Augmentation of Tumor Metastasis by Platelet-activating Factor

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ABSTRACT

The effect of platelet-activating factor (PAF) on experimental pulmonary metastasis by the B16F10 murine melanoma and the possible involvement of PAF in the activities of tumor necrosis factor α (TNF-α) and interleukin 1α (IL-1α) in tumor metastasis were investigated. i.p. injection of PAF enhanced the lung colonization in a dose- and time-dependent manner. PAF enhanced lung colonization when it was administered after, but not before, B16F10 inoculation. Multiple injections of PAF were more effective than a single injection. Neutralization of endogenous PAF with PAF antagonist BN50739 decreased lung colonization, suggesting that endogenous PAF plays an important role in pulmonary metastases. A single i.p. injection of TNF-α or IL-1α caused a marked enhancement in lung colonization. TNF-α- and IL-1α-mediated enhancement in lung colonies was significantly inhibited by BN50739. These results demonstrate that PAF has a metastasis-enhancing effect and is a mediator of the metastatic activities of TNF-α and IL-1α.

INTRODUCTION

Tumor metastasis is a major cause of death in cancer patients and is one of the most important obstacles to successful treatment. The array of mediators involved in tumor metastasis remains to be precisely defined. It has been recently reported that proinflammatory cytokines, such as IL-1β and TNF-α, have tumor metastasis-enhancing capacities in various murine and human tumor models (1–6). Several in vitro studies (7–10) have established an interaction between proinflammatory cytokines and PAF. PAF is a membrane-bound phospholipid with a wide range of potent biological activities, including a variety of inflammatory reactions (11). TNF-α and IL-1 induce PAF synthesis from several cell types (7–9), and PAF, in turn, stimulates the production of these cytokines by monocytes and macrophages (10). Furthermore, recent studies have revealed that the in vivo action of TNF-α is not only related closely to biosynthesis of PAF but is blocked by the PAF antagonist (12). This strongly suggests that PAF mediates some of the biological properties of TNF-α. Therefore, PAF may mediate, at least in part, the biological actions of proinflammatory cytokines in tumor metastasis.

PAF is produced by a variety of inflammatory cells, such as neutrophils, basophils, eosinophils, monocytes and macrophages, and platelets (13–15), which are known to infiltrate tumors (16), as well as by tumor cells themselves under certain conditions (17). In addition, PAF activates platelets (15, 18), an event central to the mechanism of tumor invasiveness and dissemination (19). All of these findings further support the possible involvement of PAF in tumor metastasis.

In this study, we investigated whether PAF enhances experimental tumor metastasis and the role of PAF in the TNF-α- and IL-1α-induced increase in metastasis.

MATERIALS AND METHODS

Animals. Specific pathogen-free female C57BL/6 mice were purchased from the Korean Research Institute of Chemistry Technology (Daejeon, Chinnam, Korea) and were housed throughout the experiments in a laminar flow cabinet and maintained on standard laboratory chow ad libitum. All mice were used at 7–8 weeks of age.

Tumor Cell Line. The B16F10 mouse melanoma metastatic to the lungs of C57BL/6 mice, which was supplied originally by the Tumor Repository of the National Cancer Institute (Bethesda, MD) was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Life Technologies, Inc., Paisley, Scotland), sodium pyruvate, nonessential amino acids, and L-glutamine. Tumor cells from a mid-log phase culture were harvested by brief exposure to a 0.05% trypsin-0.02% EDTA solution, washed twice, and resuspended in PBS (Life Technologies) at the concentration indicated for injection.

Reagents. Recombinant mouse TNF-α and IL-1α were purchased from R&D Systems, Inc. (Minneapolis, MN). PAF (1–0-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) was purchased from Sigma Chemical Co. (St. Louis, MO). The PAF antagonist BN50739 (batch 51-884, M, 596.2, 50 mg/ml in DMSO), a ginkgolide-derived synthetic PAF analogue, was a gift from Dr. Pierre Bruquet (Institut Henri Beaufour, Le Plessis Robinson, France) and was stored at −20°C.

Treatment with PAF. Mice were randomly assigned to one of seven treatment groups. Treatments were: (a) control (vehicle); (b) PAF for 5 days before B16F10 injection (days −4−0); (c) PAF for 3 days before B16F10 injection (days −2−0); (d) PAF 30 mm before B16F10 injection (day 0); (e) PAF for 2 days after B16F10 injection (days 0 and 1); (f) PAF for 3 days after B16F10 injection (days 0–2); and (g) PAF for 5 days after B16F10 injection (days 0–4). PAF (1 μg/mouse) was given i.p. daily between 10 and 11 a.m.

Lung Colonization Assay. A single-cell suspension (>95% viability by trypan blue exclusion assay) at a concentration of 1.5 × 105 in 0.2 ml PBS was injected i.v. into the lateral tail veins of C57BL/6 mice. Mice were autopsied 14 days later. Lungs were removed and fixed in Bouin’s solution (Sigma), and the number of surface lung colonies was counted under a dissecting microscope. Differences in the number of lung colonies were analyzed using the Mann-Whitney U test. Results are representative of at least two independent experiments.

