Angiogenesis **5**: 141–151, 2002.
© 2003 *Kluwer Academic Publishers. Printed in the Netherlands.*

Review

HIV-1 Tat protein and endothelium: From protein/cell interaction to AIDS-associated pathologies

Marco Rusnati & Marco Presta
General Pathology and Immunology, Department of Biomedical Sciences and Biotechnology, University of Brescia, Brescia, Italy

Received 5 August 2002; accepted 16 January 2003

Key words: AIDS, angiogenesis, cytokines, endothelium, heparan sulfate proteoglycans, HIV-1, integrins, Kaposi's sarcoma

Abstract

Tat protein, the transactivating factor of the human immunodeficiency virus type 1 (HIV-1), is a small cationic polypeptide that can be released from HIV-1 infected cells. Extracellular Tat elicits different biological responses in several types of target cells, including endothelial cells (ECs). In the present paper, we will review the various aspects from the laboratory bench to the bedside that characterize the tight relationship that exists between HIV-1 Tat and the endothelium. Tat interacts with at least three different types of receptors present on the surface of ECs. This leads to the activation of several signal transduction pathways and triggers various biological responses in the endothelium. The bioavailability, cell interaction, intracellular signaling, and biological activity of Tat are tightly regulated by components of the extracellular matrix and circulating molecules. Thus, Tat is at the center of a complex network of interactions that occur at the surface of ECs and that greatly affect the functions of the endothelium, possibly resulting in some of the pathological processes that occur in AIDS patients.

Abbreviations: ECs – endothelial cells; ELAM-1 – endothelial leukocyte adhesion molecule 1; ECM – extracellular matrix; ERK – extracellular regulated kinase; FGF2 – basic fibroblast growth factor; FN – fibronectin; HIV – human immunodeficiency virus; HSPGs – heparan sulfates proteoglycans; ICAM-1 – intercellular adhesion molecule 1; INF – interferon; IL – interleukin; K_d – dissociation konstant; KS – Kaposi's sarcoma; MAPK – mitogen activated protein kinase; MCP-1 – monocyte chemoattractant protein-1; PAF – platelet-activating factor; PKC – protein kinase C; RGD – Arg–Gly–Asp; TGF-β – transforming growth factor-β; TNF-α – tumor necrosis factor-α; TSP – thrombospondin; VCAM-1 – vascular cell adhesion molecole 1; VEGF – vascular endothelial growth factor; VEGFR – vascular endothelial growth factor receptor

Introduction

The clinical features that characterize AIDS cannot be ascribed to the simple CD4+ cell infection by the human immunodeficiency virus (HIV). Indeed, several distinct pathological processes take place in HIV-infected individuals: central and peripheral neuropathies [1], early lymph node hyperplasia followed by lymphoid depletion [2, 3], pulmonary complications [4], nephritis [5, 6], vasculopathies [7], heart diseases [8], and an increased incidence of tumors [9]. This suggests that cell types other than CD4+ can be infected by HIV and/or can be altered in their functions by viral products.

Correspondence to: Dr Marco Rusnati, General Pathology and Immunology, Department of Biomedical Sciences and Biotechnology, Via Valsabbina 19, 25123 Brescia, Italy. Tel: +39-030-3717318; Fax: +39-030-3701157; E-mail: rusnati@med.unibs.it

Endothelial cells (ECs) are involved in different important stages of HIV biology:

(i) HIV can directly bind to the surface of ECs [10] and penetrate the endothelium *via* transcytosis reaching susceptible organs [11].
(ii) ECs have been proposed as progenitors of the spindle cells that characterize Kaposi's sarcoma (KS), a hypervascularized lesion frequently found in male AIDS patients (discussed later).
(iii) HIV-infected patients show retinal microangiopathy, microaneurism formation, and loss of capillary cells [12, 13]. Also, the aortic endothelium of HIV-infected patients shows pyknotic nuclei, bare patches, syncytium formation, and increased expression of surface adhesion proteins [14]. These endothelial injuries and/or dysfunctions may favor

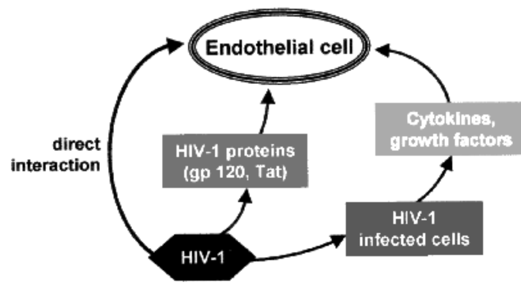

Figure 1. HIV-1 and ECs. HIV-1 can directly interact with (and infect?) ECs. Alternatively, viral products can induce EC dysfunctions. Finally, cytokines and growth factors aberrantly produced by HIV-infected CD4+ cells can target the endothelium altering its structural and functional integrity.

the extravasation of HIV-infected lymphocytes and macrophages and can contribute to the pathogenesis of AIDS-associated diseases [7, 15, 16].

In turn, HIV influences EC biology in different ways (Figure 1):

(i) HIV may productively infect ECs, even though this is strongly debated [15, 16]. The data point to a variable HIV permissivity of ECs from different anatomical districts [17–19].
(ii) Endothelial functions can be altered by cytokines and growth factors aberrantly produced by HIV-infected lymphocytes and/or monocytes [20].
(iii) HIV can alter EC functions by means of viral products that act as extracellular 'toxins'. In this regard, the HIV-1 transactivating factor (Tat) may play a major role [21].

