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HIV-1 Tat increases the adhesion of monocytes and T-cells to the endothelium in vitro and in vivo: implications for AIDS-associated vasculopathy☆

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Abstract

HIV-1-infected patients exhibit severe damages of the aortic endothelium, develop angioproliferative lesions such as Kaposi's sarcoma (KS), and have an increased risk of cardiovascular diseases and atherosclerosis. An increased adhesion of leukocytes to the endothelium is a common pathogenic parameter of AIDS-associated vascular diseases. Here we show that the HIV-1 Tat protein, a regulatory protein of HIV-1 released by infected cells, and TNF-α, a cytokine increased in sera and tissues of HIV-1-infected patients, activate synergistically the adhesion of leukocytes to endothelial cells both in vitro and in vivo. This effect is selectively mediated by HIV-1 Tat, since HIV-1 Nef, another HIV-1 regulatory protein, and the HIV-1 envelope protein gp41, had no effect. In vitro adhesion assays with PBMC and quantitative cell type analysis of adherent cells by FACS demonstrated that HIV-1 Tat selectively activates the adhesion of T-cells and monocytes but not of B-cells. Intravital microscopic studies in mice confirmed the synergistic activity of HIV-1 Tat and TNF-α on leukocyte adhesion to the endothelium in vivo. These data indicate that HIV-1 Tat in cooperation with TNF-α may contribute to the vascular damage and cardiovascular diseases observed in AIDS patients but also to the prominent extravasation of T-cells and monocytes which is a key process in the formation and progression of KS lesions.

Keywords: AIDS; HIV-1 Tat; Monocytes/macrophages; T-lymphocytes; Cytokines

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1. Introduction

HIV-1 infection is accompanied by vascular diseases. Until recently, the clinically most evident manifestation of AIDS-associated vasculopathy was Kaposi's sarcoma (AIDS-KS). KS is an angio-proliferative tumor with prominent infiltration of monocytes and T-cells that is closely associated with human herpesvirus-8 (HHV-8) infection (Stürzl et al., 2001). Of note, in HHV-8 and HIV-1 coinfected patients KS incidence is more than 20,000-fold increased (Beral et al., 1990). In the era of the highly active antiretroviral therapy (HAART), also other diseases have been recognized that indicate chronic vessel activation and dysfunction in HIV-1 infection. For example, cardiovascular diseases account for up to 10% of the current death cases in HIV-1-infected patients (Bonnet et al., 2002), evidence of atherosclerosis has been found in 75% of dyslipidemic HIV-1 patients (Acevedo et al., 2002), and an increasing frequency of pulmonary hypertension has been reported (Pellicelli et al., 2001). These findings are in agreement with earlier observations by us demonstrating severe alterations of the structural integrity of the aortic endothelium of HIV-1-infected patients, concomitant with an increased adhesion of leukocytes (Zietz et al., 1996).

HIV-1 Tat is a transcriptional activator of viral gene expression produced early after infection and is essential for virus replication (Arya et al., 1985; Chang et al., 1995; Ensoli et al., 1993). During acute infection of T-cells by HIV-1, HIV-1 Tat is released from the cells in an active form and via a leaderless secretory pathway (Chang et al., 1997; Ensoli et al., 1990, 1993). Consistent with these observations, HIV-1 Tat has been detected in sera of HIV-1-infected individuals (Westendorp et al., 1995) and in AIDS-KS lesions particularly in vessels (Ensoli et al., 1994a, 2001).

Extracellular HIV-1 Tat released by infected cells can enter infected or non-infected cells (Chang et al., 1997; Ferrari et al., 2003) and can activate a variety of genes regulated by specific viral and cellular promoters (Fanales-Belasio et al., 2002; Kumar et al., 1998; Vaishnav and Wong-Staal, 1991). Of note, HIV-1 Tat also increases angiogenesis mediated by bFGF and is capable of inducing the growth, migration, and invasion of endothelial cell-like cultures derived from KS lesions (Albini et al., 1995; Barillari et al., 1992; Ensoli et al., 1990, 1993, 1994b) and of normal endothelial cells that have been activated by inflammatory cytokines (IC) including TNF-α (Albini et al., 1995; Barillari et al., 1992, 1993; Fiorelli et al., 1995). This is due to the HIV-1 Tat molecular mimicry of extracellular matrix proteins and is mediated by two regions of the protein. The basic region that retrieves bFGF from heparan sulfate proteoglycans of the extracellular matrix (Barillari et al., 1999a,b; Chang et al., 1997) and the RGD region that binds to the α5β1 and αvβ3 integrins (Barillari et al., 1993) and through this mediates cell adhesion, migration, and cell invasion, which in turn is associated with the induction of matrix metalloproteinase (MMP)-1 by HIV-1 Tat (Albini et al., 1995; Ensoli et al., 1994a). In addition, HIV-1 Tat induces the cell surface expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and endothelial leukocyte adhesion molecule-1 (E-selectin) in endothelial cells (Dhawan et al., 1997), suggesting that it may induce the interaction of endothelial cells with leukocytes. In fact, it has been shown that HIV-1 Tat enhances the migration and the invasion of monocytes into reconstituted basement membranes (Lafrenie et al., 1996b). In addition, HIV-1 Tat up-regulates the expression of β2-integrins in these cells and increases their adhesion to TNF-α-treated endothelial cell monolayers (Lafrenie et al., 1996a). The latter is accompanied by a substantial disruption of the endothelial monolayer and it has been proposed that a HIV-1 Tat-induced up-regulation of MMP-9 may contribute to this effect (Lafrenie et al., 1996a). The stimulation of monocyte adhesion has been attributed to the induction by HIV-1 Tat of autocrinely active cytokines, namely IL-1β and TNF-α (Lafrenie et al., 1997). Moreover, it has been shown that HIV-1 Tat can bind to and activate VEGF receptor-1 (Flt-1) in monocytes (Mitola et al., 1997) and VEGF receptor-2 (KDR/FLK) in endothelial cells (Albini et al., 1996).

All the effects of HIV-1 Tat on endothelial cells and their interaction with monocytes are significantly increased by or require a previous exposure of the cells to IC, such as IFN-γ, IL-1β, and TNF-α. Of note, these same cytokines are present at increased concentrations in the sera of HIV-1-infected patients and in both sera and tissues of patients with KS or at risk of KS (Kobayashi et al., 1990; Lahdevirta et al., 1988; Pugliese et al., 2002). In particular, IL-1β and IFN-γ are more closely associated with acute infection (Rinaldo et al., 1990; Sinicco et al., 1993; Ullum et al., 1997), whereas increasing serum concentrations of TNF correlate with disease progression (Kobayashi et al., 1990; Lahdevirta et al., 1988; Medrano et al., 1998).

Here we show that TNF-α and HIV-1 Tat increase synergistically the adhesion of leukocytes to endothelial cells in vitro and that this effect is selectively mediated by HIV-1 Tat and not by other HIV-1-encoded proteins, including the envelope protein gp41 and HIV-1 Nef, another regulatory factor of HIV-1. Further, adhesion experiments with PBMC and FACS analysis of the adherent cells demonstrated that HIV-1 Tat selectively increases the adhesiveness of monocytes and T-cells but not of B-cells. Finally, an intravital microscopic study in mice demonstrated for the first time that HIV-1 Tat and TNF-α cooperate in the activation of leukocyte adhesion to the endothelium also in vivo.