Measurement of Plasma PAF. Blood was collected by retro-orbital bleeding under ether anesthesia, mixed with a 0.1 volume of 3.8% cold citrate solution, and centrifuged immediately using an Eppendorf microfuge for 20 s. The plasma was mixed with an equal volume of cold methanol (pH 4.0) to inhibit the action of plasma acetylhydrolase (20) and was stored at −20°C until use. Each plasma was applied to a SI silica column (Amersham International Plc., Bucks, United Kingdom). The column was washed with 1 ml 50% methanol solution and was eluted with 1 ml chloroform:methanol:water (65:35:6) as the manufacturer recommended. The eluates were evaporated under nitrogen. PAF was quantified using a RIA-SPA (scintillation proximity assay) (Amersham) according to the protocol of the manufacturer. Results were expressed as a concentration from a standard curve of known concentrations of PAF.

Organ Distribution Analysis. Cultures of B16F10 melanoma cells were labeled with 125I (DuPont Co., Boston, MA) using the Iodo-Gen iodination reagent (Pierce Chemical Co., Rockford, IL) according to the protocol of the manufacturer. Viability of the cells was >90% as judged by trypan blue dye exclusion assay. Labeled tumor cells (2 × 107/0.2 ml) were injected i.v. At the
RESULTS

Effect of PAF on Experimental Pulmonary Metastasis. We investigated whether exogenous PAF influenced experimental pulmonary metastasis of B16F10 melanoma cells. As shown in Table 1, injection of PAF for 3 or 5 days before B16F10 injection or single injection on day 0 did not influence the colony formation. In contrast, multiple injections of PAF after B16F10 injection significantly increased the number of lung colonies. The increase was maximal in mice receiving PAF for 3 days. Injection of PAF for longer than 3 days did not increase the number of colonies further. As the concentration of PAF increased, the number of lung colonies increased (Table 2). Complete inhibition of PAF-induced experimental metastasis was achieved when BN50739 (100 μg/mouse) was given i.p. 30 min before each PAF (1 μg) injection (Fig. 1). Furthermore, the number of lung colonies in mice receiving 400 μg BN50739 (median number, 30) was lower than that of the control group (median number, 67). Photographs of increased lung colonization by PAF and its blocking by BN50739 were shown in Fig. 2.

Because exogenous PAF increased experimental metastasis, the influence of endogenous PAF on the tumor metastasis was examined. Mice received one (day 0), two (days 0 and 1), or three (days 0–2) i.p. injections of BN50739 (200 μg). B16F10 cells were injected i.v. on day 0. As shown in Table 3, a single administration of BN50739 did not influence lung colonization, whereas multiple injections of BN50739 decreased the experimental metastasis.

Inhibition of TNF-α- or IL-1α-induced Increase in Lung Colonization by BN50739. Proinflammatory cytokines, such as TNF-α and IL-1α, have been reported to enhance experimental tumor metastases (1–6). In light of the positive feedback network between PAF and TNF-α and IL-1α, it was tempting to investigate whether these cytokines exerted their activities via PAF. First, we examined the effect of TNF-α and IL-1α on lung colonization of B16F10 melanoma cells. TNF-α (0.6 μg/mouse) or IL-1α (0.5 μg/mouse) was injected i.v. in a bolus 1 h before B16F10 injection. As reported by other investigators (1–6), these cytokines enhanced the number of lung colonies; TNF-α and IL-1α increased the colonies 2.9- and 3.8-fold, respectively (Fig. 3). Significant, but not complete, inhibition of the metastases-enhancing activities of these regimens was observed by pretreatment with BN50739. The pretreatment inhibited experimental metastases 43.9 and 37.1% for TNF-α and IL-1α, respectively (Fig. 3).

These results imply that TNF-α or IL-1α treatment of mice enhances the release of PAF in vivo. To clarify this, we determined whether administration of TNF-α or IL-1α result in the release of PAF.
EFFECT OF PAF ON TUMOR METASTASIS

Table 3  Inhibition of experimental pulmonary metastasis by neutralization of endogenous PAF with PAF antagonist

<table>
<thead>
<tr>
<th>Treatment of BN50739(a)</th>
<th>Median no. of lung colonies(b)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>93</td>
<td>65–134</td>
</tr>
<tr>
<td>Day 0</td>
<td>90</td>
<td>82–102</td>
</tr>
<tr>
<td>Days 0 and 1</td>
<td>64</td>
<td>52–80</td>
</tr>
<tr>
<td>Days 0–2</td>
<td>55</td>
<td>44–65</td>
</tr>
</tbody>
</table>

\(a\) BN50739 (200 \(\mu\)g) was given i.p. (single injection, daily) at different frequencies as indicated; B16F10 melanoma cells (\(1.5 \times 10^8\)) were injected i.v. on day 0 (n = 5).

\(b\) Lungs were removed on day 14, and the number of surface colonies was counted. * \(P < 0.05\) compared with mice receiving vehicle (DMSO).

\(d\) \(P < 0.05\).