Tat protein is a cationic 86–101 amino acid polypeptide that acts as the main transactivator of HIV [22]. Tat can be actively released by HIV-infected cells [20] and can be detected in the serum of HIV-infected individuals at concentrations equal to 0.1–1.0 ng/ml [23]. Extracellular Tat targets different types of uninfected cells causing a variety of biological effects, possibly related with distinct AIDS-associated pathologies. For instance, Tat is considered to be a neurotoxin implicated in the pathogenesis of AIDS dementia [24] and contributes to the immune suppression in AIDS patients by interfering with the function of different cells of immunity [9, 20]. Also, Tat plays a pivotal role in tumorigenesis and leads to KS in AIDS patients [9].

In the present paper, we will review the various aspects from the laboratory bench to the bedside that characterize the tight relationship that exists between extracellular Tat and ECs.

Interaction of extracellular Tat with ECs

Heparan sulfates proteoglycans (HSPGs) are composed of anionic glycosaminoglycan chains covalently bound to a core protein. HSPGs are expressed on the EC surface where they act as low affinity receptors for circulating growth factors and chemokines [25]. Binding to HSPGs induces growth factor oligomerization which, in turn, favors the dimerization of the cognate tyrosine kinase receptors and intracellular signaling [26]. Also, HSPGs mediate internalization of growth factors, their protection from lysosomal degradation [27], and possibly their nuclear delivery [28]. Finally, growth factors immobilized to HSPGs of the extracellular matrix (ECM) and cell surface may act as localized, persistent stimuli for ECs [25].

The Tat protein contains the so-called 'basic domain' composed of a stretch of positively charged amino acids (position 46–61). Tat binds through this basic domain to negatively charged sulfated groups of free heparin and heparan sulfates and to cell-surface HSPGs [29, 30, L. Tassone, personal communication]. As already demonstrated for other growth factors, the interaction with heparin leads to Tat oligomerization [31]. Also, the interaction of Tat with cell-surface HSPGs is required for Tat internalization and transactivating activity [32, L. Tassone, personal communication]. Finally, Tat binding to HSPGs leads to its accumulation in the ECM [33]. Likely, these phenomena may also occur at the surface of ECs (Figure 2). Accordingly, low affinity, high capacity binding sites for Tat have been identified on ECs [34].

Two types of signaling receptors activated by extracellular Tat have been identified so far on the surface of ECs: integrins and the vascular endothelial growth factor receptor-2 (VEGFR-2)/KDR.

Integrins are a family of heterodimeric receptors that promote EC adhesion to extracellular components and modulate EC proliferation [35]. Tat interacts with integrins present on ECs [34, 36, 37, C. Urbinati et al., in preparation]. The molecular bases of integrin/Tat interaction have been studied: The amino acid sequence Arg–Gly–Asp (RGD) represents a well known integrin recognition motif. This sequence is present at position 78–80 in the Tat protein and mediates specific interactions with integrins [38, C. Urbinati et al., in preparation]. However, the basic domain of Tat also contributes significantly to integrin interaction [34, C. Urbinati et al., in preparation].

Among the various integrins, $\alpha_v\beta_3$ and $\alpha_5\beta_1$, but not of $\alpha_v\beta_5$, promote EC adhesion to immobilized Tat [39, C. Urbinati et al., in preparation]. In contrast, $\alpha_v\beta_5$ integrin is responsible for the adhesion of rat skeletal muscle myoblast to Tat [40], suggesting that distinct integrins are involved in the adhesion of different cell types to the immobilized Tat. As stated before, Tat can be found in the ECM as an immobilized protein, thus suggesting that EC adhesion to Tat may be physiologically relevant.

Tat/integrin interaction leads to biological consequences other than cell adhesion. Indeed, we have recently observed that EC adhesion to immobilized Tat induces phosphorylation of the p125 focal adhesion kinase (p125FAK). Interestingly, p125FAK activation is also induced when Tat is administered as a free molecule to ECs adherent to tissue culture plastic (C. Urbinati et al., in preparation). Relevant to this point, $\alpha_v\beta_3$

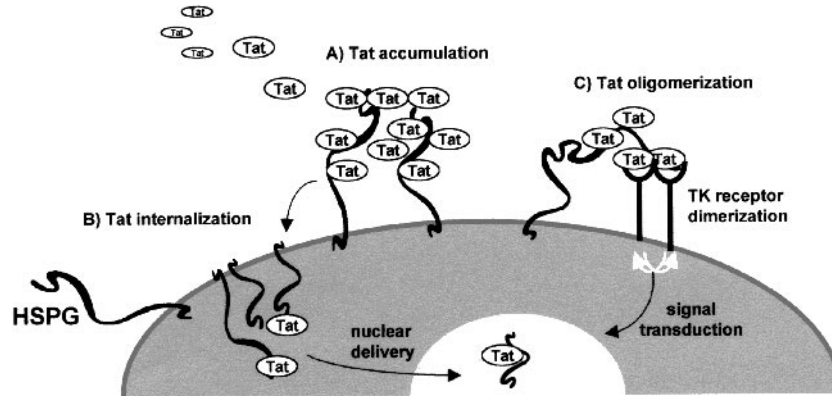

Figure 2. HSPG/Tat interaction. (A) Tat binds with a high capacity to HSPGs of the EC surface, thus increasing its concentration in the microenvironment. (B) HSPGs mediate cell internalization of Tat (and its nuclear delivery?). (C) HSPGs favor Tat oligomerization which, in turn, promotes tyrosine kinase (TK) receptor dimerization and signal transduction.

integrin is expressed both at the basal and luminal aspects of ECs [41].

