Motility Induced by Human Immunodeficiency Virus-1 Tat on Kaposi's Sarcoma Cells Requires Platelet-Activating Factor Synthesis

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In the present study, we evaluated whether motility of Kaposi's sarcoma (KS) spindle cells induced by HIV-1 Tat protein is dependent on the synthesis of platelet-activating factor (PAF). The results obtained indicate that Tat induced a dose-dependent synthesis of PAF from KS cells at a concentration as low as 0.1 ng/ml. PAF production started rapidly after Tat stimulation, peaking at 30 minutes and declining thereafter. Tat-induced cell migration was also a rapid event starting at 30 minutes. The motility was abrogated by addition of a panel of chemically unrelated PAF receptor antagonists (WEB 2170, CV 3988, CV 6209, and BN 52021), suggesting that the synthesized PAF mediates the mitogenic effect of Tat. This effect was also present on cells plated on a type-I collagen-, fibronectin-, or basement membrane extract-coated surface. Expression of PAF receptor-specific mRNA was detected in KS cells. In addition, examination of the cytoskeletal organization showed that Tat-mediated KS cell redistribution of actin filaments and shape change was also inhibited by a PAF receptor antagonist. Moreover, PAF receptor blockade prevented the up-regulation of $\beta 1$ integrin and the down-regulation of $\alpha v\beta 3$ observed after stimulation of KS cells with Tat. In conclusion, the results of the present study indicate that Tat-induced PAF synthesis plays a critical role in triggering the events involved in motility of KS cells. (*Am J Pathol* 1999, 155:1731-1739)

Kaposi's sarcoma (KS) is one of the most common cancers affecting patients with human immunodeficiency virus-1 (HIV-1) infection. KS is a hemoangiosarcoma containing spindle-shaped cells, vascular smooth cells, endothelial cells, and fibroblasts.¹⁻³ The growth and diffusion of KS have been ascribed to an imbalance in the

network of soluble mediators caused by HIV-1 infection.⁴ We have recently observed that platelet-activating factor (PAF), produced *in vitro* by KS-derived spindle cells, induces and sustains *in vivo* angiogenesis in a murine model.⁵ Indeed, PAF is a phospholipid mediator of cell-to-cell communication that belongs to the structurally related family of acetylated phosphoglycerides.⁶ Recently, it has been found that a mutation of a PAF-specific acetylhydrolase is the underlying defect of a congenital neurological disorder named Miller-Dieker lissencephaly, characterized by impaired migration of central neurons.⁷ Indeed, several lines of evidence provide support for a role of this agent in regulating cell contraction, migration, and adhesion.^{8,9} A number of factors such as tumor necrosis factor- α (TNF α), hepatocyte growth factor (HGF), and interleukin-12, able to induce these events, were shown to act at least in part through the rapid synthesis of PAF.¹⁰⁻¹²

A number of cell surface structures were shown to interact with Tat. First, $\alpha 5\beta 1$ and $\alpha v\beta 3$ integrins may bind to Tat through its arginine-glycine-aspartic acid (RGD) sequence.¹³ Moreover, we found that HIV-1 Tat protein may interact with endothelial cells through binding to the mitogenic vascular endothelial growth factor-A (VEGF-A) receptor Flk-1.¹⁴ Furthermore, the chemokine receptors CCR2 and CCR3 may act as additional Tat receptors on monocytes.¹⁵ Finally, it has been shown that HIV-1 Tat may interact with Flk-1 on KS 38 cells, activating a number of signal transduction pathways.¹⁶

The aim of the present study was to evaluate whether HIV-1 Tat can stimulate the synthesis of PAF by KS cells and whether the newly synthesized PAF mediates the mitogenic activity of Tat on these cells.

