregation, and adherence to artificial surfaces,18–24 especially in the context of inflammatory reactions. However, a role for these metabolites in modulating the adhesion of PMN to endothelial cell surfaces under normal circumstances has not been clearly defined.25 As an approach to this question, we have used a quantitative monolayer adhesion assay to evaluate the effects of prostacyclin (PGI2) and various inhibitors of arachidonate metabolism on PMN attachment to cultured endothelial cells under...
"basal" conditions, i.e., in the absence of exogenous activating stimuli. Our results suggest that arachidonate metabolism by non-cyclooxygenase-dependent mechanisms plays a role in the regulation of leukocyte–endothelial interactions.

MATERIALS AND METHODS

Prostacyclin (PGI₂), 6-keto-prostaglandin F₆ (6-keto-PGF₆), and 4,7,10,13-eicosatetraynoic acid (4,7,10,13-ETYA) were obtained from Dr. J. Pike of the Upjohn Co., Kalamazoo, MI, and 5,8,11,14-eicosatetraynoic acid (5,8,11,14-ETYA) was a gift of Dr. W. E. Scott, Hoffmann-LaRoche, Inc., Nutley, NJ. A stock solution of PGI₂ was prepared in Tris-HCl (0.05 M) at pH 8.6 immediately before use, and its potency assessed by inhibition of platelet aggregation, as previously described.⁶ ETYA was first solubilized in a small volume of 100% ethanol, and then diluted to the desired ETYA concentration in a final mixture of 10% ethanol and 50 µM sodium carbonate. This was further diluted 40-fold when added to the leukocyte and endothelial cell media. Indomethacin, obtained from Sigma Chemical Corp., St. Louis, MO, was also dissolved in 10% ethanol containing 50 µg sodium carbonate. Hanks' balanced salt solution (HBSS), Dulbecco's modified Eagle's medium (DME), and bovine sera were obtained from M. A. Bioproducts, Walkersville, MD. ¹H-adenine (0.1 µg/ml, specific activity, 100 µCi/mg, New England Nuclear, Boston, MA) was used in combination with unlabeled adenine (Sigma Chemical Corp.). ¹¹Indium oxine solution (carrier-free, buffered) was obtained from Amersham Corp., Arlington Heights, IL. Aspirin (crystalline acetylsalicylic acid, Sigma Chemical Corp.) was dissolved in equimolar concentrations of sodium carbonate as previously described.²⁸ Dextran (500,000 mol wt) was obtained from Sigma Chemical Corp. Ficoll-diatrizoate (Lymphocyte Separation Medium) was obtained from Litton Bionetics, Kensington, MD. Ultrafluor (liquid scintillation counting fluid) was obtained from National Diganostics, Somerville, NJ. All cell culture plastic containers were obtained from Costar, Cambridge, MA; the coverslips on which endothelial cells were grown were obtained from Lux Scientific Co., Newbury Park, CA (Thermanox plastic) or Belco Glass, Inc., Vineland, N.J. Collagenase was obtained from Worthington Millipore Corp., Freehold, N.J. Scanning electron microscopy chemicals were obtained from Polysciences Inc., Warrington, PA.

Isolation and Radiolabeling of Leukocytes

Whole blood was collected from healthy volunteers into acid-citrate-dextrose (8:16) and centrifuged at 2,000 g for 3 min at 22°C. The platelet-rich plasma was discarded, and the remaining buffy coat and erythrocytes were resuspended in an equimolervol of 6% dextran in HBSS and processed according to standard methods.²⁹ Residual erythrocytes were lysed by a 20-sec hypotonic exposure, and the leukocytes were then passed through a Ficoll-diatrizoate gradient at 400 g for 20 min. The supernatant and Ficoll gradient were discarded, and the remaining cell pellet was resuspended in 5 ml of calcium-free Tyrode's solution containing 150 µCi of ¹H-adenine and incubated for 30 min at 22°C. The cells were washed twice in calcium-free Tyrode's solution and finally suspended in 4% Tyrode's albumin (2 mM Ca⁺⁺, 1 mM Mg⁺⁺) at a concentration of 5,000 leukocytes/µl. Unlabeled adenine (1 µg/ml) was added and the cell suspension stored on ice until used. This technique routinely yielded >95% pure polymorphonuclear leukocyte populations (by Wright-Giemsa staining), which contained <1 platelet/100 leukocytes (as monitored by phase-contrast and scanning electron microscopy). In certain experiments, washed PMN cell suspensions also were labeled with ¹¹In, using a modification of published methods.⁰