2. Materials and methods

2.1. Recombinant HIV-1 proteins

Tat from HIV-1 type IIIB-BH-10 (subtype B) was expressed in *Escherichia coli* and purified to homogeneity by

heparin-affinity chromatography and HPLC, as described (Fanales-Belasio et al., 2002). The purified HIV-1 Tat protein was fully biologically active, as tested by various assays, including rescue assays on the HLM-1 cell line carrying a Tat-defective HIV-1 provirus, or the induction of chloramphenicol acetyl transferase activity in HL3T1 cells containing a HIV-1-long terminal repeat chloramphenicol acetyl transferase construct and by uptake studies with dendritic cells (Fanales-Belasio et al., 2002). To prevent oxidation, the HIV-1 Tat protein was stored lyophilized at −80 °C and reconstituted in degassed buffer before use (Fanales-Belasio et al., 2002). To prevent attachment of the protein to surfaces, plastic tips and vials before use were rinsed in 0.1% PBS–BSA or in RPMI 1640 (Invitrogen, Karlsruhe, Germany) supplemented with 20 mM HEPES (Sigma–Aldrich, St. Louis, MO), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (Invitrogen) and 15% fetal bovine serum (FBS) (Invitrogen). In addition, because HIV-1 Tat is also photo- and thermosensitive (Chang et al., 1997; Ensoli et al., 1993), the handling of the protein was always performed in the dark and on ice. In all cases endotoxin concentration was below the detection limit (<0.05 EU/μg), as determined by the Limulus Amoebocyte Lysate analysis (Pyrochrome, Associates of Cape Cod, Falmouth, MA).

Nef from HIV LAV-1 Bru was a gift from V. Erfle (GSF-National Research Center for Environment and Health, Germany). It was purified as described in detail by Kohleisen et al. (1996) and handled as described above for HIV-1 Tat.

Since gp41 is rather hydrophobic, a recombinant fusion protein between maltose-binding protein (MBP) and an 82-amino-acid-long extracellular region (amino acids 565–647) of gp41 was used for the experiments (Intracel, Cambridge, MA) (Speth et al., 2002). To rule out possible side effects of MBP, recombinant MBP (New England Biolabs, Beverly, MA) was used as the negative control (Speth et al., 2002).

2.2. Cell cultures

PBMC were isolated from fresh blood of three different healthy donors by Percoll (Amersham Biosciences, Freiburg, Germany) density gradients (d=1.080) at 800 × g for 30 min. The PBMC were collected at the interface and washed twice with PBS. Then the cells were suspended at a concentration of 5×10^5 cells/ml and maintained in RPMI 1640 containing 5% FBS. The U937 monocytic cell line was obtained from ATCC (CRL-1593.2) and was grown at a density of 5–10 × 10^5 cells/ml in RPMI 1640-10% FBS.

Human microvascular endothelial cells (HMVEC) were obtained from Bio Whittaker (Verviers, Belgium) and maintained further in endothelial basal medium (EBM-2 MV, Bio Whittaker) supplemented with 5% FBS as has been described (Guenzi et al., 2001, 2003).

2.3. Cell adhesion assay

Cell adhesion experiments were carried out as described (Guenzi et al., 2001). HMVEC (passage 5) were seeded at 7 × 10^4 cells/well in eight-well microchamber slides (Nunc, Wiesbaden, Germany) and incubated overnight in EBM-2.5% FBS. Human recombinant TNF-α (Roche Diagnostic, Mannheim, Germany) was added in EBM–0.5% FBS for 6 h at the concentrations indicated in the figures. Subsequently, 500 μl of a suspension of U937 cells (5×10^5 cells/ml) or of PBMC (1×10^6 cells/ml) were added, and after 15 min non-adherent cells were gently removed by dipping the slides into RPMI 1640–1% FBS. Afterwards, the cells were fixed (30 min, RPMI 1640–1% FBS–5% glutaraldehyde) and the adherent cells were counted using a photo-imaging system (Optimas, Stemmer Imaging, Puchheim, Germany). Each experiment was carried out in triplicate and eight different optical fields were counted of each well. Results are expressed as the mean (±S.D.) of three independent experiments. Statistical significance was assessed using the Student's t-test. A P value of ≤0.05 was regarded as statistically significant.

For FACS analysis HMVEC were seeded at 1 × 10^6 cells in culture dishes (8 cm^2) and, as described above, incubated overnight in EBM–2.5% FBS and stimulated in EBM–0.5% FBS containing TNF-α for 6 h. Afterwards, 2 ml of a suspension of PBMC (1 × 10^6 cells/ml) were added, and after 15 min non-adherent cells were removed by gentle washing of the cells twice with PBS. Subsequently, the adherent cells were harvested by treatment with Accutase (PPA Laboratories, Coelbe, Germany) and re-suspended in PBS–1% BSA–0.01% NaAz. Then the cells (1 × 10^6 cells/100 μl) were incubated with FITC-conjugated anti-CD45 mAb (μg/ml, IgG1), anti-CD3 mAb (5 μg/ml, IgG1), anti-CD20 mAb (5 μg/ml, IgG1) or PerCP-conjugated anti-CD14 mAb (0.5 μg/ml, IgG2b) for 30 min at RT. To prove staining specificity the cells were incubated for 30 min at RT with FITC-conjugated IgG1 (5 μg/ml) and PerCP-conjugated IgG2b (5 μg/ml) control antibodies. All mAbs were purchased from BD Biosciences (Heidelberg, Germany). After washing the cells twice they were fixed with 2% paraformaldehyde and analyzed (10,000 events/measurement) with a FACSCalibur (BD Biosciences). The data were processed using the CellQuest programm (BD Biosciences). Experiments were repeated six times and the results are presented as the mean (±S.D.). Statistical significance was assessed using the Student's t-test. A P value of ≤0.05 was regarded as statistically significant.

2.4. Intravital microscopy

The experiments were approved by the Ethical Committee on Animal Experiments of Maastricht University (The Netherlands). Intravital microscopy was performed with C57Bl/6 mice of a body weight varying from 25 to

30 g. On the day of the experiments the mice were injected i.p. with either 100 ng ($n = 3$) recombinant murine TNF-α (R&D Systems, Abingdon, England), 20 μg HIV-1 Tat ($n = 3$), 20 μg HIV-1 Tat and 100 ng recombinant murine TNF-α ($n = 2$) or PBS–0.1% BSA ($n = 2$) as a control. Four hours later the mice were anesthetized by subcutaneous injection of a mixture of ketamine (0.1 mg/g body weight (b.w.), Nimatek, Ad Usem Veterinarium, Cuijk, The Netherlands) and xylazine (0.02 mg/g b.w., Sedamun, Ad Usem Veterinarium, Cuijk). To enable intravital microscopic fluorescence-based observation of leukocytes, 10–20 μl Rhodamine-6G (Sigma–Aldrich) dissolved in NaCl 0.9%-solution (1 mg/ml) and filtered through a 0.22 μm filter (Millipore, Etten-Leur, The Netherlands) were injected through a PE-10 catheter (Portrex, Kent, England) into the tail vein. Ear skin venules in both ears were visualized using a Leitz intravital microscope carrying a Leitz × 1.25 Ploemopak 2.2 for interchangeable filter sets and a Leitz × 25 salt water immersion objective (numerical aperture 0.60). To this purpose, the mouse was positioned on a preheated platform (3 °C), the ear was placed on a glass ring with the dorsal side up and covered with a drop of paraffin oil and a cover slip. Trans-illumination was performed using a 60 W tungsten lamp, while for fluorescence microscopy a Leitz N2.1 filter set (excitation filter BP 515–560, dichroic mirror RHP 580, and barrier filter LP580) was used in combination with a 100 W mercury lamp. Images were projected on a charge coupled device camera (Hamamatsu Photonics, C2400, Hamamatsu City, Japan), coupled to an intensifier unit (Hamamatsu Photonics A4405), and recorded on videotape (sVHS recorder, Panasonic, Japan) for off-line analysis. The final optical magnification at the front plane of the camera was 40×. In all experiments the mid-plane of a vessel was kept in focus.