Tat/$\alpha_v\beta_3$ interaction is required for the angiogenic, chemotactic, and mitogenic activities exerted by Tat on ECs [34, 36, 39]. Accordingly, $\alpha_v\beta_3$ integrin co-localizes with the Tat-activated vessels *in vivo* [39]. Thus, EC integrins may play an important role in Tat biology, and may mediate distinct effects that depend on their engagement by the free or the immobilized form of Tat (Figure 3).

Extracellular Tat also binds to the tyrosine-kinase VEGFR-2/KDR present on the surface of ECs [42]. This interaction leads to KDR autophosphorylation and is required for Tat-induced EC proliferation and chemotaxis *in vitro* and for neovascularization [42] and vascular permeability *in vivo* [43]. The region of Tat involved in KDR interaction has been identified as the basic domain [42].

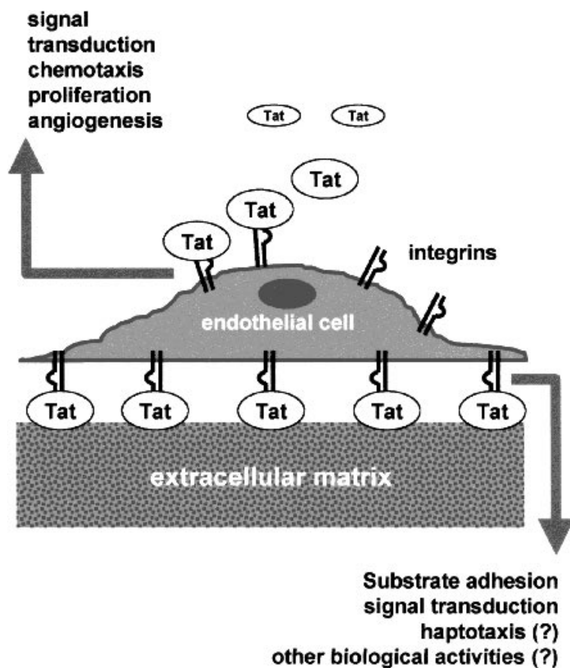

Figure 3. Interaction of EC $\alpha_v\beta_3$ integrin with free/immobilized Tat in ECs. Integrin engagement by free or immobilized extracellular Tat leads to different biological consequences.

Both integrins and VEGFR-2/KDR expression are upregulated by inflammatory cytokines, and this appears to confer Tat responsiveness to ECs [37, 44].

In conclusion, a complex network of interactions among Tat and different receptors can be envisaged at the surface of ECs. This may account for the multifaceted effects exerted by Tat in this cell type.

Modulation of the bioavailability, EC interaction, and biological activity of extracellular Tat by ECM

Various circulating molecules and ECM components regulate the bioavailability of extracellular Tat, its interaction with ECs, and biological activity. Among them, heparin binds Tat with high affinity [dissociation constant (K_d) equal to 10–30 nM] [31, 45]. Accordingly, heparin modulates several biological activities exerted by Tat in different cell types including ECs (Table 1). Interestingly, as observed for fibroblast growth factor-2 (FGF2)/heparin interaction [25], the glycosaminoglycan

Table 1. Biological activities of Tat modulated by heparin.

Cell	Biological activity	Reference
Endothelial cells	Chemotaxis ↑	[42]
	Proliferation ↑	[42]
	Cell adhesion ↑	Rusnati et al., unpublished data
	$ERK_{1/2}$ activation ↑	Rusnati et al., unpublished data
	Angiogenesis ↑	[42]
	MCP-1 production ↑	[61]
Epithelial cells	PA production ↑	[128]
	LTR transactivation ↓	[30]
	Cell proliferation ↓	[30]
T cells	Cell proliferation ↓	[74]

↑ and ↓ indicate an enhancement and an inhibition of the biological activity of Tat, respectively.

can exert either agonist or antagonist effects on Tat activity. Indeed, heparin induces Tat oligomerization [31] that may favor the clustering and activation of tyrosine-kinase receptors. Also, heparin protects Tat from proteolytic degradation [33]. On the other hand, high concentrations of free heparin may compete with cellular receptors for Tat interaction. Indeed, free heparin mobilizes Tat from a heparin-coated surface that mimics cell-surface HSPGs [45] and mobilizes Tat from cell-associated HSPG storage [33].

Thrombospondin-1 (TSP) is another ECM component that has been demonstrated to bind Tat and to affect its biological activity in ECs. TSP is a heparin-binding trimeric protein of 440 kDa secreted by several cell types including endothelial and inflammatory cells [46]. *In vivo* TSP is found as a free molecule or in a cell-associated, HSPG-bound form [47]. TSP modulates in vitro EC adhesion, motility, and proliferation [48], and inhibits angiogenesis *in vivo* [49]. These effects are related to the binding of TSP to EC receptors, including integrin $\alpha_v\beta_3$ and HSPGs [46], and to angiogenic growth factors (e.g. FGF2) [50].