Materials and Methods

Reagents

Synthetic C16 PAF (1-hexadecyl-2-acetyl-sn-glycerol-3-phosphorylcholine) was obtained from Bachem

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Feinchemikalien (Bubendorf, Switzerland). CV 3988 was from Takeda Chemical Industries (Kyoto, Japan).¹⁵ CV 6209 and BN 52021 were purchased from Biomol (Plymouth Meeting, PA). WEB 2170 was obtained from Boehringer Ingelheim KG, Germany.¹⁶ Silica gel 60F254 thin-layer chromatography (TLC) plates were obtained from Merck (Darmstadt, Germany). mPorasil high-performance liquid chromatography (HPLC) columns were provided by Millipore Chromatographic Division (Waters, Milford, MA). RPMI 1640 medium was from GIBCO (Grand Island, NY) and bovine calf serum (BCS) was from Hyclone Lab (Logan, UT).

Recombinant Tat was obtained from Intracell (London, UK). Polymyxin B, phospholipase A2, phospholipase A1, bovine serum albumin (BSA) fraction V (tested for not more than 1 ng endotoxin per mg), FMLP, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine were purchased from Sigma Chemical Company (St. Louis, MO). Rabbit polyclonal IgG anti-human flk-1 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). [³H]acetate ([³H]CH₃CO₂Na; 2.5 Ci/mmol) was obtained from NEN Life Science Products (Boston, MA).

In Vitro PAF Synthesis by KS Cells

KS cell line derived from a HIV-1 patient and spontaneously immortalized was propagated as previously described.¹⁷ In standard PAF synthesis assays, confluent KS cells maintained for 24 hours in DMEM without fetal calf serum (FCS) were stimulated in 1 ml Iscove's medium containing 0.25% BSA for various period of times with different doses of Tat. PAF released into the medium and cell associated was extracted and purified as previously described.¹² PAF extracted and purified by KS cells was quantified by bioassay on washed rabbit platelets.¹² PAF bioactivity, tested after extraction and purification by TLC and HPLC, was characterized by comparison with synthetic PAF according to the following criteria: i) induction of platelet aggregation by a pathway independent of both ADP- and arachidonic acid/thromboxane A2-mediated pathway; ii) specificity of platelet aggregation as inferred from the inhibitory effect of PAF receptor antagonist WEB 2170 (3 μ mol/L); and iii) TLC and HPLC chromatographic behavior and physicochemical characteristics such as inactivation by base-catalyzed methanolysis or phospholipase A2 treatment and resistance to phospholipase A1 or treatment with weak base and acids.¹⁸

To study the incorporation of radioactive precursors, 5×10^5 KS cells were incubated in 1 ml RPMI 1640 for 30 minutes with 30 μ Ci [³H]acetate before stimulation.¹⁹ The cell pellets were extracted with formic acid added to lower the pH of the aqueous phase to 3.0 and lipids were fractionated by TLC on aluminum-sheet silica-gel plates (silica gel 60, F254, 0.2 mm thickness, Merck) using a solvent chloroform/methanol/acetic acid/water 50:25:8:4 by volume.¹⁹ The plates were cut into 1-cm sections and the radioactivity of each was measured. Radiolabeled C16-PAF was used as a standard.

In Vitro KS Cell Migration

Cells (10^5 /well) were plated and rested for 12 hours with medium M199 containing 1% FCS, then washed three times with PBS and incubated with RPMI and the agonist. Cell division did not start to any significant degree during the experiments. Cell migration was studied over a 20-hour period under a Nikon Diaphot inverted microscope with a 10 $\times$ phase-contrast objective in an attached, hermetically sealed Plexiglas Nikon NP-2 incubator at 37°C. Cell migration was recorded using a JVC-1CCD video camera. Image analysis was performed with a MicroImage analysis system (Cast Imaging, Venice, Italy) and an IBM-compatible system equipped with a video card (Targa 2000, Truevision, Santa Clara, CA). Image analysis was performed by digital saving of images at intervals of 30 minutes. Migration tracks were generated by marking the position of nucleus of individual cells on each image. The net migratory speed (velocity straight line) was calculated by the MicroImage software based on the straight line distance between the starting and ending points divided by the time of observation. Migration of at least 30 cells was analyzed for each experimental condition. Values are given as mean $\pm$ SD. In selected experiments, KS cells were seeded on plates previously coated with 10 μ g/ml bovine fibronectin (Sigma), bovine type-I collagen (Sigma), or reconstituted basement membrane (Matrigel, Sigma) overnight at 37°C.

