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*J Immunol* 1998; 161:3064-3070; ;
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Triple Role of Platelet-Activating Factor in Eosinophil Migration Across Monolayers of Lung Epithelial Cells: Eosinophil Chemoattractant and Priming Agent and Epithelial Cell Activator

Lixin Liu,* Åstrid E. M. Zuuriber,* Frederik P. J. Mul,* Arthur J. Verhoeven,* René Lutter,† Edward F. Knol,* and Dirk Roos 2*

Infiltration of eosinophils into the lung lumen is a hallmark of allergic asthmatic inflammation. To reach the lung lumen, eosinophils must migrate across the vascular endothelium, through the interstitial matrix, and across the lung epithelium. The regulation of this process is obscure. In this study, we investigated the migration of human eosinophils across confluent monolayers of either human lung H292 epithelial cells or primary human bronchial epithelial cells. Established eosinophil chemoattractants (IL-8, RANTES, platelet-activating factor (PAF), leukotriene B4, and complement fragment 5a (C5a)) or activation of the epithelial cells with IL-1β induced little eosinophil transmigration (<7% in 2 h). In contrast, addition of PAF in combination with C5a induced extensive (>20%) transepithelial migration of unprimed and IL-5-primed eosinophils. Eosinophil migration assessed in a Boyden chamber assay, i.e., without an epithelial monolayer, was only slightly increased upon addition of PAF and C5a. Preincubation of eosinophils with the PAF receptor antagonist WEB 2086 only inhibited migration of unprimed eosinophils toward PAF and C5a, whereas preincubation of epithelial cells with WEB 2086 abolished migration of both IL-5-primed and unprimed eosinophils. This latter result indicated the presence of PAF receptors on epithelial cells. Indeed, addition of PAF to epithelial cells induced an increase in cytosolic free Ca2+, which was blocked by the PAF receptor antagonists WEB 2086 and TCV-309. Our results show that PAF induces permissive changes in epithelial cells, and that PAF acts as a chemoattractant and priming agent for the eosinophils. The Journal of Immunology, 1998, 161: 3064–3070.

Allergic asthma is characterized by massive infiltration of eosinophils into the lung. Eosinophils are considered to play an important role in the pathogenesis of allergic asthma (1, 2). After infiltration of the allergic reaction sites, eosinophils release toxic granule proteins, causing tissue damage, and generate lipid mediators that can influence the behavior of surrounding vasculature and airway smooth muscle cells. To reach the airway lumen, circulating eosinophils must first extravasate; i.e., the cells initially roll on the endothelium, followed by firm adhesion to and migration across endothelial cells (1, 3). Thereafter, the eosinophils migrate through the interstitial matrix and across the epithelium into the lung lumen (1, 4, 5). The migration to the sites of inflammation is presumed to be regulated at the levels of 1) adhesion receptors that mediate transient or firm adhesion to inflamed vascular endothelium and to extracellular matrix molecules, 2) activating factors (cytokines and chemokines) that induce migration and expression of adhesion molecules and their ligands, and 3) cells that are present at the inflammatory site and regulate the release of these activating factors.

The first step in the migration process is the interaction of circulating eosinophils with the vascular endothelium at the allergic reaction site. Endothelial cells actively regulate leukocyte infiltration in inflammatory tissues through different mechanisms, such as vasodilatation, expression of adhesion molecules, opening of intercellular junctions, and secretion of chemotactic factors (6). One can imagine that selective up-regulation and/or activation of adhesion molecules on eosinophils and endothelial cells by cytokines and the production of chemokines and/or chemoattractants may promote specific eosinophil migration and their subsequent accumulation in tissues. However, migration studies have shown that the so-called allergy-related cytokines or chemokines are not selective in attracting eosinophils (3, 7–9). These findings imply that the selective activation and attraction of eosinophils toward sites of allergic inflammation are the results of a complex interplay among different cell types, mediators, and adhesion molecules.