TSP binds Tat with high affinity (K_d equal to 10–30 nM) [51]. Also, TSP inhibits Tat-induced chemoattraction of ECs *in vitro* [52] and Tat-dependent neovascularization *in vivo* [51, 52]. Even though TSP may exert its inhibitory effects on ECs by sequestering Tat in the extracellular environment [51], alternative mechanisms of action may also be possible, including the competition for the binding of Tat to HSPGs and integrin $\alpha_v\beta_3$. Indeed, TSP inhibits the stimulation of ECs by Tat at concentrations equal to 0.002–0.2 nM, significantly lower than the K_d value for Tat/TSP interaction.

Fibronectin (FN) shares with TSP several biological features. Indeed, like TSP, FN can be found both as free and ECM-associated molecules; it binds integrins and heparin/HSPGs; it regulates the biological effects exerted by Tat on ECs [53]. These effects appear to be mediated by the concerted action of FN and Tat on integrin interaction and signal transduction. Interestingly, Tat regulates the synthesis of FN in different cell types [54–56] including ECs [53], thus suggesting that a tight cross-talk may exist between Tat and FN.

Epithelin/granulin growth factors have been demonstrated to bind to Tat [57]. Being produced and released as paracrine/autocrine growth factors by hemic cells, they may regulate the activity of Tat on ECs [58].

In conclusion, Tat can be envisaged at the center of a complex network of interactions in which the alternative binding of different ECM components to Tat itself or to its cell-surface receptors modulates its bioavailability, cell interaction, and biological activity (see Figure 4).

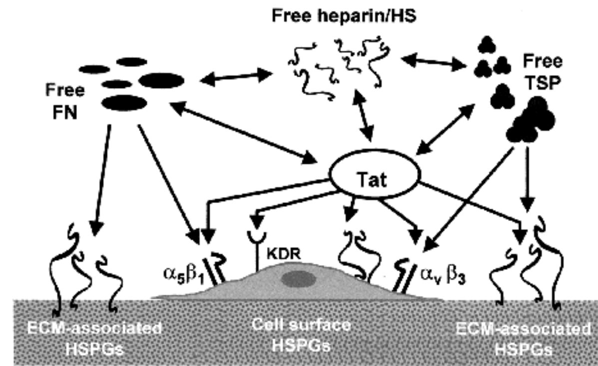

Figure 4. The network of interactions between extracellular Tat and free or ECM-associated molecules. Tat can bind various ECM components (including FN, TSP, and HSPGs) both in their free and immobilized forms. Also, Tat and ECM components compete for the interaction with various receptors (including integrins and transmembrane HSPGs).

Extracellular Tat and signal transduction in ECs

The signal transduction pathways generated by the engagement of EC tyrosine-kinase VEGFR-2/KDR and integrins by extracellular Tat are far from being fully elucidated. Figure 5 represents an attempt to summarize the current knowledge about intracellular second messengers triggered by the interaction of Tat with the EC cell surface.

Phosphoinositide 3-kinase (PI3K) is activated by extracellular Tat in ECs following VEGFR-2/KDR engagement [34]. Accordingly, VEGF similarly triggers PI3K activation under the same experimental conditions. Also, a recombinant form of Tat mutated in its basic domain loses the capacity to activate VEGFR-2/KDR and PI3K. In contrast, amino acid substitutions R78K and D80E in Tat protein disrupt the RGD sequence and impair the capacity of Tat to mediate EC adhesion, proliferation and chemotaxis, without affecting PI3K activation [34].

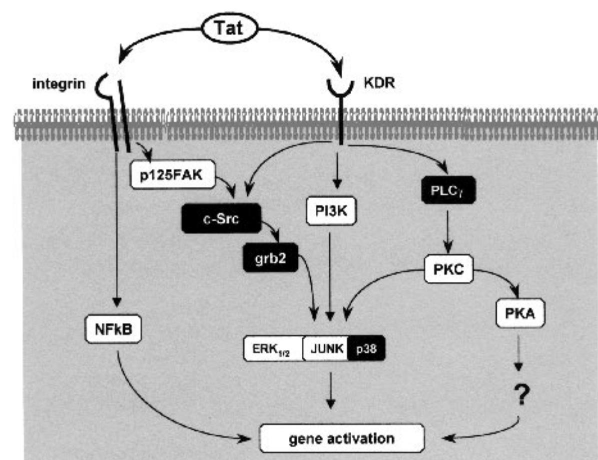

Figure 5. Signal transduction pathways activated by Tat in ECs. Interaction of extracellular Tat with integrins and VEGR-2/KDR leads to the activation of several intracellular second messengers. Pathways depicted in white boxes have been shown to occur in Tat-activated ECs, those in the black boxes are activated by Tat in different cell types and may also occur in ECs (see text for further details).

P125FAK is an integrin-coupled intracellular kinase involved in EC proliferation [59]. We have recently observed that Tat induces p125FAK phosphorylation in ECs (C. Urbinati et al., in preparation).