PAF Receptor mRNA Expression

PAF receptor-specific mRNA was detected in total RNA extracted from cells by guanidinium thiocyanate phenol-chloroform precipitated with isopropanol. One microgram of RNA was treated with 6 U of RNase-free DNase for 1 hour at 37°C and then for 5 minutes at 94°C. Complementary DNA was obtained by using random hexamer primers (Perkin-Elmer Cetus, Norwalk, CT). Reverse transcription was carried out at 42°C for 60 minutes; in addition to 1 μ g of RNA, the reaction mixture (20 μ l) contained 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 5 mmol/L MgCl₂, 1.0 mmol/L dNTPs, 20 U ribonuclease inhibitor, and 50 U of Moloney murine leukemia virus reverse transcriptase (Perkin-Elmer Cetus). cDNA was then subjected to 35 cycles of amplification by the polymerase chain reaction in an automated DNA thermal cycler (Perkin-Elmer Cetus) by using these human PAF receptor mRNA-specific primer pairs: forward, 5' CAC GGG CTC GAG ACC AAC ACA GTG CCC GAC AGT GCT 3'; reverse, 5' CGC GGG ATC CCG GGT GAC CTG ATG TGC ATC ATT AAT 3'.

The PCR reaction mixture (50 μ l) contained 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.2 mmol/L dNTPs, 20 pmol of (+) and (–) primers, and 2 U thermostable DNA polymerase (Perkin-Elmer Cetus). Times and temperatures for denaturation, annealing, and extension were 30 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C, respectively. Amplification product (262 bp) was analyzed in 2% agarose gels containing 0.5 μ g/ml of ethidium bromide. As control, B16

Figure 1. PAF synthesis by KS cells. **A** shows the time course of PAF synthesis by KS cells stimulated with 1 ng/ml Tat (□, cell-associated PAF; ■, released PAF) or with vehicle alone (▲, cell-associated PAF; △, released PAF). Numbers are the mean $\pm$ SD of three individual experiments. **B** shows the effect of different doses of Tat on PAF synthesis by KS cells and of thrombin (2 U/ml) used as positive control. The numbers are the mean $\pm$ SD of five individual experiments. **C** shows the incorporation of radiolabeled acetate in the newly synthesized PAF after stimulation of KS cells with Tat. Representative TLC analysis of radiolabeled lipids extracted from 5×10^5 KS cells stimulated with 1 ng/ml Tat (solid line). Dotted line shows the chromatographic behavior of radiolabeled lipids extracted from unstimulated cells. The chromatographic behavior of synthetic C16 PAF is indicated at the top of the figure. This figure is representative of four different experiments performed with similar results.

cells (American Type Culture Collection, Manassas, VA) untransfected or transfected with human PAF receptor-specific cDNA (kindly provided by Dr. R. D. Ye, La Jolla, CA) were used.

Figure 2. Expression of mRNA for human PAF-receptor by B16 cells (lane 2), PAF receptor-transfected B16 cells (lane 3) or KS cells (lane 4) detected by RT-PCR. Empty plasmid vector (lane 5) or plasmid vector containing PAF-receptor cDNA (lane 6) detected by PCR. Lane 1 shows DNA markers.

Cell Cytoskeleton Studies

Cytoskeleton alterations were studied on fixed KS after permeabilization with 0.1% Triton X-100 in PBS and stained for F-actin with 2 μ g/ml FITC-conjugated phalloidin (F-PHD; Sigma).