Recently, attention has focused on the role of the epithelium in leukocyte infiltration into inflammatory sites (10–13). Besides the barrier function for protection against pathogen invasion, epithelial cells play an active role in the induction of inflammation through the expression of cellular adhesion molecules, such as ICAM-1 (14–16) and integrins (17). Another role of epithelial cells in lung inflammation involves the synthesis of a variety of proinflammatory mediators, including RANTES, PAF,3 and IL-8 after IL-1β or...
TNF-α stimulation (18), and granulocyte-macrophage CSF after stimulation with eosinophil peroxidase (19).

Whereas eosinophil migration across endothelial monolayers has been described extensively (1, 3, 6, 8, 9), little is known about the mechanisms regulating eosinophil migration across epithelial monolayers. In the present study, we investigated human eosinophil migration across confluent monolayers of lung epithelial cell line cells (H292) and primary human bronchial epithelial cells (HBEC) in vitro. Substantial eosinophil transmigration was observed with combinations of PAF and C5a, or PAF and LTB4, but not with these chemooattractants alone. Besides the conventional potent chemotactic role of PAF and the priming effect of PAF on eosinophils, we also found that a permissive change in the epithelial monolayers induced by PAF is pivotal for efficient eosinophil transepithelial migration.

Materials and Methods

Reagents

Recombinant human (rh) TNF-α was a gift from Dr. A. Creasy (Cetus, Oakland, CA). PAF, C5a, FMLP, and LTB4 were purchased from Sigma (St. Louis, MO). rhRANTES was purchased from Life Technologies (Gaithersburg, MD). The PAF receptor antagonist WEB 2086 was a gift from Dr. H. Heuer (Boehringer Ingelheim, Ingelheim, Germany), and the PAF receptor antagonist TCV-309 was purchased from Takeda (Osaka, Japan) (20). RhIL-1β and rhIL-8 were purchased from Pepro Tech (Rocky Hill, NJ), and rhIL-8 and rhIFN-γ were obtained from Boehringer Mannheim (Mannheim, Germany). WEB 2086 was dissolved in DMSO at 1000 times the final concentration and was stored at −20°C. C5a, PAF, FMLP, RANTES, LTB4, IL-1β, IL-5, IL-8, and IFN-γ were dissolved in PBS supplemented with 0.5% (w/v) human serum albumin (HSA; Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) and were stored at −20°C. HEPES medium contained 132 mM NaCl, 6.0 mM KCl, 1.0 mM CaCl2, 1.0 mM MgSO4, 1.2 mM KH2PO4, 20 mM HEPES, 5.5 mM glucose, and 0.5% (w/v) HSA. The activity of trypsin was inhibited immediately after the confluence of the monolayers was confirmed by 3H]inulin (Amersham, Aylesbury, U.K.) leakage experiments (24).

Granulocyte isolation

Blood was obtained from healthy volunteers. Granulocytes were purified from a buffy coat of 500 ml of blood by density gradient centrifugation on Histopaque Percoll (Pharmacia, Uppsala, Sweden) (25). After lysis of the erythrocytes in the pellet fraction with a cold lysis buffer containing 155 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA (pH 7.4), the granulocytes were washed twice in PBS and resuspended in HEPES medium without CaCl2.

Eosinophil purification

Human eosinophils were purified by means of the FMLP method (26). In brief, granulocytes in HEPES medium without CaCl2 were incubated for 30 min at 37°C to restore the initial density of the cells. The cells were washed, resuspended in PBS supplemented with 0.5% (w/v) HSA and 13 mM trisodium citrate, and incubated for 5 min in a shaking water bath at 37°C. The incubation was continued for 10 min after the addition of 10 nM FMLP to the cell suspension. Thereafter, the eosinophils were purified by centrifugation (15 min, 1000 × g) over isotonic Percoll (1.082 g/ml, pH 7.4), washed, and resuspended in HEPES medium. The purity and viability of the eosinophils were >95%. Contaminating cells were mostly neutrophils. In some experiments, eosinophils were isolated by means of immunomagnetic removal of CD16-expressing cells (27).