Zidovetzki et al. [60] demonstrated that extracellular Tat induces a fast and transient increase in protein kinase C (PKC) activity in ECs, with consequent increase in intracellular cAMP levels and cAMP-dependent PKA activation. Interestingly, the activation of the PKC/cAMP/PKA signal transduction pathway represents an absolute requirement for Tat-mediated interleukin (IL)-6 up-regulation in ECs. Also, PKC activation leads to up-regulation of monocyte chemoattractant protein-1 (MCP-1) secretion by lung microvascular ECs [61].

NF-κB is activated by extracellular Tat in several cell types [20]. We recently observed that Tat activates NF-κB in ECs [C. Urbinati et al., in preparation]. Also, $\alpha_v\beta_3$ integrin engagement, but not $\alpha_v\beta_5$ integrin engagement, appears to be implicated in NF-κB activation by extracellular Tat (C. Urbinati et al., in preparation). In turn, NF-κB activation by extracellular Tat mediates up-regulation of E-selectin [62], endothelial leukocyte adhesion molecule 1 (ELAM-1), vascular cell adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1) [63] in ECs.

Also, the mitogen activated protein kinases (MAPKs)/extracellular regulated kinases 1/2 ($ERK_{1/2}$) are activated by extracellular Tat in a wide spectrum of cell types including ECs [see Ref. 64 and references therein]. Studies performed with ECs overexpressing VEGFR-2/KDR implicated this receptor in $ERK_{1/2}$ phosphorylation [64, 65]. However, our unpublished experiments performed with anti-integrin antibodies also indicate that this class of receptors contributes to $ERK_{1/2}$ activation. This suggests that the signal transduction pathways generated by integrins and VEGFR-2/KDR engagement by Tat converge on $ERK_{1/2}$ (see Figure 5). Similar conclusions have been drawn for $ERK_{1/2}$ activation following FGF2 interactions with integrins [66] and its tyrosine-kinase receptors [67]. $ERK_{1/2}$ activation mediates the increase in endothelium permeability triggered by Tat [68], but it is not implicated in Tat-induced neovascularization [64] and MCP-1 up-regulation [61].

Also, the c-jun amino-terminal kinase (JNK1) is rapidly activated by Tat in human ECs [69]. This activation has been tentatively associated with Tat-induced angiogenesis and EC surface expression of adhesion proteins [69].

More second messengers triggered by extracellular Tat in various cell types may also play a role in EC activation (see Figure 5): phospholipase C gamma (PLCγ) [70] and the growth factor receptor bound protein-3 (grb3) [71] are activated by extracellular Tat in T cells; MAPK p38 in microglia/astrocytes [72]; focal tyrosine kinase (RAF-TK), paxillin, Src kinase and p130^{cas} in KS-derived 'spindle cells' [73]. This latter observation is particularly relevant due to the tight correlation existing between ECs and KS cells (see below).

Biological activity exerted by extracellular Tat in ECs

Given the variety of receptors engaged and activated by extracellular Tat, it can be anticipated that Tat may induce a broad spectrum of biological effects in ECs.

As already described, Tat induces EC adhesion to the substrate, mimicking classical ECM proteins such as FN and vitronectin [36]. Since Tat can be found in an immobilized form in ECM [33], it is tempting to hypothesize that ECM-bound Tat may induce EC adhesion and may act at the same time as a localized, persistent stimulus for EC stimulation (Figure 3). Indeed, immobilized Tat retains its biological activity under defined experimental conditions [74, 75].

Extracellular Tat plays a dual role in EC proliferation (Figure 6). It promotes the transition from G1 to S phase of the EC cycle [76]. Accordingly, Tat and Tat-derived peptides promote the proliferation of ECs in culture [34, 36, 65, 77–79]. Interestingly, Tat exerts its mitogenic effect in the presence of heparin [77, 79, 80]. In contrast, Tat inhibits the growth of ECs adherent to FN [53], thus confirming the role of ECM components in mediating the biological activity of Tat (see Figure 4).

In turn, Tat influences the mitogenic activity of other growth factors. Barillari et al. [39] demonstrated that Tat retrieves soluble bioactive FGF2 from ECM storage that, in turn, stimulates ECs proliferation. In contrast, Jia et al. [81] observed that Tat peptides inhibit signal transduction, mitogenesis and angiogenesis by FGF2 and VEGF in ECs.

As observed for EC proliferation, extracellular Tat also exerts a dual role in EC apoptosis (Figure 6). Cantaluppi et al. [82] demonstrated that Tat acts as a survival factor for human umbilical vein ECs subjected to serum withdrawal. This effect correlates with the decrease of caspase-3 activity and with the upregulation of Bcl-X_L but not with Fas, Bcl-2 or Bax expression. On the contrary, other groups demonstrated that Tat induces apoptosis in primary human microvascular ECs [81, 83]. Tat-driven apoptosis depends upon the

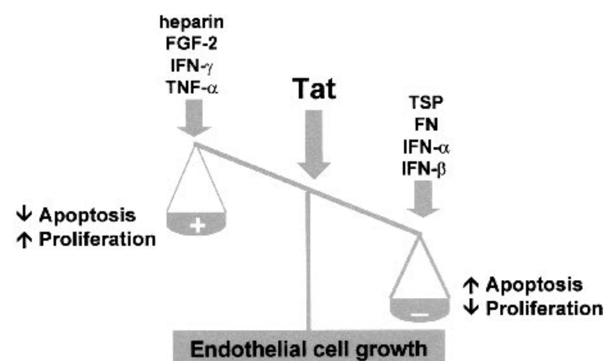

Figure 6. Dual role of extracellular Tat in EC proliferation and apoptosis: modulation by cytokines and ECM components. Tat may stimulate or inhibit EC growth depending on the modulation exerted by different molecules that affect its mitogenic and apoptotic balance. ↑ and ↓ indicate an enhancement and an inhibition of the biological activity of Tat, respectively.