Apoptosis Assays

In selected experiments apoptosis was evaluated by the XTT-based assay (Sigma). Briefly, cells were cultured in 96-well flat-bottomed microtiter plates (Falcon Labware, Oxnard, CA) at a concentration of 5×10^4 cells/well in RPMI. At different periods of time, cells were washed and incubated in serum-free Dulbecco's modified minimum essential medium containing 250 μ g/ml XTT at 37°C.

Apoptosis was also evaluated by cytofluorimetric analysis of DNA after propidium iodide (PI) staining. Briefly, 10^6 cells were incubated for 4 hours at 4°C in 2 ml hypotonic solution containing 50 μ g/ml PI, 0.1% sodium citrate, 0.1% Triton X-100, and 20 μ g/ml DNase-free RNase A. Cells with subdiploid DNA content (sub-G₀/G₁ peak) were considered apoptotic cells. All cultures were done in triplicate.

Cytofluorimetric Analysis

After appropriate stimulation (at 37°C for 1, 4, or 12 hours) with Tat (10 ng/ml), Tat plus CV 3988 (3 μ M), or vehicle alone, cells were detached from plates with 0.02% EDTA at 4°C, washed, resuspended in PBS containing 0.25% BSA, and incubated at 4°C for 30 minutes with containing 1 μ g/ml of monoclonal anti- β 1 or anti- α v β 3 integrin antibodies (Chemicon, Temecula, CA) or with anti-PAF-R antibody (kind gift of Dr. M. Rola-Pleszczynski, Sherbrooke, PQ). As a second step reagent, FITC-conjugated anti-mouse IgG (Sigma) was used. Cells were analyzed on a FACS (Becton Dickinson, Mountain View, CA).

Statistical Analysis

All data are expressed as mean $\pm$ SD. Statistical analysis was performed by analysis of variance (ANOVA) with Neumann-Keul's multiple comparison test or Kolmogorov-Smirnov where appropriate.

Results

Synthesis of PAF by Tat-Stimulated KS Cells

Unstimulated KS cells produced a small amount of PAF that in short-term experiments was detected associated with the cells. When stimulated with Tat, KS cells showed a rapid increase of PAF synthesis, which peaked at 15 minutes and decreased thereafter and was followed by a partial release into the medium (Figure 1A). As shown in Figure 1B, the effect of Tat on PAF synthesis was dose-dependent. Tat induced the synthesis of PAF at doses as low as 0.1 ng/ml. Using radioactive acetate as substrate for PAF synthesis, we found that PAF detected after stimulation with Tat was newly synthesized. The TLC analysis of lipid fractions extracted 15 minutes after addition of Tat to KS cells preincubated with [3 H]-acetate demonstrated the presence of one main peak of radioactivity that comigrated with synthetic [3 H]-C16-PAF (Figure 1C). This peak was lower in the lipid fractions extracted from unstimulated KS cells. PAF-bioactive material extracted and purified from KS cells was insensitive to treatment with phospholipase A1 that cleaves the acyl- but not the alkyl-PAF.^{18,20} The amount of PAF-bioactive material treated with phospholipase A1 did not show a significant reduction of its biological activity ($93 \pm 1.9\%$ recovered activity). To evaluate the efficiency of phospholipase A1 treatment 4 samples containing PAF-bioactive material were added with [14 C]-acyl-PAF before treatment with phospholipase A1. The amount of [14 C]-acyl-PAF hydrolyzed (recovered as a free fatty acid) was $82 \pm 4.2\%$, whereas the biological activity was not significantly reduced ($92 \pm 2.8\%$ recovered activity).

PAF Receptor Expression

PAF receptor-specific mRNA was detected by RT-PCR in total RNA extracted from KS cells. Analysis of mRNA from CHO cells transfected with human PAF-receptor specific cDNA but not untransfected cells displayed identical amplification product (Figure 2) to the one from KS cells thus providing additional control. Moreover, we evaluated by cytofluorimetric analysis whether Tat stimulation increased the surface expression of PAF receptor during experiments of cell motility. The results obtained indicated that the basal expression of PAF receptor was not enhanced by Tat at 4 and 12 hours (data not shown).