Eosinophil transmigration

Fresh medium was added to the transwells 4 h before the start of the transmigration assay, and the transwells were washed twice just before starting the experiment. The lower compartment was filled with prewarmed incubation medium with or without chemotactants. Eosinophils (5 × 106 cells in 0.5 ml of prewarmed incubation medium) were placed in the upper compartment, and the transwells were incubated for 2 h at 37°C with 5% CO2 and maximal humidity. Whenever indicated, eosinophils and/or epithelial cells were preincubated with 10 μM WEB 2086 for 5 min before the start of the transmigration assay. When epithelial cells were treated with WEB 2086, WEB 2086 was added to the lower compartment. WEB 2086 remained present throughout the experiment. After the 2-h incubation, the upper and lower compartments were washed separately with incubation medium and ELISA sample buffer, respectively, and the fluids were collected. The cells in the collected fluids and in the excised membranes were lysed in ELISA sample buffer. The percentage of eosinophils that had transmigrated was determined by means of an ELISA for eosinophil cationic protein (ECP).

ECP quantification

The amount of ECP as a measure for the number of eosinophils in different cell preparations was determined by means of a slightly modified, previously described ELISA (28). In brief, specific polyclonal rabbit antiserum (poAb) against human ECP was obtained by immunization of a rabbit with highly purified human ECP (29). Human ECP was a gift from Prof. I. Olsson (Lund, Sweden). The Ig fraction of the rabbit serum was isolated by ammonium sulfate precipitation and was biotinylated (30).

Culture plates with 96 wells (Maxisorb, Nunc, Roskilde, Denmark) were coated overnight at 4°C with 100 μl of 3 μg/ml rabbit anti-ECP poAb diluted in 0.1 mM NaHCO3. After each incubation step, the plates were washed three times with PBS containing 0.1% (v/v) BSA and Tween-20. The wells were blocked with blocking buffer, consisting of PBS supplemented with 0.2% (w/v) BSA and 0.1% (v/v) Tween-20, for 1 h at 37°C. Highly purified ECP and samples were diluted in ELISA sample buffer, added at a volume of 100 μl/well, and incubated for 2 h at 37°C. The wells were subsequently incubated with 100 μl of 1 μg/ml biotin-conjugated anti-ECP poAb diluted in block buffer for 90 min at 37°C. The biotin-labeled ECP was allowed to bind avidin-biotinylated alkaline phosphatase conjugate (Dako, Glostrup, Denmark) diluted in Tris-buffered saline supplemented with 0.1% (v/v) Tween-20 and 0.2% (w/v) BSA for 30 min at room temperature according to the manufacturer’s description. Enzymatic activity was detected with 1 mg/ml phosphate substrate (Sigma) 104 dissolved in 1 M diethanolamine, 0.5 mM MgCl2, and 0.02% NaN3 (pH 9.8). The absorbance was measured after 60 min in a Multiscan Multisoft microplate reader (Labsystems Oy, Helsinki, Finland) at 405 nm. The sensitivity of this assay ranged from 0.1 to 10 ng/ml of ECP. We confirmed that this ECP ELISA assay was highly specific for eosinophils, i.e., no reaction was measured in lysates of human monocytes, lymphocytes, or neutrophils. In addition, eosinophil migration quantified by means of the ECP ELISA and that determined by cell counting in the lower compartment were comparable. The total amount of ECP added to the transwell system as well as that in each compartment separately (upper compartment, lower compartment, and membrane) were determined. The percentage of

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recovery was always > 85%. The percentage of eosinophils that had transmigrated was calculated from the amount of ECP detected in the lower compartment in relation to the total amount of added ECP. Alternatively, transepithelial migration of calcine-AM (Molecular Probes, Eugene, OR)-labeled eosinophils was measured by both ECP detection and calcine quantification.