Throughout the experiment, body temperature was kept at 37 °C with an infra-red heating lamp controlled by a thermo-analyzer system connected to a rectal probe.

2.4.1. Experimental parameters

Venules were selected within the diameter range of 15–45 μm. Leukocyte-vessel wall interactions were analyzed as described (Tromp et al., 2000). In short, leukocytes were considered as rolling when their velocity along the vessel wall was at least 10-fold lower than that of the free flowing blood cells. The level of leukocyte rolling was measured off-line by counting in duplicate the number of cells that rolled through a predefined segment of the vessel during a period of 120 s. Data were expressed as the number of rolling cells passing a vessel segment per minute.

The level of leukocyte adhesion was assessed in a 100 μm segment of the venule and expressed as the number of cells per endothelial surface area. Leukocytes were considered adherent when they remained stationary for at least 30 s.

2.4.2. Statistics

Because of their non-symmetrical distribution, data are presented as medians with interquartile ranges (i.e. the spread from 25th to 75th percentile). Differences between two independent data groups were tested with the Mann–Whitney *U*-test.

3. Results

3.1. HIV-1 Tat and TNF-α activate synergistically the adhesion of U937 cells and PBMC to endothelial cells

In vitro adhesion assays were performed with primary human microvascular endothelial cells and the promonocytic cell line U937. Activation of HMVEC with sub-optimal concentrations of TNF-α (0.5 ng/ml) resulted in a three-fold increase of U937 cell adhesion to endothelial cells (Fig.1A, ctrl., compare white and black columns). Incubation of U937 cells with increasing concentrations of the HIV-1 Tat protein stimulated the adhesion of these cells to HMVEC only slightly (1.7–2-fold) in an non significant manner (Fig. 1A, white columns). Simultaneous stimulation of U937 cells with HIV-1 Tat (10 and 100 ng/ml) and HMVEC with TNF-α (0.5 ng/ml) resulted in a strong synergistic increase (10-fold) of U937 cell adhesion to HMVEC (Fig. 1A, middle and right black columns). The differences of the results obtained by stimulation with either TNF-α or HIV-1 Tat alone as compared to the simultaneous stimulation with both factors were significant [$P \leq 0.007$ (10 ng/ml HIV-1 Tat), $P \leq 0.003$ (100 ng/ml HIV-1 Tat)].

Adhesiveness of U937 cells to TNF-α-stimulated endothelial cells was significantly ($P \leq 0.01$) and selectively activated by the HIV-1 Tat protein but not by the HIV-1 envelope protein gp41 or by Nef, another regulatory protein of HIV-1 (Fig. 1B). Similarly to U937 cells, also adhesion of PBMC was significantly ($P \leq 0.03$) and selectively increased (1.6-fold) by HIV-1 Tat but not by gp41 and by Nef (Fig. 1C).

3.2. HIV-1 Tat preferentially activates adhesion of primary monocytes and T-lymphocytes to HMVEC

In order to examine which cell type (B-cells, T-cells or monocytes) present in PBMC is preferentially activated by HIV-1 Tat to adhere to the endothelium, adhesion experiments were performed with PBMC. Leukocytes bound to endothelial cells were stained with different cell-type associated markers and quantified by FACS analysis (Figs. 2 and 3).

TNF-α treatment of HMVEC increased the numbers (within 10,000 counted cells) of adherent $CD45^+$ leukocytes (Fig. 2A versus B), $CD3^+$ T-cells (Fig. 2E versus F), $CD20^+$ B-cells (Fig. 2G versus H) and $CD14^+$ monocytes (Fig. 2I versus K). The relative number of $CD45^-$ endothelial cells was decreased (Fig. 2C versus D), due to increased

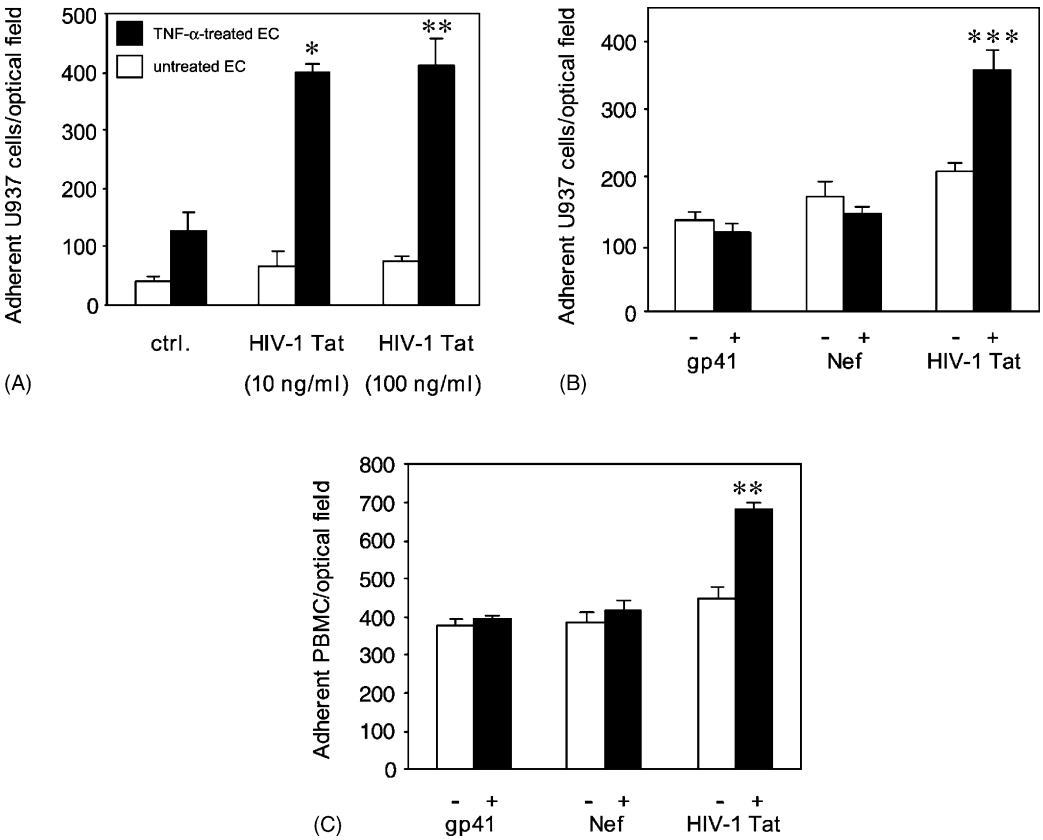

Fig. 1. HIV-1 Tat and TNF-α increase synergistically the adhesion of leukocytes to HMVEC in vitro. In vitro adhesion of U937 cells (A, B) and PBMC (C) to HMVEC. (A) HMVEC were either non-stimulated (white columns) or stimulated with TNF-α (0.5 ng/ml, black columns). U937 cells were either non-stimulated (ctrl.) or activated with increasing concentrations of HIV-1 Tat. (∗) $P \leq 0.007$ and (∗∗) $P \leq 0.003$ for the differences of the results obtained by stimulation with either TNF-α or HIV-1 Tat alone as compared to the simultaneous stimulation with both factors. (B) HMVEC were treated with TNF-α (0.1 ng/ml). U937 cells were either treated with gp41 (182 ng/ml, left, black column), Nef (100 ng/ml, middle, black column) or HIV-1 Tat (100 ng/ml, right, black column) or were non-stimulated (white columns). (∗∗∗) $P \leq 0.01$ compared to non-stimulated U937-cells. (C) The same conditions as described in (B) but PBMC were used instead of U937 cells. (∗∗∗) $P \leq 0.03$ compared to non-stimulated PBMC. In each experiment stimulation of HMVEC was carried out for 6 h and stimulation of U937 cells and PBMC for 24 h. In each case the mean values and standard deviations of three different experiments are shown.

numbers of $CD45^+$ cells in the 10,000 cells evaluated. Staining specificity was demonstrated with isotype-matched IgG1 and IgG2b control antibodies (Fig. 2L–O). Calculation of the number of adherent cells per 1000 endothelial cells confirmed that TNF-α treatment of HMVEC increased (two-fold) the adhesiveness to endothelial cells of all leukocyte populations examined, including T-cells ($P \leq 0.02$), B-cells ($P \leq 0.02$) and monocytes ($P \leq 0.05$) (Fig. 2P).