In Vitro Migration of KS Cells

The baseline migration rate of KS cells corresponding to the spontaneous motility of rested, unstimulated cells was first measured and found to remain steady for the whole period of observation, never exceeding 5 to 6 μ m/hour speed (Figures 3A and 4A). Incubation with Tat stimulated a marked dose-dependent acceleration of cell motility, peaking as early as 30 minutes after addition and maintaining a significantly higher speed compared to unstimulated KS cells for the time of observation (Figures

Figure 3. Motility of KS cells measured as described in the Materials and Methods section. **A** shows incubation of KS cells with vehicle alone or with different doses of Tat or with PAF (10 ng/ml). **B** shows the inhibitory effect of specific PAF receptor antagonists WEB2170, CV 3988, CV 6209, or BN 52021, on Tat-induced KS motility. Cells were incubated with Tat (10 ng/ml) in the presence or absence of 3 μ mol/L WEB2170, 3 μ mol/L CV 3988, 0.17 μ mol/L CV 6209, or 0.34 μ mol/L BN 52021. Data are expressed as mean $\pm$ SD of three different experiments. ANOVA with Newmann Keul's multicomparison test was performed: cells incubated with vehicle alone (control) versus cells incubated with Tat (* $P < 0.05$); cells incubated with Tat versus cells incubated with Tat + WEB2170, Tat + CV 3988, Tat + CV 6209, or Tat + BN 52021 ($^{\$}P < 0.05$).

3A and 4B). Similar enhancement of cell motility was observed after stimulation of KS cells with 10 ng/ml PAF (Figure 3A). Addition of the specific PAF receptor antagonists, WEB 2170,²¹ CV 3988,²² CV 6209,²³ or BN 52021,²⁴ which completely abrogated PAF-induced motility (not shown), also significantly inhibited motility stimulated by Tat (Figures 3B and 4C). No significant difference in motility was observed among unstimulated cells and cells stimulated with Tat and treated with PAF receptor antagonist. Moreover, PAF receptor antagonist alone did not reduce the baseline migration of untreated KS cells (data not shown). These results suggest that the enhanced motility induced by Tat but not the spontane-

Figure 4. Micrographs representative of time-lapse analysis of KS cell motility performed by digital saving at 30-minute intervals. Migration tracks were generated by marking the position of the nucleus of individual cells of each image (see Materials and Methods). KS cells were incubated with vehicle alone (A), with 10 ng/ml Tat (B), or with Tat (10 ng/ml) in the presence of 3 μ mol/L WEB2170 (C). Original magnification, $\times 120$.

ous KS cell motility was PAF-dependent. As shown in Figure 5, we have also evaluated the role of different matrix substrates on Tat-induced KS cell motility. The results obtained indicate that Tat-induced motility was inhibited by the PAF receptor antagonist WEB 2170 not only on plastic but also on type 1 collagen (Figure 5A), fibronectin (Figure 5B), and reconstituted basement membrane (Matrigel; Figure 5C).

Cell Shape and Cytoskeleton Changes

The incubation of KS cells with Tat induced shape changes (Figure 6) and modified the normal distribution of actin-containing stress fibers (Figure 7). After exposure of KS cells to 10 ng/ml Tat for 1 hour at 37°C cells change shape (Figure 6D) and stress fibers tend to axially condense, retract, and appear to fuse (Figure 7B). Such alterations are observed also as a result of incubation with PAF (10 ng/ml; data not shown). Incubation with WEB 2170 as well as with CV 3988, CV 6209, or BN

Figure 5. Evaluation of the role of different matrix substrates on Tat-induced KS cell motility. KS cells plated on type 1 collagen (A), fibronectin (B), and Matrigel (C) were stimulated with vehicle alone or with of Tat (10 ng/ml) or Tat plus PAF-receptor antagonists WEB2170 (3 μ mol/L). Three experiments were performed with similar results.