**Calcein labeling**

Eosinophils were labeled with calcine-AM before the onset of the transmigration assay. The cells (10 × 10^6/ml) were labeled with 4 μg/ml calcine-AM diluted in HEPES medium for 45 min at 37°C. After labeling, the cells were washed three times with HEPES medium. The transmigration assay with calcine-AM-labeled eosinophils was performed as described above, except that HEPES medium was used instead of incubation medium. The concentrations of calcine-AM in the upper compartment, lower compartment, and membrane were measured with a spectrofluorometer (model RF-540, Shimadzu, Kyoto, Japan). The percentage of eosinophils that had transmigrated was calculated from the amount of fluorescence detected in the lower compartment in relation to the fluorescence of the total added calcine-AM-labeled eosinophils.

**Chemotaxis assay**

Chemotaxis in Boyden chambers was measured with a computerized image analyzer (Quantimet 600, Leica, Cambridge, U.K.) to quantify eosinophil migration into filters after an incubation period of 1 h (31).

**Intracellular free Ca^{2+} concentration ([Ca^{2+}]_i) measurement**

For the [Ca^{2+}]_i measurement, cells (6–10 × 10^6/ml in HEPES medium) in suspension were loaded with 1 μM indo-1/AM (Molecular Probes) for 40 min at 37°C (32). The cells were washed, resuspended in HEPES medium to the previous concentration, and kept in the dark at room temperature. Before being transferred to a cuvette, the indo-1/AM-loaded cells were diluted 10 times in HEPES medium and were prewarmed for 5 min at 37°C. Fluorescence changes in the magenta in the Indo-1 fluorescence as a function of [Ca^{2+}]_i, all trapped Indo-1 was saturated with Ca^{2+} by addition of 10 μM digitonin, after which Indo-1 fluorescence was quenched with 0.5 mM MnCl_2. A dissociation constant of 250 nM for the Indo-1-Ca^{2+} complex was used to calculate [Ca^{2+}]_i (33).

**Statistical analysis**

Results were expressed as the mean ± SEM of the number of different experiments mentioned in the figure and table legends. Results were analyzed using Student’s t test. One-sided p values were calculated, and p values exceeding 0.05 were considered not significant.

**Results**

**Low eosinophil transepithelial migration induced by individual chemoattractants**

Human eosinophil migration across confluent monolayers of lung epithelial H292 cell line cells was analyzed in the physiologic basolateral to apical direction (with the epithelial cells growing underneath a filter membrane). Eosinophil transepithelial migration reached its plateau after 2 h of incubation (our unpublished observations). The monolayers remained intact during the transmigration assay, as checked by examination of the filter by light microscopy and by determination of [3H]insulin diffusion through the monolayer before and after the transmigration assay.

Individual chemoattractants (C5a, PAF, IL-8, RANTES, or LTB_4) induced little transmigration of IL-5-primed eosinophils, i.e., maximally 7% (Table I). Pretreatment of the epithelial monolayers with 5 ng/ml IL-1β for 4 h (Table I) or with TNF-α and/or IFN-γ for 4 or 24 h (not shown) did not induce this migration either. In these studies eosinophils that were isolated by means of removal of CD16-expressing cells showed a similar response. Moreover, quantification of eosinophil migration by means of ECP measurement yielded results similar to those obtained by calcine fluorescence measurement.

**Table I. Eosinophil transepithelial migration induced by individual chemoattractants**

<table>
<thead>
<tr>
<th>Chemoattractants</th>
<th>Migration (% ECP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>0.40 ± 0.16 (4)</td>
</tr>
<tr>
<td>C5a (10^{-8} M)</td>
<td>4.69 ± 1.19 (4)*</td>
</tr>
<tr>
<td>PAF (10^{-6} M)</td>
<td>6.48 ± 0.82 (4)**</td>
</tr>
<tr>
<td>IL-8 (10^{-8} M)</td>
<td>0.61 ± 0.14 (3)</td>
</tr>
<tr>
<td>RANTES (50 ng/ml)</td>
<td>0.50 ± 0.32 (4)*</td>
</tr>
<tr>
<td>LTB_4 (10^{-6} M)</td>
<td>0.60 ± 0.14 (3)</td>
</tr>
<tr>
<td>IL-1β (4 h) (5 ng/ml)</td>
<td>0.46 ± 0.09 (6)</td>
</tr>
</tbody>
</table>