Endothelial Cell Cultures

Bovine aortic endothelial cells (BAEC) were isolated from the intimimal lining of calf thoracic aortae and cultured as previously described.³⁰ One strain of these cells (11-BAEC) was utilized for essentially all the studies reported here. For monolayer adhesion assays, BAEC were replicate-plated on 15-mm round Thermox plastic coverslips or 12-mm round glass coverslips in Dulbecco's Modified Eagle's medium (DME) supplemented with 10% calf sera and allowed to grow to a stable confluent density. The intactness of each endothelial monolayer was monitored by phase-contrast microscopy before use and confirmed by scanning electron microscopy of representative coverslips at the end of experimental incubations. In certain studies, confluent monolayers of primary human umbilical vein endothelial cells³¹ were examined in parallel with BAEC monolayers.

Determination of 6-Keto-PGF₁α

To document inhibition of prostacyclin production in endothelial monolayers, 6-keto-PGF₆ levels were determined in culture media using a modification of published techniques.³² Briefly, purified 6-keto-PGF₆ standard (Upjohn) was dissolved in acetonitrile and then coupled to free-base histamine in the presence of 1-ethyl-3(3-dimethylaminopropyl carbodiimide). A small aliquot of this conjugate was iodinated (¹²⁵I) using the chloramine-T procedure.³³ Equivolumes of tracer, standard (or test sample), and specific antisera were incubated together for 2 hr at 22°C. The bound and free moieties were then separated using a double-antibody technique.³⁴ The bound fraction was then counted in a Beckman 7000 gamma counter and the 6-keto-PGF₁α concentrations were calculated by a computer-optimized Scatchard analysis.³⁵ The assay sensitivity was 30 pg/ml, with an operating range of 30–2,000 pg/ml. The antibody, obtained from Seragen (Dorchester, MA), cross-reacted with PGF₁α, PGE₂, PGD₂, PGA₂, and TxB₂ at 4.5%, 2.1%, 0.3%, <0.1%, and <0.1%, respectively.

Leukocyte–Endothelial Cell Adhesion Assay

The quantitative monolayer adhesion assay used in this study was a modification of a procedure, previously described in this laboratory, for the study of platelet–endothelial interactions.²⁶,²⁷ Coverslips with confluent endothelial monolayers were removed from culture medium, rinsed in calcium-free HBSS, and then incubated in 750 µl of ¹H-adenine-labeled PMN suspension for 3 min at 37°C under static conditions in 16-mm plastic culture wells. After the incubation period, the coverslips were removed and again rinsed by immersion in a series of beakers containing HBSS²⁷ to remove nonadherent leukocytes. In preliminary experiments, we found that a series of 3 washes effectively removed all loosely attached leukocytes. The washed coverslips were then placed in 10 ml of Ultrafluor, and tritium activity was determined in a Beckman liquid scintillation counter. In experiments with ¹¹In-labeled PMN, washed coverslips were counted in a Beckman gamma counter.

In each experiment, representative coverslips were stained with a Wright-Giemsa stain and examined by light microscopy. In certain experiments, monolayers were also fixed in 3% glutaraldehyde (in 0.1 M sodium cacodylate, 0.2 mM calcium chloride, pH 7.35), dehydrated in a series of graded ethanol, gold-platinum sputter-coated, and examined in an AMR-1000A scanning electron microscope, as previously described.¹³
Experimental Design