Figure 6. Micrographs representative of shape change of KS cells as observed by comparison of the time 0 (**A**, **C**, and **E**) and 1-hour frames (**B**, **D**, and **F**). **A** and **B** show unstimulated KS cells; **C** and **D** show KS cells stimulated with 10 ng/ml Tat; **E** and **F** show KS cells stimulated with 10 ng/ml Tat in the presence of 3 μmol/L WEB 2170. Three experiments were performed with similar results. Original magnification, ×120.

52021 (data not shown) prevented both the change in cell shape (Figure 6F) and cytoskeleton changes (Figure 7C) induced by Tat. Tat-induced alterations were reversible after washing and culturing cells overnight (Figure

7B). In selected experiments to evaluate whether rounding up of the cells induced by Tat precluded apoptosis, we tested cell viability by XTT-based and subdiploid DNA content methods. Cell survival was $96 \pm 5\%$ on vehicle-

Figure 7. Micrographs representative of F-actin distribution in fixed and permeabilized KS cells after incubation for 1 hour in the following experimental conditions: **A**, unstimulated KS cells (vehicle alone); **B**, KS cells stimulated with 10 ng/ml Tat; **C**, KS cells stimulated with 10 ng/ml Tat in the presence of 3 $\mu\text{mol/L}$ WEB 2170; **D**, KS cells stimulated with Tat 10 ng/ml and then washed and cultured for 12 hours with RPMI supplemented with 10% FBS to evaluate reversal of cytoskeletal changes induced by Tat. Three experiments were performed with similar results. Original magnification, $\times 400$.

treated cells and $98 \pm 2\%$ on 10 ng/ml Tat-treated cells for 12 hours. Cells with subdiploid DNA content accounted for $5 \pm 3\%$ of the vehicle-treated cells and $4 \pm 3\%$ of the 10 ng/ml Tat-treated cells. These results indicate that changes in cell shape and cytoskeleton were not associated with apoptosis.

Analysis of Integrin Expression

The effect of PAF and Tat stimulation on integrin expression in the presence or absence of PAF receptor antagonist CV3988 was evaluated after 1, 4, and 12 hours. Two distinct trends of expression were observed for $\alpha\text{v}\beta 3$ and $\beta 1$ integrins. PAF as well as Tat stimulation induced a progressive down-regulation of $\alpha\text{v}\beta 3$, which was already detectable at 4 hours (data not shown) and maximal at 12 hours (Figure 8, A and C). In contrast, both agonists induced up-regulation of $\beta 1$, which was evident after 12 hours' stimulation (Figure 8, E and G). Treatment with the PAF receptor antagonist CV3988 abrogated both the down-regulation of $\alpha\text{v}\beta 3$ (Figure 8, B and D) and the up-regulation of $\beta 1$ (Figure 8, F and H) integrins induced by PAF and Tat.

Discussion

The results of the present study indicate that Tat induces synthesis of PAF by KS cells and that PAF, in turn, me-

Figure 8. Detection of $\alpha\text{v}\beta 3$ and $\beta 1$ integrins on KS cells by cytofluorimetry after 12 hours stimulation with PAF (10 ng/ml) or Tat (10 ng/ml) in the presence or absence of PAF receptor antagonist CV3988 (3 $\mu\text{mol/L}$). PAF-stimulated (dark line, **A**) as well as Tat-stimulated (dark line, **B**) KS cells showed a down-regulation of $\alpha\text{v}\beta 3$ in respect to the unstimulated KS cells (gray line, **A** and **B**). In contrast, PAF-stimulated (dark line, **E**) as well as Tat-stimulated (dark line, **G**) KS cells showed an up-regulation of $\beta 1$ in respect to the unstimulated KS cells (gray line, **E** and **G**). Treatment with the PAF receptor antagonist CV3988 abrogated both the down-regulation of $\alpha\text{v}\beta 3$ (**B** and **D**) and the up-regulation of $\beta 1$ (**F** and **H**) integrins induced by PAF and Tat. Three experiments were carried out with similar results. The Kolmogorov-Smirnov statistical analysis performed between unstimulated cells and cells stimulated with Tat or PAF was significant for the changes in the expression of both $\alpha\text{v}\beta 3$ and $\beta 1$ integrins ($P < 0.05$). Moreover, the inhibitory effect of CV 3988 on Tat- and PAF-stimulated KS cells was statistically significant ($P < 0.05$).