*a Chemoattractants were added to the lower compartment of the transwell system, except for IL-1β, which was used for pretreatment of the epithelial cells for 4 h. The eosinophils were incubated with IL-5 (10^{-5} M) for 30 min at 37°C before addition to the upper compartment of the transwell system. Values are given in percentage of input ECP measured in the lower compartment (mean ± SEM; numbers in parentheses = number of experiments). Asterisks indicate significance of difference with medium value (* p < 0.05; ** p < 0.01).

**Enhancement of eosinophil transmigration by PAF**

In general, we observed that neither individual chemoattractants nor most combinations of chemoattractants induced >10% of eosinophil transepithelial migration (Tables I and II). Only when PAF was combined with C5a, LTB_4, or RANTES did higher percentages of eosinophils migrate across the monolayers (Table II). The enhanced migration toward PAF/C5a (up to 25%) was not only observed with IL-5-primed eosinophils but also with unprimed cells (Fig. 1). In addition, PAF/C5a enhanced the migration of IL-5-primed eosinophils across primary HBEC (C5a, 6.5%; PAF, 9.5%; C5a/PAF, 34.6%). This synergistic response was not observed when C5a was combined with LTB_4 (Table II); thus, the enhancement must be due to the effect of PAF. Both PAF and C5a play a chemotactic role in these transmigration assays, because the addition of either of these agents to the upper compartment just before starting the migration assay resulted in a dose-dependent inhibition of eosinophil transmigration toward PAF/C5a in the lower compartment (not shown). PAF did not induce damage to the epithelial cell layers, as measured by light microscopy and determination of [3H]insulin diffusion through the monolayer.

**Eosinophil chemotaxis in a modified Boyden chamber assay**

To investigate the effect of PAF on eosinophil migration toward C5a in the absence of epithelial cells, chemotaxis of eosinophils

**Table II. Eosinophil transepithelial migration induced by combinations of chemoattractants**

<table>
<thead>
<tr>
<th>Chemoattractants</th>
<th>Migration (% ECP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>0.38 ± 0.06 (13)</td>
</tr>
<tr>
<td>PAF + C5a</td>
<td>24.85 ± 3.84 (5)*</td>
</tr>
<tr>
<td>PAF + LTB_4</td>
<td>14.49 ± 4.45 (3)*</td>
</tr>
<tr>
<td>PAF + IL-1β (4 h)</td>
<td>2.32 ± 0.50 (3)</td>
</tr>
<tr>
<td>PAF + IL-8</td>
<td>6.18 ± 2.17 (4)</td>
</tr>
<tr>
<td>PAF + RANTES</td>
<td>12.46 ± 2.33 (4)*</td>
</tr>
<tr>
<td>RANTES + IL-8</td>
<td>1.10 ± 0.40 (3)</td>
</tr>
<tr>
<td>C5a + LTB_4</td>
<td>8.28 ± 2.61 (3)</td>
</tr>
<tr>
<td>C5a + IL-1β (4 h)</td>
<td>2.30 ± 1.38 (3)</td>
</tr>
</tbody>
</table>

*a For chemoattractants and eosinophil treatment, see Table I. Values are given in percentage of input ECP measured in the lower compartment of the transwell system (mean ± SEM; numbers in parentheses = number of experiments). The chemoattractants were added to the lower compartment of the transwell system. Asterisks indicate significance of difference between eosinophil transmigration in response to a combination of chemoattractants in comparison to the sum of the responses to the individual chemoattractants (Table I); * p < 0.05; ** p < 0.01.
toward PAF, C5a, or combinations was assessed. The total distance migrated by eosinophils in filters was determined in a Boyden chamber assay. Addition of PAF or C5a to the lower compartment as well as IL-5 priming of eosinophils were found to enhance migration, but these three factors together appeared to render the strongest migration stimulus (Fig. 2).