Leukocyte suspensions and endothelial monolayers were treated with various concentrations of indomethacin, sodium salicylate, or ETYA prior to the adhesion assay. After 30-min preincubation, the cells were washed and then incubated together (as described above) in the presence of the same concentration of the inhibitor. In the aspirin experiments, either the leukocyte suspension and/or the endothelial monolayer were pretreated with 100 μM aspirin for 30 min, washed, and then allowed to interact in the absence of aspirin during the adhesion assay. In most experiments with exogenous leukocyte suspension and/or endothelial monolayers were pretreated with 100 μM aspirin for 30 min, washed, and then allowed to interact in the absence of aspirin during the adhesion assay. In most experiments with exogenous leukocyte suspension and/or endothelial monolayers were selectively pretreated with 100 μM aspirin for 30 min prior to the adhesion assay. This concentration of aspirin essentially completely blocked PGI2 production by BAEC monolayers for the duration of the assay, as assessed by 6-keto-PGF1α radioimmunoassay (data not shown), and presumably had a similar effect on the production of PGE2 and other cyclooxygenase-derived metabolites by the PMN.18 As seen in Table 1, inhibition of cyclooxygenase activity by aspirin pretreatment, in either or both cell types, had little, if any, influence on basal PMN–endothelial adhesion. Preincubation of both leukocytes and endothelial cells for 30 min with PGI2, over a broad concentration range, also did not significantly alter their adhesive interaction (Table 2A). Furthermore, addition of 0.25 μM PGI2 to PMN suspensions 5 min before, or immediately before, their use in adhesion assays did not significantly alter their interaction with bovine, or human, endothelial monolayers (Table 2B). Finally, the combination of aspirin pretreatment (both PMN suspensions and bovine endothelial monolayers) and addition of PGI2 (10^{-9} - 10^{-7} M) to the assay did not significantly reduce PMN–endothelial adhesion (data not shown).

Results similar to those seen with 100 μM aspirin treatment were also obtained when both cell types were incubated with relatively low concentrations of indomethacin. Thus, 1 μM and 5 μM indomethacin (concentrations that inhibited PGI2 production in BAEC monolayers >60% and >95%, respectively, as assessed by 6-keto-PGF1α radioimmunoassay) had no effect on

| Table 1. Effect of Aspirin Treatment on Leukocyte–Endothelial Adhesion

<table>
<thead>
<tr>
<th>Leukocyte Suspensions</th>
<th>Endothelial Monolayers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>Untreated</td>
<td>100% ± 7%</td>
</tr>
<tr>
<td>Treated</td>
<td>78% ± 6%</td>
</tr>
<tr>
<td>Treated</td>
<td>89% ± 13%</td>
</tr>
</tbody>
</table>

*The leukocyte suspensions and/or endothelial monolayers were selectively pretreated with 100 μM aspirin for 30 min prior to the adhesion assay, as described in text.
†Data are expressed as percent of control values for untreated leukocytes interacting with untreated endothelial monolayers (4,376 ± 332 PMN/sq mm BAEC monolayer surface = 100% ± 7%; mean ± SEM, n = 8).
Table 2. Effect of Exogenous PGI₂ on Leukocyte-Endothelial Adhesion

(A) Preincubation of Both Cell Types with PGI₂* 

<table>
<thead>
<tr>
<th>PGI₂ Added (µM)†</th>
<th>Percent Control Adhesion‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 ± 13 (6)</td>
</tr>
<tr>
<td>0.001</td>
<td>95 ± 13 (2)</td>
</tr>
<tr>
<td>0.01</td>
<td>97 ± 19 (4)</td>
</tr>
<tr>
<td>0.1</td>
<td>91 ± 16 (4)</td>
</tr>
<tr>
<td>1.0</td>
<td>99 ± 20 (6)</td>
</tr>
</tbody>
</table>

(B) Addition of PGI₂ to PMN Suspensions§

<table>
<thead>
<tr>
<th>Endothelial Monolayer</th>
<th>PGI₂ Treatment</th>
<th>Percent Control Adhesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine aorta</td>
<td>None</td>
<td>100 ± 13</td>
</tr>
<tr>
<td></td>
<td>t₀ addition</td>
<td>96 ± 29</td>
</tr>
<tr>
<td></td>
<td>5-min preincubation</td>
<td>121 ± 29</td>
</tr>
<tr>
<td>Human umbilical vein</td>
<td>None</td>
<td>100 ± 8</td>
</tr>
<tr>
<td></td>
<td>5-min preincubation</td>
<td>98 ± 4</td>
</tr>
</tbody>
</table>