The treatment of PBMC with the HIV-1 Tat protein further increased the relative numbers (within 10,000 counted cells) of $CD45^+$ leukocytes (Fig. 3A and B) adhering to the TNF-α-stimulated HMVEC. However, HIV-1 Tat selectively increased the numbers of $CD3^+$ T-cells (Fig. 3E and F) and $CD14^+$ monocytes (Fig. 3I and K) adherent to endothelial cells, but not of $CD20^+$ B-cells (Fig. 3G and H). Again and in agreement with the experimental set-up the increase in leukocyte adhesion was paralleled by a relative decrease of the numbers of $CD45^-$ endothelial cells (Fig. 3C and D). Staining specificity was demonstrated whith isotype matched IgG1 and IgG2b control antibodies (Fig. 3L–O). Calculation of the number of adherent cells per 1000 endothelial cells indicated that HIV-1 Tat increases adhesion of leukocytes by 1.8-fold ($P \leq 0.02$) (Fig. 3P) which is consistent with the previous adhesion experiments (Fig. 1C). Within the adherent cells the numbers of monocytes were increased 2.2-fold ($P \leq 0.02$) and those of $CD3^+$ T-cells 1.8-fold ($P \leq 0.02$). In contrast, the number of adherent B-cells was not significantly increased by HIV-1 Tat stimulation (Fig. 3P).

3.3. HIV-1 Tat and TNF-α activate synergistically the adhesion of leukocytes to endothelial cells in vivo

To investigate the effect of HIV-1 Tat and TNF-α on leukocyte adhesion in vivo an intravital microscopy study was performed. In the mouse model used TNF-α at concentrations of 500 ng significantly increases adhesion of leukocytes (Dirkx et al., 2003). In order to evaluate potential synergistic effects of HIV-1 Tat and TNF-α sub-optimal concentrations of TNF-α (100 ng) were used that still significantly

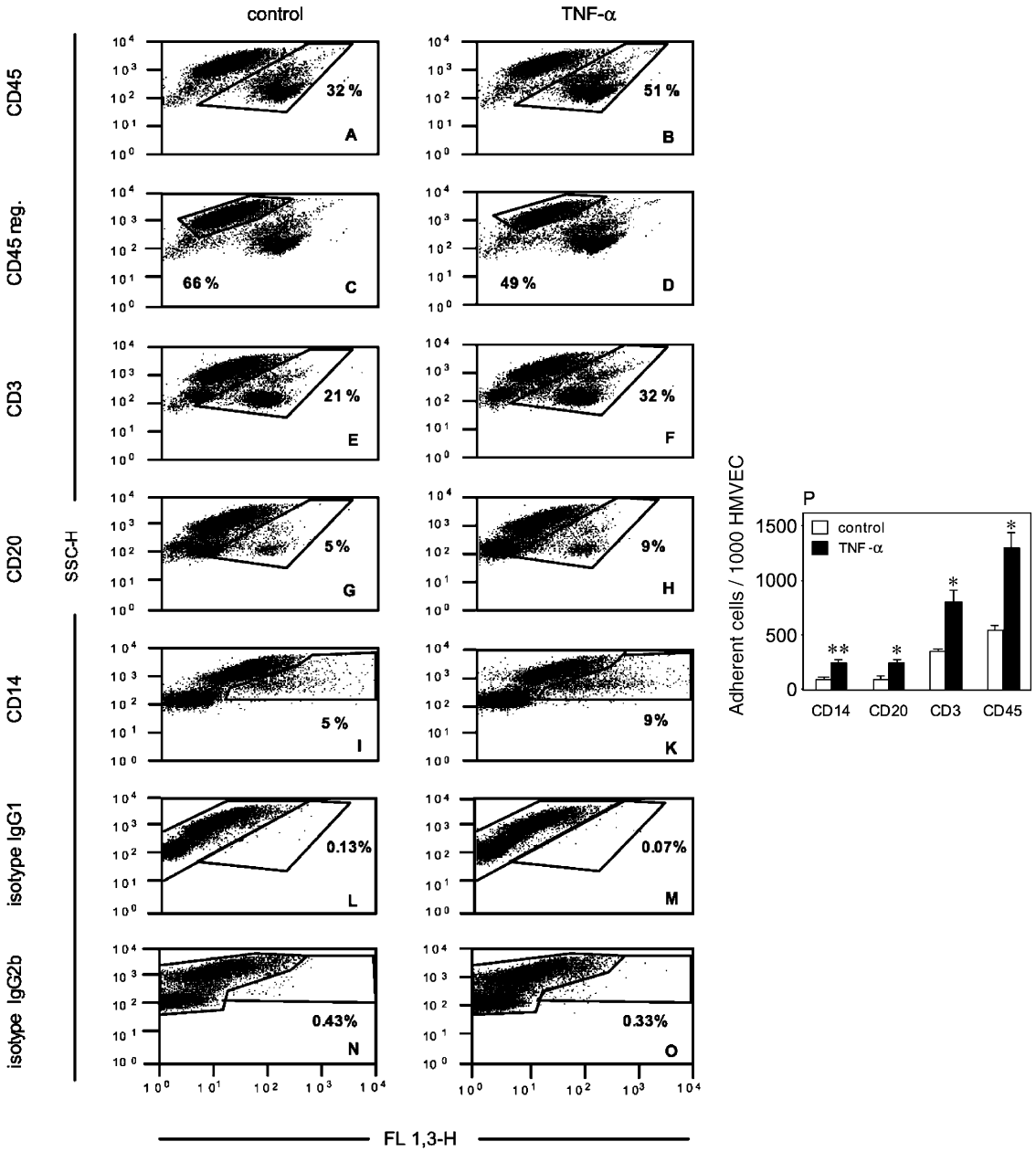

Fig. 2. TNF-α stimulation of HMVEC increases the adhesion of T-cells, B-cells and monocytes. HMVEC that were either non-stimulated (control) or treated for 6 h with TNF-α (0.1 ng/ml) were over-laid with freshly isolated PBMC. Non-adherent cells were removed by washing. Adherent cells and HMVEC were harvested, stained with FITC (FL1-H)-coupled antibodies to CD45 (A and B), CD3 (E and F), CD20 (G and H) and a PE (FL3-H)-coupled antibody to CD14 (I and K) and quantitatively determined by FACS analysis of 10,000 cells. CD45 negative cells (CD45 neg.) represent the HMVEC (C and D). To prove staining specificity the cells were incubated with isotype matched control antibodies (L–O). A representative result of three independent experiments is shown. In (P) the relative numbers of $CD45^+$, $CD3^+$, $CD20^+$ and $CD14^+$ cells that bound to 1000 HMVEC are graphically displayed. The mean values and standard deviations were calculated from three different experimental points. (∗) $P \leq 0.02$ and (∗∗) $P \leq 0.05$ compared to unstimulated HMVEC.