**Effect of WEB 2086 on eosinophil transepithelial migration toward PAF and C5a**

The role of PAF in inducing eosinophil migration across epithelial monolayers toward C5a was further analyzed with the PAF receptor antagonist WEB 2086. When eosinophils were treated with WEB 2086, transmigration of unprimed eosinophils was inhibited, whereas transmigration of IL-5-primed cells remained unaffected, even when higher concentrations (up to 25 μM) of WEB 2086 were used (Fig. 3A). In contrast, the transmigration of both IL-5-primed and unprimed eosinophils was impaired when the epithelial monolayers were treated with WEB 2086 (Fig. 3A). WEB 2086 treatment of both eosinophils and epithelial cells did not yield more inhibition than WEB 2086 treatment of the epithelial cells alone. Eosinophil migration across monolayers of primary HBEC was comparably affected by WEB 2086 (Fig. 3B).

**Increase in cytosolic free Ca^{2+} concentration in epithelial cells induced by PAF**

The inhibitory effect of WEB 2086 treatment of epithelial cells on transmigration of eosinophils indicated the existence of PAF receptors on lung epithelial cells. To investigate the response of epithelial cells to PAF binding, we measured the change in the intracellular free Ca^{2+} concentration in epithelial cells. Both H292 epithelial cells and primary HBEC showed a rapid [Ca^{2+}]_{i} increase upon addition of 10^{-6} M PAF (Fig. 4), and this response was completely abolished by pretreatment of the cells with two distinct PAF receptor antagonists, i.e., 10 μM WEB 2086 (Fig. 4) and 0.1 μM TCV-309 (not shown). Addition of C5a (10^{-8} M), IL-8 (10^{-8} M), FMLP (10^{-7} M), WEB 2086 (10 or 25 μM), or TCV-309 (0.1 μM) to epithelial cells did not cause a Ca^{2+} response (not shown).
Thus, we found no indication for ECP release during eosinophil migration or selective migration of ECP-rich or ECP-poor eosinophils.

Chemotactic and priming role of PAF

Transepithelial migration of primed eosinophils was low. Even PAF, the most potent chemoattractant, attracted <7% of the eosinophils. Resnick et al. (39) also observed low eosinophil migration across human intestinal epithelium toward individual chemoattractants, but, in contrast, relatively high migration toward PAF. Apparently, PAF induces more eosinophil migration across intestinal epithelium than across bronchial epithelium.

We show that eosinophil migration across bronchial epithelium toward C5a, LTβ, or RANTES is synergistically enhanced by PAF. This stimulatory effect was specific for PAF, but did not occur when PAF was combined with other chemoattractants. On the basis of these results, we predict that in human eosinophils, PAF activates a signaling pathway that differs from those activated by C5a, LTβ, and RANTES.

Eosinophils need to be primed (e.g., by IL-5) for induction of migration across epithelial cells; i.e., unprimed eosinophils hardly migrated across epithelial monolayers. However, massive migration of unprimed eosinophils across epithelial monolayers was induced by PAF/C5a, and this migration could be inhibited by treatment of the eosinophils with the PAF receptor antagonist WEB 2086. In contrast, treatment of IL-5-primed eosinophils with WEB 2086 did not inhibit transepithelial migration toward PAF/C5a. This reveals the priming role of PAF that had diffused from the lower compartment to unprimed eosinophils in the upper compartment. In addition, PAF and C5a both induce chemotaxis of eosinophils in a Boyden chamber assay. Thus, C5a is a chemoattractant for eosinophils, and PAF is not only a chemoattractant but also a primer for eosinophils.