activation of caspase-3 but not of Fas, Bcl-2, Bax or Bad. These data give rise to the hypothesis that the pro- or anti-apoptotic activity exerted by Tat in ECs may depend upon their origin. Indeed, brain endothelium, but not bone marrow endothelium is susceptible to Tat-mediated apoptosis (unpublished data from T. Kim and S. Avraham reported in [83]). Also, distinct EC receptors may transduce pro- or anti-apoptotic signals. Indeed, both VEGFR-2/KDR [82] and integrin receptors [84, 85] mediate the protective effect exerted by Tat against EC apoptosis. Finally, extracellular Tat internalized through HSPGs may control apoptosis by intracellular mechanisms. This possibility is supported by the observation that intracellular Tat affects apoptosis in T cells [86].

Whatever be the mechanism of action, an uncontrolled apoptotic process may have relevant consequences at a clinical level. For instance, it may cause vessel damage in the lung or brain endothelium, thus facilitating the transit of HIV virions and HIV-infected cells [83]. Conversely, inhibition of the apoptotic process may sustain EC viability during angiogenesis or during their transition to the KS cell phenotype [82].

Tat induces EC motility, accelerating the repair of a wounded EC monolayer [87]. Accordingly, Tat induces the chemotactic migration of ECs [34, 36, 42, 52, 79, 88]. The basic domain and the RGD sequence contribute to the chemotactic activity of Tat, suggesting that both VEGFR-2/KDR and integrin receptors are involved in this process [34].

Tat upregulates the proteolytic capacity of ECs. Indeed, it enhances the production and secretion of matrix-metalloproteinase-2 (MMP-2) [39, 78, 89], MMP-9 [89] and inhibits the secretion of tissue inhibitor of metalloproteinases-1 (TIMP-1) and TIMP-2 [89]. As a result, Tat-treated ECs degrade collagen IV more efficiently than untreated control cells [88]. At variance, Tat does not affect urokinase-type plasminogen activator (uPA) production in ECs [53], whereas it increases the release of plasminogen activator inhibitor-1 (PAI-1) [90].

Cell migration and protease production contribute to the increased invasive capacity of ECs stimulated by Tat. Indeed, Tat-treated cells invade collagen and/or Matrigel-coated filters [39, 79, 88] and form tube-like structures in tridimensional collagen gels or Matrigel [65, 80, 88].

Proliferation, protease production and morphogenesis are part of the so-called 'angiogenic program' activated in ECs during neovascularization. Accordingly, Tat is angiogenic *in vivo* [91]. This has been demonstrated in the rabbit cornea assay [79], in the s.c. murine Matrigel plug assay [34, 42, 52, 77, 78, 80, 87], and in the chick embryo chorioallantoic membrane assay [45, 64, 65]. In the latter two models, neovascularization can be also observed in the presence of Tat-overexpressing cells [51, 92–94]. The angiogenic activity of Tat is mediated by VEGFR-2/KDR [42], even though integrin $\alpha_v\beta_3$ engagement may also contribute to the process [36, C. Urbinati et al., in preparation]. In apparent discrepancy with these observations, the possibility that Tat may exert anti-angiogenic effects has been suggested [81, 95].

By competing for the binding to HSPGs, Tat retrieves FGF2 from the ECM in a bioactive form [96]. Thus, it is possible to hypothesize that the angiogenic activity of Tat is the indirect consequence of the release of canonic angiogenic growth factors, including FGF2 [78]. In contrast with this hypothesis, we have observed that inhibitors of $ERK_{1/2}$ activation inhibit FGF2-dependent neovascularization without affecting Tat-induced angiogenesis [64]. Accordingly, VEGFR-2/KDR activation is essential for the angiogenic activity of Tat but not of FGF2 [42].

Neovascularization induced by Tat may account for the microangiopathy and related visual defects [97] and for the increased incidence of tumors, in particular lymphomas, described in AIDS patients [98, 99]. Accordingly, *tat*-transgenic mice develop different tumors, including lymphomas [100, 101].

Overexpression of Tat *in vivo* has been associated with the increase of hypervascularized lesions that resemble KS [36, 76–78, 101–103]. Even though a direct correlation between Tat and KS is strongly debated [104], it is very likely that Tat contributes to the intense neovascularization of AIDS-associated KS by stimulating ECs. More disputed is the possibility that Tat may induce normal ECs to acquire the functional features of AIDS-KS spindle cells [76, 78, 105]. Indeed, the origin of KS-spindle cells is uncertain since they express mixed markers of macrophage/dendritic cells and vascular/lymphatic ECs [106, 107]. Whatever be the origin of KS-spindle cells, it is interesting to note that they share with ECs the capacity to respond to extracellular Tat (Table 2). Thus, despite the topic still being debatable, several observations point to a role for Tat in AIDS-KS. This likely occurs with the co-operation of several inflammatory cytokines and human herpes virus 8 co-infection [108].