diates the motogenic activity of Tat on these cells. PAF is a phospholipid mediator with multiple biological activities that has been shown to directly stimulate *in vitro* migration of endothelial cells and promote *in vivo* angiogenesis.⁹ Recently, we found that KS cells synthesize PAF after stimulation with cytokines and that PAF released in the supernatant of KS cells accounts, at least in part, for its angiogenic activity *in vivo*.⁵

Several studies indicate that Tat has a relevant role in the pathogenesis of Kaposi's sarcoma. Indeed, Tat transgenic mice develop Kaposi's sarcoma-like lesions.²⁵ Tat may interact with cells either through $\alpha 5\beta 1$ and $\alpha\text{v}\beta 3$ integrins via its RGD sequence,¹³ through Flk-1/KDR via its VEGF-like sequence,¹⁴ and/or through chemokine receptors CCR2 and CCR3.¹⁵

Previous studies have shown that PAF mediates some of the biological properties of certain polypeptide mediators. In particular, PAF was found to mediate directional

migration of endothelial cells induced by tumor necrosis factor^{10,26} and hepatocyte growth factor.¹¹ In the present study we demonstrate that a viral protein such as HIV-1 Tat induces synthesis of PAF by KS cells. PAF is newly synthesized, as shown by the incorporation of labeled acetate in the molecule of PAF and is released in the supernatant. Moreover, we found that PAF is instrumental in the motility of KS cells elicited by HIV-1 Tat. Indeed, *in vitro* KS cell migration induced by Tat was inhibited by a panel of chemically different PAF receptor antagonists. Therefore, one can envisage that PAF produced by KS cells has an autocrine and/or paracrine effect on the motility of these cells. The characteristics of the motility assay used in the present study exclude a gradient for both Tat and PAF, suggesting a chemokinetic effect. In this contest, PAF may act either as mediator of cell-to-cell communication involved in the amplification of the signal triggered by Tat or in the up-regulation of adhesion molecules involved in cell migration. Indeed, it has recently been shown that PAF up-regulates integrins and that the $\beta 1$ integrins are critically involved in PAF-induced leukocyte locomotion in extravascular tissue.²⁷ The results of the present study indicate that Tat induced a PAF-dependent up-regulation of $\beta 1$ integrins and down-regulation of $\alpha v \beta 3$ integrins, because PAF mimics and a PAF receptor antagonist abrogates the events elicited by Tat. It is recognized that not only the activation, but also the modulation of surface concentration of integrins is instrumental for cell motility on extracellular matrix.²⁸ In the present study, we have also evaluated the role of different matrix substrates on Tat-induced KS cell motility. The results obtained indicate that Tat-induce motility is PAF-dependent not only on plastic but also on more physiological substrates, such as type 1 collagen, fibronectin, and reconstituted basement membrane (Matrigel).

Taken together, these results suggest that PAF synthesized by KS cells after stimulation with either HIV-1-derived Tat or cytokines may be instrumental in the infiltration of KS cells in tissues and in the interaction with the neoformed vessels that characterize this angiogenic tumor. In fact, PAF is able to trigger *in vivo* a neoangiogenic response by stimulating endothelial cell recruitment.⁹ In conclusion, our observations that Tat stimulates the synthesis of PAF by KS cells and that a panel of specific PAF receptor antagonists inhibits the migration of KS cells triggered by Tat suggest that this phospholipid may act as a secondary mediator for the motility induced by this HIV-1 protein.

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