Epithelium-activating role of PAF

Our results indicate that the synergistic transepithelial migration of eosinophils toward C5a/PAF is partly due to the direct action of PAF on the epithelial cells. We found that the synergistic enhancement by PAF was not observed when the eosinophils migrated in the absence of epithelial cells. Moreover, treatment of the epithelial monolayer with the PAF receptor antagonist WEB 2086 strongly decreased the transepithelial migration toward C5a/PAF. Expression of PAF receptors in bronchial epithelial cells was confirmed by the finding that PAF induces a rapid increase in [Ca2+]i, in epithelial cells, a response that was prevented by pretreatment of epithelial cells with the PAF receptor antagonists WEB 2086 and TCV-309. PAF receptors have previously been identified in epithelial cells derived from the chinchilla middle ear (40); feline, canine, guinea pig, and cow trachea (41–44); and rabbit cornea (45). PAF induces an increase in [Ca2+]i, acts as a mucous secretagogue, and decreases the ciliary beat frequency of the tracheal epithelial cells (41–43, 46). Moreover, PAF receptors have been identified on human primary bronchial epithelial cells (47), and it has been shown that PAF induces up-regulation of the nuclear transcription factor activator protein-1 in these cells (47). Binding of PAF to the receptors in bronchial epithelial cells may cause transduction of signals leading to functional changes, such as the ability to permit eosinophil transmigration.

Together, these data strongly suggest that PAF induces permissive changes in epithelial cells that favor eosinophil transmigration. However, the exact nature of the epithelial changes that allow eosinophil transmigration remains to be elucidated. The role of PAF is currently under investigation. Preliminary results suggest that the morphology of bronchial epithelial cells is unaffected by
PAF, and that the monolayers do not become more “leaky” when PAF is present. Moreover, we found that ICAM-1 expression is not up-regulated by PAF (4-h incubation with PAF; unpublished observation). Thus, PAF-treated bronchial epithelial cells do not appear to be activated or morphologically changed. PAF possibly induces more subtle changes in the epithelial cells. One possible explanation might be that tight junction resistance is decreased as a result of the PAF-induced [Ca\textsuperscript{2+}]i elevation has been shown in human intestinal epithelial T84 cell line cells (48). Tight junction resistance in T84 cells is unaffected by PAF (39), because these epithelial cells do not express PAF receptors (49). However, inulin leakage experiments did not show increased permeability of the monolayers. Another possibility is that PAF induces changes in cortical actin, which affects cell-cell adhesion of epithelial cells and may result in augmented transmigration (50).

Differences between endothelial and epithelial cells

PAF did not induce an increase in [Ca\textsuperscript{2+}]i, in endothelial cells from umbilical veins (HUVEC), and PAF did not enhance eosinophil migration across HUVEC toward CSa (6, 51) (not shown). Thus, HUVEC probably lack a functional PAF receptor. However, endothelial cells are capable of PAF production after incubation with IL-1β or TNF-α (52, 53), in contrast to epithelial cells (23). These results confirm the importance of the stimulatory effects of PAF on eosinophil transepithelial migration. Moreover, this demonstrates that different mechanisms control eosinophil migration across the endothelium and epithelium.

PAF did not enhance eosinophil transepithelial migration toward IL-8. It is known that IL-8 is a very poor chemoattractant for endothelium and epithelium. It is known that different mechanisms control eosinophil transepithelial migration. Moreover, this demonstrates that different mechanisms control eosinophil migration across the endothelium and epithelium.

Conclusion

Together, our results show that human eosinophils migrate massively across lung epithelial monolayers in response to a chemotactic gradient of PAF in combination with a potent chemoattractant (CSa or LTβ3). In this process, PAF acts as a priming agent and as a chemoattractant for the eosinophils. In addition, PAF induces transmigration-permissive changes of the epithelial cells. Clarification of this epithelial activation may help in the development of drugs to prevent eosinophil migration into the lungs and concomitant damage to the epithelium.

Acknowledgments

We thank Dr. P. S. Hiemstra and S. van Wetering for providing the primary human bronchial epithelial cells, and Drs. Arne Egesten and Anton T. J. Tool for helpful discussions and technical assistance. We also thank Prof. Inge Olsson (Lund University, Lund, Sweden) for providing purified ECP.

References