*Both leukocytes and bovine aortic endothelial monolayers were preincubated with the indicated concentrations of PGI₂ for 30 min, washed, and the same concentration of PGI₂ added to the assay buffer.
†The potency of PGI₂ stock solutions was assessed by inhibition of platelet aggregation prior to their use in these adhesion assays.
‡Data are expressed as percent of control values for untreated leukocytes and bovine aortic endothelial cell monolayers obtained in 6 separate experiments (mean ± SEM); 3H-adenine-labeled PMN suspensions, 30-min adhesion assay. The number of separate experiments performed at each PGI₂ concentration are indicated in parentheses; in each experiment, determinations were performed in triplicate and averaged.
§Washed PMN suspensions, labeled with ¹¹¹In, were treated with 0.25 µM PGI₂ 5 min before, or immediately before (t₀), incubation with confluent monolayers of bovine aortic endothelial cells (BAEC) or human umbilical vein endothelial cells (HUVEC) in 10-min adhesion assays; data expressed as in part A.

basal PMN adhesion (Fig. 1). In contrast, higher concentrations (10–1,000 µM) of indomethacin, which, among other actions, can block the release of arachidonic acid from cellular phospholipid stores and its metabolism via lipoxygenase pathways, significantly inhibited PMN adhesion in a dose-related manner (p < 0.001 versus control for 10 µM and higher concentrations) (Fig. 1).

To determine the influence of endogenous non-cyclooxygenase-derived arachidonic acid metabolites on PMN adhesion, leukocyte suspensions and endothelial monolayers were preincubated for 30 min in various concentrations of either the relatively nonselective cyclooxygenase/lipoxygenase inhibitor, 5,8,11,14-ETYA, or the more selective lipoxygenase inhibitor, 4,7,10,13-ETYA. Each compound was also present at the same concentration during the adhesion assay. Both inhibitors decreased PMN adhesion in a dose-related manner (Fig. 2). However, 4,7,10,13-ETYA was approximately 1,000-fold more potent than 5,8,11,14-ETYA (threshold concentrations <0.1 and >100 µM, respectively; half-maximal inhibitory concentrations <1.0 µM and >1,000 µM, respectively) and gave essentially complete inhibition.

To compare the effect of a non-fatty-acid inhibitor of arachidonate metabolism, leukocyte suspensions and endothelial monolayers were treated with increasing concentrations of sodium salicylate. When used at millimolar concentrations, sodium salicylate has been shown to inhibit peroxidase activity and the subsequent conversion of hydroperoxy-eicosatetraenoic acid (HPETE) to hydroxy-eicosatetraenoic acid (HETE). As seen in Fig. 3, sodium salicylate effectively inhibited basal PMN-endothelial adhesion, in a dose-related manner, at concentrations ≥0.5 mM (p < 0.005).

DISCUSSION

The adherence of polymorphonuclear leukocytes to the vascular endothelial lining appears to be a necessary antecedent to their migration into the extracellular space. This cell–cell interaction occurs under physiologic conditions, is markedly enhanced during inflammatory reactions, and can be inhibited by vari-
ous antiinflammatory drugs.\textsuperscript{1,4,8} Furthermore, leukocyte adhesion to the endothelial lining typically is well demarcated, suggesting that local, rather than systemic, factors are involved.\textsuperscript{1,8} While the molecular mechanisms responsible for PMN recognition and binding by endothelial cell surfaces remain unclear,\textsuperscript{5,25} the data presented here suggest that non-cyclooxygenase-derived products of arachidonate may play a regulatory role in this process.