($P \leq 0.001$) increased leukocyte rolling (Fig. 4A) but had no effect on the adhesion of leukocytes to the vessel wall (Fig. 4B). HIV-1 Tat was also used in a concentration (20 μg) that significantly ($P \leq 0.001$) stimulated rolling (Fig. 4A) but only slightly stimulated cell adhesion (Fig. 4B). The combined application of HIV-1 Tat and TNF-α significantly increased adhesion of leukocytes ($P \leq 0.001$, Fig. 4B) at the expense of the number of rolling cells (Fig. 4A). From earlier studies it is known that the rolling of leukocytes along the endothelium is the necessary step preceding leukocyte adhesion (von Andrian et al., 1991). The present data strongly suggest that the combination of HIV-1 Tat and TNF-α significantly upregulates expression of adhesion molecules in endothelial cells, resulting in a fast transition of rolling to adhesion. This is illustrated by photographs of microvessels of the different groups (Fig. 4C–F). In the control situation a

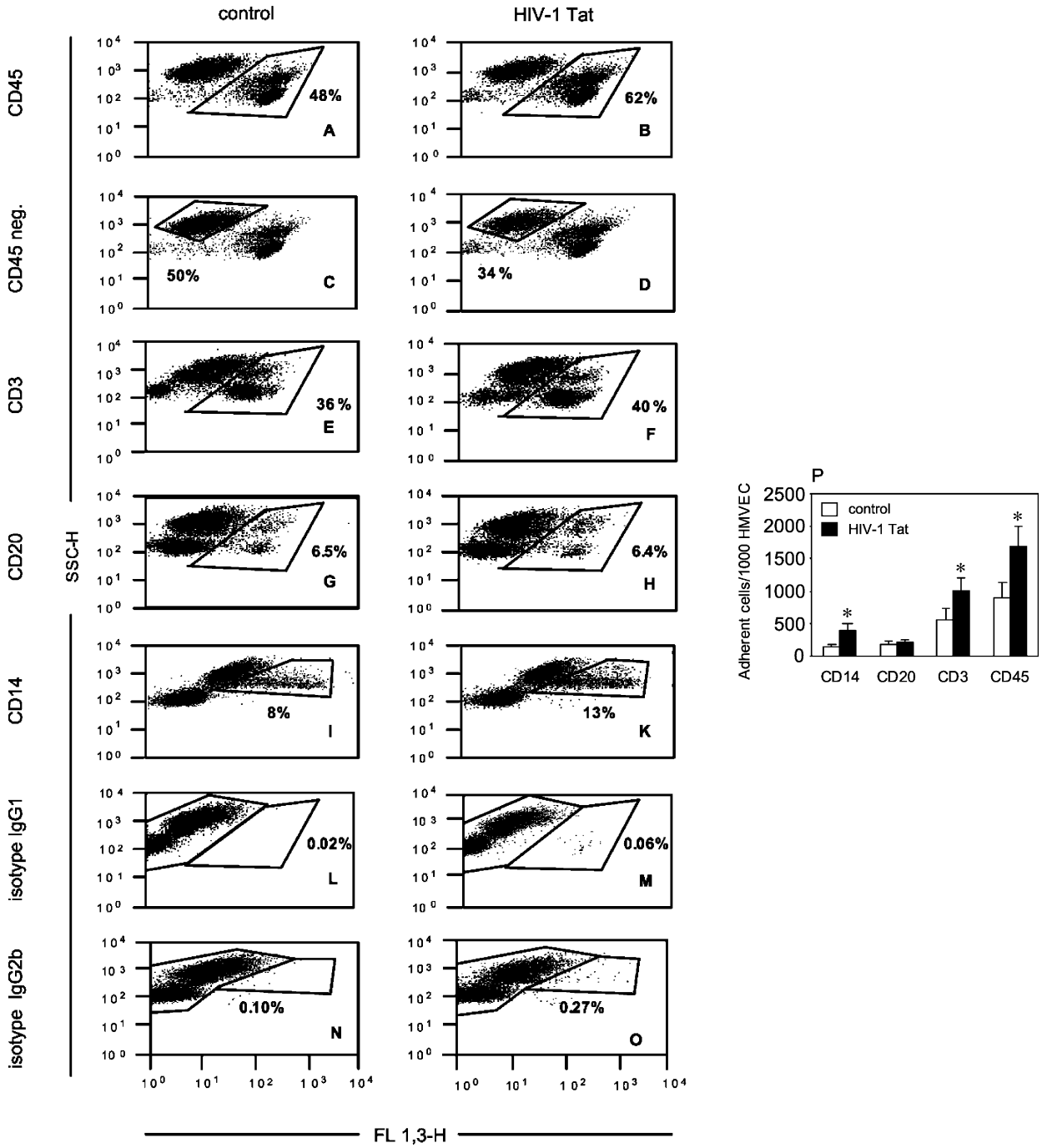

Fig. 3. HIV-1 Tat stimulation of PBMC increases selectively the adhesion of monocytes and T-cells. Freshly isolated PBMC were either non-stimulated (control) or treated for 24 h with HIV-1 Tat (100 ng/ml) and applied onto TNF-α (0.1 ng/ml, 6 h)-treated HMVEC. Non-adherent cells were removed by washing. Adherent cells and HMVEC were harvested, stained with FITC (FL1-H)-coupled antibodies to CD45 (A and B), CD3 (E and F), CD20 (G and H) and a PerCP (FL3-H)-coupled antibody to CD14 (I and K) and quantitatively determined by FACS analysis of 10,000 cells. CD45 negative cells (CD45 neg.) represent the HMVEC (C and D). To prove staining specificity the cells were incubated with isotype matched control antibodies (L–O). A representative result of three independent experiments is shown. In (P) the relative numbers of $CD45^+$, $CD3^+$, $CD20^+$ and $CD14^+$ cells that bound to 1000 HMVEC are graphically displayed. The mean values and standard deviations were calculated from three different experimental points. (∗) $P \leq 0.02$ compared to control.