Tat decreases the electrical resistance of the endothelium [68] and increases its permeability to albumin [43, 68, 89]. The mechanism(s) of Tat-induced blood vessel permeability have not been fully elucidated even though its capacity to mimic the vascular permeability factor VEGF may play a role. Also, it is interesting to note that Tat up-regulates EC production of IL-6 (see below) that is known to increase endothelial permeability [109].

Table 2. Biological responses to extracellular Tat shared by ECs and KS-derived spindle cells.

Biological activity	Endothelial cells	KS-derived spindle cells
KDR activation	[42, 43]	[129]
Integrin activation	[34, 36–38]	[38]
PI3K activation	[34]	[130]
$ERK_{1/2}$ activation	[64, 65, 68]	[73]
Cell adhesion	[34, 36–38]	[38]
Cell migration	[34, 36,42, 52, 79, 88]	[76, 82]
Cell proliferation	[34, 36, 65, 77–79]	[76, 82]
Cytokines upregulation	[43, 60, 61, 87, 90, 111, 113]	[131]

Tat induces the expression of ELAM-1, VCAM-1, ICAM-1 [63, 110] and of E-selectin [62, 90, 110, 111] in ECs. The up-regulation of these leukocyte receptors, as well as an increased permeability, may favor leukocyte extravasation. Indeed, Tat increases the adhesion of human monocytes to an EC monolayer [63, 110] and promotes their trans-endothelial migration [61]. This process may be relevant in the dissemination of viral infection in different tissues and leads to lymphomas often observed in HIV-infected individuals. Also, extravasation of HIV-infected monocytes may play a pivotal role in the damage of the central nervous system and cause AIDS-associated neuropathies [24].

Tat and inflammatory cytokines cross-talk

Many of the biological effects exerted by extracellular Tat occur only (or are potentiated) in ECs that have been preactivated by inflammatory cytokines [38, 76, 88, 105, 112].

Tumor necrosis factor-α (TNF-α) synergizes with Tat in inducing ICAM-1, VCAM-1 and ELAM-1 expression [63] and in up-regulating IL-6 [90, 111] and IL-8 [113] in ECs. Platelet activating factor (PAF) mediates the capacity of Tat to induce migration of ECs *in vitro* [87]. Also, PAF synergizes with Tat in inducing vascular permeability [43] and neovascularization [87] *in vivo*. Interestingly, PAF has been already demonstrated to also be a mediator of the angiogenic activity of hepatocyte growth factor [114] and VEGF [115]. Also, interferon-γ (IFN-γ) increases the responsiveness of ECs to Tat [37] and a cocktail of IL-1β, TNF-α and IFN-γ synergizes with Tat in inducing angio-proliferative KS-like lesions in nude mice [36]. The synergic action exerted by inflammatory cytokines on Tat activities may be relevant *in vivo* where their levels increase in the tissues and the blood of HIV-infected individuals [116].

The mechanism(s) by which inflammatory cytokines increase ECs' responsiveness to Tat depends, at least in part, on their capacity to increase the expression of Tat receptors: For instance TNF-α and IL-1β up-regulate the expression of VEGFR-2/KDR [44, 117], whereas INF-γ and IL-1β also affect $\alpha_v\beta_3$ integrin expression [37, 118] (see also Figure 7). An alternative mechanism has been proposed in which inflammatory cytokines up-regulate FGF2 that act as the true mediator of the biological activity of Tat in ECs [36, 78]. Indeed, FGF2 potentiates the capacity of Tat to induce MMP-2 expression, proliferation, chemotaxis, vascular permeability, angiogenesis and angioproliferative KS-like lesions in nude mice [36, 78, 89]. Interestingly, the cooperative effect of FGF2 is specific, since it is not shared by VEGF, and may depend, at least in part, on its capacity to increase the expression of $\alpha_v\beta_3$ integrin [36].

Some cytokines antagonize the biological activity exerted by extracellular Tat in ECs. Indeed, transforming growth factor-β (TGF-β) inhibits Tat-dependent up-regulation of IL-8 [113], and INF-α and INF-β inhibit

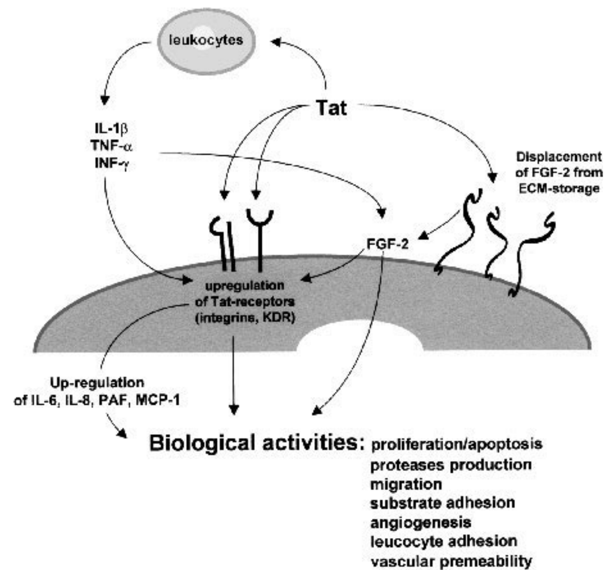

Figure 7. Tat, angiogenic growth factors and inflammatory citokines/chemokines cross-talk in ECs.