The effects of various exogenous agents, including the cyclooxygenase products PGE\textsubscript{2} and PGI\textsubscript{2}, on PMN adhesiveness have been evaluated previously in different in vitro systems,\textsuperscript{25,38-41} using both artificial surfaces (e.g., nylon fibers) and endothelial cell monolayers; however, the results have been highly variable. For example, exogenous PGI\textsubscript{2} was found to inhibit IgG-stimulated PMN adhesion to dialysis membranes,\textsuperscript{39} while in other studies, PGE\textsubscript{2} and PGI\textsubscript{2} appeared to enhance PMN adhesion to cultured endothelial cells.\textsuperscript{25} In yet other studies, the inhibitory effect of PGI\textsubscript{2} on PMN adhesion to nylon wool and endothelial cells was observed to be relatively short-lived.\textsuperscript{41} This lack of agreement may reflect the fact that the potential effects of exogenous prostaglandins on both PMN and endothelial cell function are multiple and complexly interrelated. For example, in addition to its direct effects on leukocyte function, PGI\textsubscript{2} can increase adenylate cyclase activity in cultured endothelial cells\textsuperscript{42} and consequently decrease their endogenous arachidonate metabolism.\textsuperscript{43} It has also been suggested that cyclic AMP released by stimulated endothelial cells can influence PMN reactivity.\textsuperscript{11} The situation is further complicated by the tendency for platelets to be present in significant numbers in standard PMN leukocyte preparations (often \textgtr 1-3 platelets/leukocyte).\textsuperscript{29} As recent studies indicate, platelet-derived thromboxane-A\textsubscript{2} can mediate increased PMN adhesiveness\textsuperscript{44}, thus, in leukocyte preparations where there are significant numbers of contaminating platelets,
inhibition of platelet activation by PGI₂ might result secondarily in decreased PMN adhesion. In the in vitro adhesion assays reported here, platelet contamination was monitored microscopically (as well as by radioimmunoassay of beta-thromboglobulin, a platelet-specific protein, data not shown) and typically was less than 1% (i.e., <1 platelet/100 leukocytes). Addition of exogenous PGI₂ to this system did not alter basal PMN–endothelial adhesion (Table 2). This lack of effect of added PGI₂ was observed over a broad concentration range (10⁻⁸–10⁻⁶ M), with and without preincubation, using both human and bovine endothelial cells. Furthermore, aspirin and indomethacin (at concentrations that significantly inhibited endothelial prostacyclin production, as assessed by 6-keto-PGF₁α, radioimmunoassay) had no appreciable effect on PMN–endothelial adhesion. We interpret these observations as suggesting that cyclooxygenase-derived arachidonic metabolites are not significantly involved in regulating PMN–endothelial cell adhesion, under unstimulated conditions, in our in vitro model system.

Lipoxygenase-derived arachidonic metabolites have been shown to influence a broad range of PMN functions. In particular, leukotriene B₄, a potent chemotactic agent, has been shown to increase PMN adhesion to nylon fibers in vitro, and to reversibly augment PMN margination in postcapillary venules in vivo. In our in vitro assay system, pretreatment of neutrophils with 4,7,10,13-ETYA resulted in a marked inhibition of basal PMN–endothelial cell adhesion, in striking contrast to 5,8,11,14-ETYA, a less selective lipoxygenase inhibitor. Furthermore, both sodium salicylate and indomethacin, which at high concentrations can block peroxidation of HPETE to HETE, also inhibited PMN–endothelial cell adhesion in a dose-related manner. It must be noted that none of these inhibitors are sufficiently selective in their mechanism of action to directly implicate the involvement of a lipoxygenase product. However, given the lack of effect of cyclooxygenase inhibitors and the dose-related patterns of inhibition obtained with 4,7,10,13-ETYA versus 5,8,11,14-ETYA and high versus low dose indomethacin treatment, we interpret these data as suggesting that endogenous lipoxygenase-derived arachidonic metabolites may be involved in the regulation of PMN adhesion to endothelial cell surfaces in our in vitro model system.

The identity and cellular origin(s) of the putative regulatory substances in this in vitro model system are not yet clear. Although the production of lipoxygenase products by various blood cells, including platelets, PMN, and monocytes, has been extensively studied, the existence and precise end products of these pathways in vascular cells, especially endothelium, have not yet been explored in detail. Given the complex nature of the regulation of arachidonic metabolism in intact cell systems, the potential for cell–cell metabolic interactions, and the current lack of highly selective irreversible lipoxygenase pathway inhibitors, this problem clearly will require further study. The in vitro model system described in this report should be useful for evaluating the direct effects of various purified lipoxygenase products on PMN–endothelial adhesion, as well as for probing the molecular mechanisms of this cell–cell interaction.

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REFERENCES

13. Buchanan MR, Crowley CA, Rosin RE, Gimbrone MA Jr,
39. Chuang HYK, Mohammad SF, Mason RG: Prostacyclin (PGI2) inhibits the enhancement of granulocyte adhesion to cuprophane induced by immunoglobulin G. Thromb Res 19:1, 1980
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