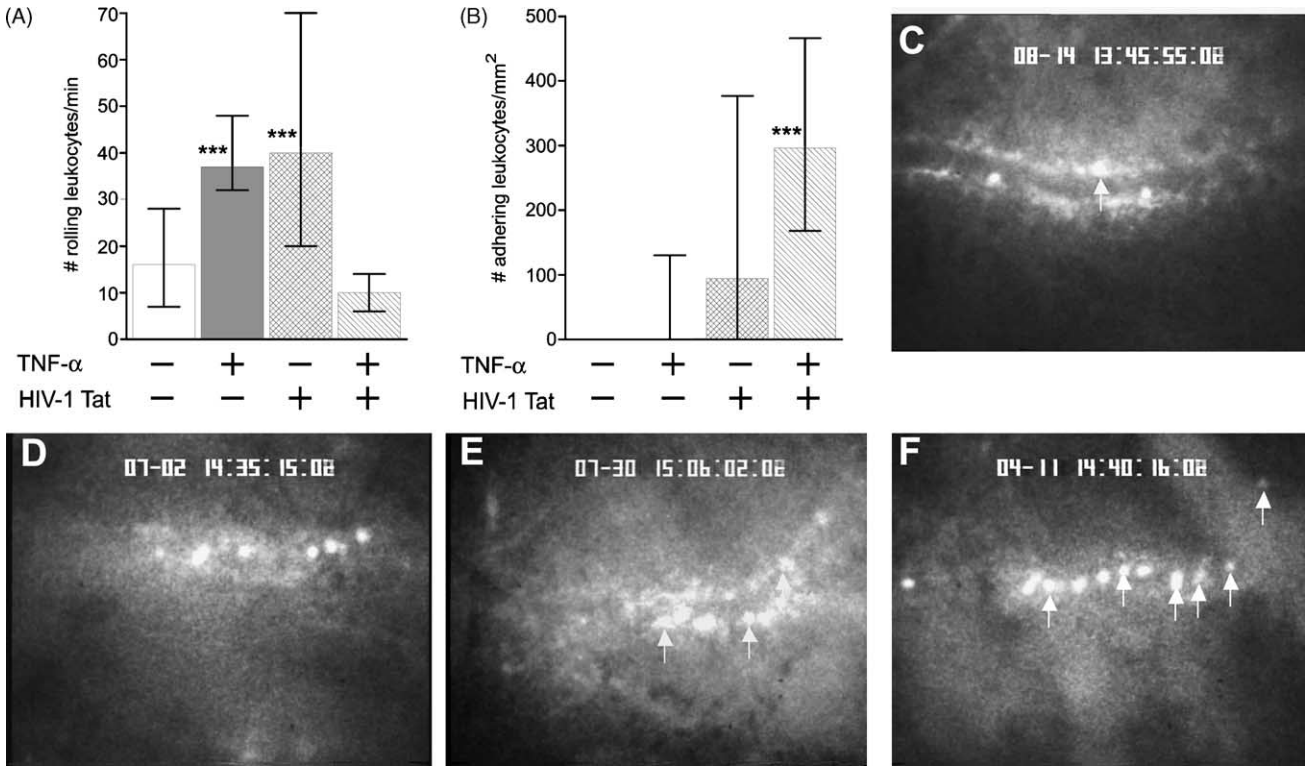

Fig. 4. HIV-1 Tat and TNF-α increase synergistically the adhesion of leukocytes to the endothelium in vivo. C57Bl/6 mice were injected i.p. with either TNF-α (100 ng, $n = 3$), HIV-1 Tat (20 μg, $n = 3$), a combination of both factors ($n = 2$) or with an equivalent volume of dilution medium (PBS/0.1%BSA, $n = 2$). By intravital microscopy (A) the numbers of rolling cells passing a vessel segment per minute and (B) the numbers of adherent leukocytes per mm^2 endothelial surface area were determined. Because of their non-symmetrical distribution data are presented as medians and interquartile ranges. (***) $P \leq 0.001$ compared to untreated mice. Images of intravital fluorescence microscopy are shown of ear venules in mice treated with (C) PBS/0.1% BSA, (D) 100 ng TNF-α, (E) 20 μg Tat and (F) a combination of both factors. Adhering leukocytes are indicated by arrows. All other leukocytes were rolling.

low level of leukocyte-vessel wall interactions was observed (Fig. 4C), which increased after administration of TNF-α (Fig. 4D), or HIV-1 Tat (Fig. 4E). In all of these cases most leukocytes rolled along the vessel wall, whereas only few leukocytes adhered stationary (Fig. 4C–E, indicated by an arrow). Administration of both substances together increased leukocyte-vessel wall interactions further, but most importantly, now most cells adhered stationary (white arrows) and fewer cells were rolling (Fig. 4F).

4. Discussion

The endothelium of HIV-1-infected patients is characterized by increased numbers of adherent leukocytes as compared to non-infected patients (Zietz et al., 1996). This may support the recruitment of lympho-/monocytes and of human herpesvirus-8 into AIDS-KS lesions (Blasig et al., 1997; Stürzl et al., 2001). In addition, in the era of HAART, with significantly elongated survival times of the patients, this may contribute to the increased risk of cardiovascular diseases and atherosclerosis in HIV-1-infected individuals (Acevedo et al., 2002; Bonnet et al., 2002).

The HIV-1 Tat protein in vitro has been shown to affect the monocyte/endothelial cell interaction. HIV-1 Tat up-regulates the expression of β2-integrin adhesion molecules and MMP-9 expression in monocytes, which increases monocyte/endothelial cell adhesion, enhances monocyte dependent damage of endothelial cell monolayers, and promotes invasive behavior of monocytes (Lafrenie et al., 1996a,b, 1997).

HIV-1 Tat has also been shown to be active in mice. Transgenic mice expressing HIV-1 Tat develop tumors (Corallini et al., 1993; Vogel et al., 1988), and inoculation into mice of purified HIV-1 Tat in combination with IC or bFGF can lead to the formation of vascular lesions (Albini et al., 1994; Barillari et al., 1999b; Ensoli et al., 1994a; Fiorelli et al., 1998). In the present study we demonstrated for the first time that HIV-1 Tat and TNF-α can synergistically increase the adhesion of leukocytes to the endothelium in vivo. Although high concentrations of HIV-1 Tat (20 μg) as compared to TNF-α (100 ng) were used, it has to be considered that two features of HIV-1 Tat may quickly reduce the effective concentration of the protein in the circulation after intra-peritoneal injection. First, HIV-1 Tat with its basic sequence binds rapidly to heparan sulfate proteoglycans of the cell surface and the extracellular matrix (Chang et al., 1997). Second, it has been shown that 4 h after injection in mice HIV-1 Tat is delivered to all tissues and incorporated into the cells (Schwarze et al., 1999). For these reasons high

concentrations of HIV-1 Tat were used. However, in HIV-1 infected patients HIV-1 Tat is highly expressed and released by infected cells and may activate leukocytes in the near proximity, possibly in lymph nodes. Activated leukocytes after re-entering the circulation may adhere with increased efficiency to the endothelium of HIV-1-infected patients that is chronically exposed to increased concentrations of IC.

Our findings may be specifically of relevance for the pathogenesis of AIDS-KS. Early stage KS lesions are characterized by an inflammatory-granulation-type reaction with activated proliferating endothelial cells forming new vessel-like structures for review see (Ensoli and Stürzl, 1998; Stürzl et al., 2001). The nature of the inflammatory cell infiltrate of KS is important since it is the first to appear and precedes the appearance of the tumor cells, the so-called KS spindle cells. Immunohistochemical studies indicate a prevalent infiltration of monocyte-macrophages ($CD68^+$, $MAC387^+$) together with CD4-positive and CD8-positiv T-cells ($CD3^+$), whereas B-cells ($CD19^+$, $CD20^+$) are rare or absent (Fiorelli et al., 1998; MacPhail et al., 1996; Nickoloff and Griffiths, 1989; Regezi et al., 1993; Tabata et al., 1993; Uccini et al., 1997). Our findings that the co-operative activity of HIV-1 Tat and TNF-α on adhesion selectively targets monocytes and T-cells is well in agreement with the leukocyte infiltration observed in AIDS-KS lesions. The co-operative activity of both factors may explain the 20,000-fold increased incidence of KS in AIDS (Beral et al., 1990) and possibly may contribute to other vascular defects in AIDS that are associated with increased leukocyte adhesion and extravasation into tissues, including cardiovascular diseases and artherosclerosis.

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References

Acevedo, M., Sprecher, D.L., Calabrese, L., Pearce, G.L., Coyner, D.L., Halliburton, S.S., White, R.D., Sykora, E., Kondos, G.T., Hoff, J.A., 2002. Pilot study of coronary atherosclerotic risk and plaque burden in HIV patients: 'a call for cardiovascular prevention'. Atherosclerosis 163 (2), 349–354.

Albini, A., Barillari, G., Benelli, R., Gallo, R.C., Ensoli, B., 1995. Angiogenic properties of human immunodeficiency virus type 1 Tat protein. Proc. Natl. Acad. Sci. U.S.A. 92, 4838–4842.

Albini, A., Soldi, R., Giunciuglio, D., Giraudo, E., Benelli, R., Primo, L., Noonan, D., Salio, M., Camussi, G., Rockl, W., Bussolino, F., 1996. The angiogenesis induced by HIV-1 tat protein is mediated by the Flk-1/KDR receptor on vascular endothelial cells. Nat. Med. 2, 1371–1375.

Albini, A., Fontanini, G., Masiello, L., Tacchetti, C., Bigini, D., Luzzi, P., Noonan, D.M., Stetler-Stevenson, W.G., 1994. Angiogenic potential in vivo by Kaposi's sarcoma cell-free supernatants and HIV-1 tat product: inhibition of KS-like lesions by tissue inhibitor of metalloproteinase-2. AIDS 8, 1237–1244.