Fig. 3. Inhibition by PAF antagonist of the TNF-\(\alpha\) or IL-1\(\alpha\)-induced increase in lung colonization. A single injection of TNF-\(\alpha\) (0.6 \(\mu\)g) or IL-1\(\alpha\) (0.5 \(\mu\)g) was given i.v. 1 h prior to i.v. injection of \(1.5 \times 10^8\) B16F10 melanoma cells. One group of mice was pretreated by i.p. injection of 200 \(\mu\)g BN50739 30 min prior to TNF-\(\alpha\) or IL-1\(\alpha\) injections. Lungs were removed on day 14, and the number of surface colonies was counted. * \(P < 0.01\) compared with control group; ** \(P < 0.025\) compared with TNF-\(\alpha\)-treated group; *** \(0.05 < P < 0.1\) compared with IL-1\(\alpha\)-treated group. Values are expressed as means (n = 7). Bars, SD. —, no treatment; + treatment.

Fig. 4. Kinetics of plasma PAF after injection of TNF-\(\alpha\) or IL-1\(\alpha\). A single injection of TNF-\(\alpha\) (•; 0.6 \(\mu\)g; n = 3 for each point) or IL-1\(\alpha\) (○; 0.5 \(\mu\)g; n = 3 for each point) was given i.v., and blood was collected from each group of mice at the time indicated. Plasma PAF was measured as described in "Materials and Methods." Values are expressed as means. Bars, SD.

DISCUSSION

Delineating cytokines or mediators involved in tumor metastases and understanding their mechanisms of action are of considerable importance. In this study, we have shown that PAF augmented experimental pulmonary metastasis of B16F10 melanoma cells. The effect of PAF was dose and time dependent. Administration of PAF for 3 or 5 consecutive days before or in a single injection 30 min before B16F10 cell injection did not influence the number of lung colonies. The enhancement of lung colonization was seen when multiple injections of PAF were given after tumor cell injection. In addition, the inhibition of the number of lung colonies by BN50739 alone (Table 2), together with the finding that a large excess of BN50739 further inhibited the number of colonies in PAF-treated mice compared with control mice (Fig. 1), demonstrates a critical role of endogenous PAF on experimental pulmonary metastases.

Another important finding presented here is that a PAF antagonist reduced the augmentation of experimental pulmonary metastases caused by TNF-\(\alpha\) or IL-1\(\alpha\) (Fig. 3). This strongly suggests that the influence of TNF-\(\alpha\) or IL-1\(\alpha\) on tumor metastases is, at least in part, mediated by PAF. Increased tumor metastasis after TNF-\(\alpha\) or IL-1\(\alpha\) treatment has been described in various animal tumor models (1, 4, 5). Furthermore, in view of the facts that PAF and these cytokines stimulate and release each other via a feedback network (7—10), our
data showing a blocking of TNF-α or IL-1α-mediated augmentation of experimental pulmonary metastases by PAF antagonist and confirming increased PAF in the circulation by the administration of TNF-α or IL-1α identify another example of interaction between PAF and TNF-α or IL-1α.

As best as we can determine, the present study is the first to demonstrate the direct role of PAF in enhancement of experimental tumor metastasis and participation of PAF in TNF-α and IL-1α-induced increases in tumor metastasis.

The mechanism by which PAF exerts its effect is, at present, unknown. However, the addition of PAF in vitro to B16F10 melanoma cells resulted in no influence on tumor cell proliferation (data not shown). Thus, it is unlikely that PAF has a direct growth-enhancing effect on tumor cells. Moreover, no enhancement of lung colonization by the tumor cells preincubated with PAF (up to 10 μg/ml) in vitro for 24 h before injection (data not shown) rules out a direct influence of PAF on tumor cells. Metastasis of tumor cells from a primary tumor requires tumor cell attachment to the vascular bed of secondary target organs (21, 22). This process may be mediated by organ-specific endothelial adhesion molecules (23, 24) or by adhesion molecules for which expression is increased by cytokines such as TNF-α and IL-1β (25–28). If the latter is the case, an inhibition of TNF-α or IL-1β-mediated metastasis by the PAF antagonist (Fig. 3) suggests an involvement of the adhesion molecules, the expression of which is enhanced by PAF. This hypothesis is supported by the enhanced adherence of radiolabeled tumor cells to the lungs in PAF-treated mice (Fig. 5). Additional experiments are required to determine the adhesion molecule(s) critical in PAF-mediated tumor metastasis.

There is little evidence to demonstrate a direct role of PAF in the pathogenesis of tumor metastasis. Berdel et al. (29) reported that lysophospholipid inhibited the development of pulmonary metastasis of a 3-Lewis lung tumor from primary tumor transplantation. They injected tumor cells into mouse hind footpads, and the tumors were surgically removed within 6–7 days. Administration of lysophospholipid i.v. or s.c. for 21 days after removal of the primary tumor resulted in a decrease in pulmonary metastasis. However, this discrepancy between that report and our data may be due to the totally different experimental conditions and/or different regimens: PAF versus lyso-PAF.

Our data clearly establish that PAF has tumor metastasis-enhancing activity, and PAF mediates metastasis-enhancing activities of TNF-α and IL-1α.

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