Arya, S.K., Guo, C., Josephs, S.F., Wong-Staal, F., 1985. Trans-activator gene of human T-lymphotropic virus type III (HTLV-III). Science 229, 69–73.

Barillari, G., Buonaguro, L., Fiorelli, V., Hoffman, J., Michaels, F., Gallo, R.C., Ensoli, B., 1992. Effects of cytokines from activated immune cells on vascular cell growth and HIV-1 gene expression. Implications for AIDS-Kaposi's sarcoma pathogenesis. J. Immunol. 149, 3727–3734.

Barillari, G., Gendelman, R., Gallo, R.C., Ensoli, B., 1993. The Tat protein of human immunodeficiency virus type 1, a growth factor for AIDS Kaposi sarcoma and cytokine-activated vascular cells, induces adhesion of the same cell types by using integrin receptors recognizing the RGD amino acid sequence. Proc. Natl. Acad. Sci. U.S.A. 90, 7941–7945.

Barillari, G., Sgadari, C., Fiorelli, V., Samaniego, F., Colombini, S., Manzari, V., Modesti, A., Nair, B.C., Cafaro, A., Stürzl, M., Ensoli, B., 1999a. The Tat protein of human immunodeficiency virus type-1 promotes vascular cell growth and locomotion by engaging the alpha5beta1 and alphavbeta3 integrins and by mobilizing sequestered basic fibroblast growth factor. Blood 94, 663–672.

Barillari, G., Sgadari, C., Palladino, C., Gendelman, R., Caputo, A., Morris, C.B., Nair, B.C., Markham, P., Nel, A., Stürzl, M., Ensoli, B., 1999b. Inflammatory cytokines synergize with the HIV-1 Tat protein to promote angiogenesis and Kaposi's sarcoma via induction of basic fibroblast growth factor and the alpha v beta 3 integrin. J. Immunol. 163, 1929–1935.

Beral, V., Peterman, T.A., Berkelman, R.L., Jaffe, H.W., 1990. Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection. Lancet 335, 123–128.

Blasig, C., Zietz, C., Haar, B., Neipel, F., Esser, S., Brockmeyer, N.H., Tschachler, E., Colombini, S., Ensoli, B., Stürzl, M., 1997. Monocytes in Kaposi's sarcoma lesions are productively infected by human herpesvirus 8. J. Virol. 71, 7963–7968.

Bonnet, F., Morlat, P., Chene, G., Mercie, P., Neau, D., Chossat, I., Decoin, M., Djossou, F., Beylot, J., Dabis, F., 2002. Causes of death among HIV-infected patients in the era of highly active antiretroviral therapy, Bordeaux, France, 1998–1999. HIV Med. 3, 195–199.

Chang, H.C., Samaniego, F., Nair, B.C., Buonaguro, L., Ensoli, B., 1997. HIV-1 Tat protein exits from cells via a leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region. AIDS 11, 1421–1431.

Chang, H.K., Gallo, R.C., Ensoli, B., 1995. Regulation of cellular gene expression and function by the human immunodeficiency virus type 1 tat protein. J. Biomed. Sci. 2, 189–202.

Corallini, A., Altavilla, G., Pozzi, L., Bignozzi, F., Negrini, M., Rimessi, P., Gualandi, F., Barbanti-Brodano, G., 1993. Systemic expression of HIV-1 tat gene in transgenic mice induces endothelial proliferation and tumors of different histotypes. Cancer Res. 53, 5569–5575.

Dhawan, S., Puri, R.K., Kumar, A., Duplan, H., Masson, J.M., Aggarwal, B.B., 1997. Human immunodeficiency virus-1-tat protein induces the cell surface expression of endothelial leukocyte adhesion molecule-1, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 in human endothelial cells. Blood 90, 1535–1544.

Dirkx, A.E., Oude Egbrink, M.G., Kuijpers, M.J., van der Niet, S.T., Heijnen, V.V., Bouma-ter Steege, J.C., Wagstaff, J., Griffioen, A.W., 2003. Tumor angiogenesis modulates leukocyte-vessel wall interactions in vivo by reducing endothelial adhesion molecule expression. Cancer Res. 63, 2322–2329.

Ensoli, B., Barillari, G., Salahuddin, S.Z., Gallo, R.C., Wong-Staal, F., 1990. Tat protein of HIV-1 stimulates growth of cells derived

from Kaposi's sarcoma lesions of AIDS patients. Nature 345, 84–86.

Ensoli, B., Buonaguro, L., Barillari, G., Fiorelli, V., Gendelman, R., Morgan, R.A., Wingfield, P., Gallo, R.C., 1993. Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. J. Virol. 67, 277–287.

Ensoli, B., Gendelman, R., Markham, P., Fiorelli, V., Colombini, S., Raffeld, M., Cafaro, A., Chang, H.K., Brady, J.N., Gallo, R.C., 1994a. Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. Nature 371, 674–680.

Ensoli, B., Markham, P., Kao, V., Barillari, G., Fiorelli, V., Gendelman, R., Raffeld, M., Zon, G., Gallo, R.C., 1994b. Block of AIDS-Kaposi's sarcoma (KS) cell growth, angiogenesis and lesion formation in nude mice by antisense oligonucleotide targeting basic fibroblast growth factor. A novel strategy for the therapy of KS. J. Clin. Invest. 94, 1736–1746.

Ensoli, B., Sgadari, C., Barillari, G., Sirianni, M.C., Stürzl, M., Monini, P., 2001. Biology of Kaposi's sarcoma. Eur. J. Cancer 37, 1251–1269.

Ensoli, B., Stürzl, M., 1998. Kaposi's sarcoma: a result of the interplay among inflammatory cytokines, angiogenic factors and viral agents. Cytokine Growth Factor Rev. 9, 63–83.

Fanales-Belasio, E., Moretti, S., Nappi, F., Barillari, G., Micheletti, F., Cafaro, A., Ensoli, B., 2002. Native HIV-1 Tat protein targets monocyte-derived dendritic cells and enhances their maturation, function, and antigen-specific T cell responses. J. Immunol. 168, 197–206.

Ferrari, A., Pellegrini, V., Arcangeli, C., Fittipaldi, A., Giacca, M., Beltram, F., 2003. Caveolae-mediated internalization of extracellular HIV-1 tat fusion proteins visualized in real time. Mol. Ther. 8, 284–294.

Fiorelli, V., Gendelman, R., Samaniego, F., Markham, P.D., Ensoli, B., 1995. Cytokines from activated T cells induce normal endothelial cells to acquire the phenotypic and functional features of AIDS-Kaposi's sarcoma spindle cells. J. Clin. Invest. 95, 1723–1734.

Fiorelli, V., Gendelman, R., Sirianni, M.C., Chang, H.K., Colombini, S., Markham, P.D., Monini, P., Sonnabend, J., Pintus, A., Gallo, R.C., Ensoli, B., 1998. gamma-Interferon produced by CD8+ T cells infiltrating Kaposi's sarcoma induces spindle cells with angiogenic phenotype and synergy with human immunodeficiency virus-1 Tat protein: an immune response to human herpesvirus-8 infection? Blood 91, 956–967.

Guenzi, E., Töpolt, K., Cornali, E., Lubeseder-Martellato, C., Jörg, A., Matzen, K., Zietz, C., Kremmer, E., Nappi, F., Schwemmle, M., Hohenadl, C., Barillari, G., Tschachler, E., Monini, P., Ensoli, B., Stürzl, M., 2001. The helical domain of GBP-1 mediates the inhibition of endothelial cell proliferation by inflammatory cytokines. Embo. J. 20, 5568–5577.

Guenzi, E., Töpolt, K., Lubeseder-Martellato, C., Jörg, A., Naschberger, E., Benelli, R., Albini, A., Stürzl, M., 2003. The guanylate binding protein-1 GTPase controls the invasive and angiogenic capability of endothelial cells through inhibition of MMP-1 expression. Embo. J. 22, 3772–3782.

Kobayashi, S., Hamamoto, Y., Kobayashi, N., Yamamoto, N., 1990. Serum level of TNF alpha in HIV-infected individuals. AIDS 4, 169–170.

Kohleisen, B., Gaedigk-Nitschko, K., Ohlmann, M., Gotz, E., Ostolaza, H., Goni, F.M., Erfle, V., 1996. Heparin-binding capacity of the HIV-1 NEF-protein allows one-step purification and biochemical characterization. J. Virol. Methods 60, 89–101.

Kumar, A., Manna, S.K., Dhawan, S., Aggarwal, B.B., 1998. HIV-Tat protein activates c-Jun N-terminal kinase and activator protein- 1a. J. Immunol. 161, 776–781.

Lafrenie, R.M., Wahl, L.M., Epstein, J.S., Hewlett, I.K., Yamada, K.M., Dhawan, S., 1996a. HIV-1-Tat modulates the function of monocytes and alters their interactions with microvessel endothelial cells. A mechanism of HIV pathogenesis. J. Immunol. 156, 1638–1645.

Lafrenie, R.M., Wahl, L.M., Epstein, J.S., Hewlett, I.K., Yamada, K.M., Dhawan, S., 1996b. HIV-1-Tat protein promotes chemotaxis and invasive behavior by monocytes. J. Immunol. 157, 974–977.

Lafrenie, R.M., Wahl, L.M., Epstein, J.S., Yamada, K.M., Dhawan, S., 1997. Activation of monocytes by HIV-Tat treatment is mediated by cytokine expression. J. Immunol. 159, 4077–4083.

Lahdevirta, J., Maury, C.P., Teppo, A.M., Repo, H., 1988. Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome. Am. J. Med. 85, 289–291.

MacPhail, L.A., Dekker, N.P., Regezi, J.A., 1996. Macrophages and vascular adhesion molecules in oral Kaposi's sarcoma. J. Cutan. Pathol. 23, 464–472.

Medrano, F.J., Leal, M., Arienti, D., Rey, C., Zagliani, A., Torres, Y., Sanchez-Quijano, A., Lissen, E., Clerici, M., 1998. Tumor necrosis factor beta and soluble APO-1/Fas independently predict progression to AIDS in HIV-seropositive patients. AIDS Res. Hum. Retroviruses 14, 835–843.

Mitola, S., Sozzani, S., Luini, W., Primo, L., Borsatti, A., Weich, H., Bussolino, F., 1997. Tat-human immunodeficiency virus-1 induces human monocyte chemotaxis by activation of vascular endothelial growth factor receptor-1. Blood 90, 1365–1372.

Nickoloff, B.J., Griffiths, C.E., 1989. The spindle-shaped cells in cutaneous Kaposi's sarcoma. Histologic simulators include factor XIIIa dermal dendrocytes. Am. J. Pathol. 135, 793–800.

Pellicelli, A.M., Palmieri, F., Cicalini, S., Petrosillo, N., 2001. Pathogenesis of HIV-related pulmonary hypertension. Ann. N.Y. Acad. Sci. 946, 82–94.

Pugliese, A., Torre, D., Saini, A., Pagliano, G., Gallo, G., Pistono, P.G., Paggi, G.C., 2002. Cytokine detection in HIV-1/HHV-8 co-infected subjects. Cell Biochem. Funct. 20, 191–194.

Regezi, J.A., MacPhail, L.A., Daniels, T.E., DeSouza, Y.G., Greenspan, J.S., Greenspan, D., 1993. Human immunodeficiency virus-associated oral Kaposi's sarcoma. A heterogeneous cell population dominated by spindle-shaped endothelial cells. Am. J. Pathol. 143, 240–249.

Rinaldo Jr., C.R., Armstrong, J.A., Kingsley, L.A., Zhou, S., Ho, M., 1990. Relation of alpha and gamma interferon levels to development of AIDS in homosexual men. J. Exp. Pathol. 5, 127–132.

Schwarze, S.R., Ho, A., Vocero-Akbani, A., Dowdy, S.F., 1999. In vivo protein transduction: delivery of a biologically active protein into the mouse. Science 285, 1569–1572.

Sinicco, A., Biglino, A., Sciandra, M., Forno, B., Pollono, A.M., Raiteri, R., Gioannini, P., 1993. Cytokine network and acute primary HIV-1 infection. AIDS 7, 1167–1172.

Speth, C., Schabetsberger, T., Mohsenipour, I., Stockl, G., Wurzner, R., Stoiber, H., Lass-Florl, C., Dierich, M.P., 2002. Mechanism of human immunodeficiency virus-induced complement expression in astrocytes and neurons. J. Virol. 76, 3179–3188.

Stürzl, M., Zietz, C., Monini, P., Ensoli, B., 2001. Human herpesvirus-8 and Kaposi's sarcoma: relationship with the multistep concept of tumorigenesis. Adv. Cancer Res. 81, 125–159.

Tabata, M., Langford, A., Becker, J., Reichart, P.A., 1993. Distribution of immunocompetent cells in oral Kaposi's sarcoma (AIDS). Eur. J. Cancer B Oral Oncol. 29B, 209–213.

Tromp, S.C., oude Egbrink, M.G., Dings, R.P., van Velzen, S., Slaaf, D.W., Hillen, H.F., Tangelder, G.J., Reneman, R.S., Griffioen, A.W., 2000. Tumor angiogenesis factors reduce leukocyte adhesion in vivo. Int. Immunol. 12, 671–676.

Uccini, S., Sirianni, M.C., Vincenzi, L., Topino, S., Stoppacciaro, A., Lesnoni La Parola, I., Capuano, M., Masini, C., Cerimele, D., Cella, M., Lanzavecchia, A., Allavena, P., Mantovani, A., Baroni, C.D., Ruco, L.P., 1997. Kaposi's sarcoma cells express the macrophage-associated antigen mannose receptor and develop in peripheral blood cultures of Kaposi's sarcoma patients. Am. J. Pathol. 150, 929–938.

Ullum, H., Cozzi Lepri, A., Bendtzen, K., Victor, J., Gotzsche, P.C., Phillips, A.N., Skinhoj, P., Klarlund Pedersen, B., 1997. Low production of interferon gamma is related to disease progression in HIV infection: evidence from a cohort of 347 HIV-infected individuals. AIDS Res. Hum. Retroviruses 13, 1039–1046.

Vaishnav, Y.N., Wong-Staal, F., 1991. The biochemistry of AIDS. Annu. Rev. Biochem. 60, 577–630.

Vogel, J., Hinrichs, S.H., Reynolds, R.K., Luciw, P.A., Jay, G., 1988. The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. Nature 335, 606–611.

von Andrian, U.H., Chambers, J.D., McEvoy, L.M., Bargatze, R.F., Arfors, K.E., Butcher, E.C., 1991. Two-step model of leukocyte-endothelial cell interaction in inflammation: distinct roles for LECAM-1 and the leukocyte beta 2 integrins in vivo. Proc. Natl. Acad. Sci. U.S.A 88, 7538–7542.

Westendorp, M.O., Frank, R., Ochsenbauer, C., Stricker, K., Dhein, J., Walczak, H., Debatin, K.M., Krammer, P.H., 1995. Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120A. Nature 375, 497–500.

Zietz, C., Hotz, B., Stürzl, M., Rauch, E., Penning, R., Löhrs, U., 1996. Aortic endothelium in HIV-1 infection: chronic injury, activation, and increased leukocyte adherence. Am. J. Pathol. 149, 1887–1898.