Tat-dependent proliferation, migration, and morphogenesis of ECs *in vitro* and Tat-induced neovascularization *in vivo* [80, 119]. Interestingly, TGF-β inhibits VEGFR-2/KDR expression in ECs [120].

It is interesting to note that extracellular Tat can affect the production of several cytokines that, in turn, modulate the activity of Tat. Tat upregulates MCP-1 [61, 43], IL-8 [113], PAF [87] and IL-6 [60, 90, 111] production in ECs. These molecules, and IL-6 in particular, may influence the passage from latency to productive HIV-1 replication and may enhance virus spreading during physiological and/or pathological interactions of monocytes with the endothelium [121, 122]. Also, Tat induces the production and the release of TNF by monocytes/macrophages [123] and lymphocytes [124], and of IL-1 by monocytes/macrophages [125]. This suggests that a cross-talk may exist in which extracellular Tat stimulates inflammatory cells to produce cytokines/chemokines that will increase the responsiveness of ECs to Tat (Figure 7).

In conclusion, a complex interplay can be envisaged in which the capacity of Tat to induce biological effects on the endothelium depends on synergies with inflammatory cytokines that potentiate the activity of Tat. On the other hand, the capacity of Tat to induce the production of cytokines/chemokines amplifies the spectrum and intensity of biological effects (see Figure 7). Also, it will be interesting to evaluate the possibility that, based on its strong affinity for heparin, Tat may mobilize heparin-binding growth factors other than FGF2.

Concluding remarks

Tat protein is a peculiar product of HIV-1 that exerts a broad spectrum of biological effects on ECs. These effects may in turn contribute significantly to some of

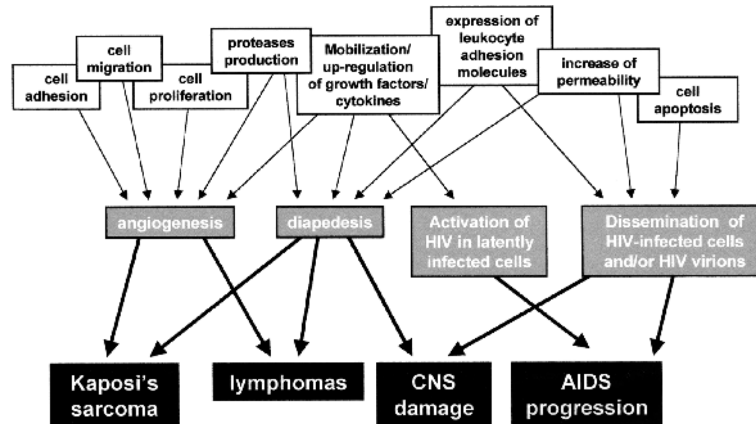

Figure 8. Biological activities exerted by extracellular Tat in ECs and associated pathological processes.

the pathological outcomes of AIDS (Figure 8). The action of Tat on ECs is regulated by its binding to several circulating molecules, ECM components, and at least three different receptor types present on the surface of ECs (Figure 4). Accordingly, Tat activates complex signal transduction pathways (Figure 5) and can be internalized by ECs to directly transactivate cellular genes (Figure 2). All these findings give rise to the suspicion that at least some of the features of Tat may be art factual and without a corresponding counterpart *in vivo*. On the other hand, the 'biochemical redundancy' displayed by Tat may be the result of a series of mimicking properties developed by this essential protein during the evolution of HIV. Moreover, the receptor-promiscuity shown by Tat is shared by eukaryotic growth factors. For instance, FGF2 binds HSPGs and tyrosine kinase FGF receptors [25, 35] and cell surface gangliosides [126]. Also, $\alpha_v\beta_3$ integrin and VEGFR-2/KDR co-operate in transducing EC activation by VEGF [127].

Based on the possible clinical outcomes derived from the interaction of Tat with the endothelium, this event can be considered as a target for the development of therapies aimed to control AIDS progression and AIDS-associated pathologies. To this purpose, it will be necessary to better characterize the complex network of interactions among extracellular Tat, circulating molecules, ECM, and EC surface.

Acknowledgements

The experimental work from our laboratory which is described in the present review was supported in part by grants from the Istituto Superiore di Sanità (AIDS Project), MURST (Cofin 2000: Project 'Trasduzione proteica attraverso le membrane biologiche: un approccio integrato di biologia molecolare e fisica biomolecolare') to M.R. and the from the European Community ('Biologically Active Novel Glycosaminoglycans' contract no. QLK3-CT-1999-00536), Associazione Italiana per la Ricerca sul Cancro, National Research Council (Target Project on Biotechnology), Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) (Cofin 2000: Project 'Meccanismi molecolari di comunicazione intracellulare: le molecole solubili') to M.P., and '60%' to M.R. and M.P.

References

1. Price RW. Neurological complications of HIV infection. Lancet 1996; 348: 445–52.
2. Burke AP, Benson W, Ribas JL et al. Postmortem localization of HIV-1 RNA by in situ hybridization in lymphoid tissues of intravenous drug addicts who died unexpectedly. Am J Pathol 1993; 142: 1701–13.
3. Fauci AS. Multifactorial nature of human immunodeficiency virus disease: Implications for therapy. Science 1993; 262: 1011–18.
4. Hopewell PC, Luce JM. Pulmonary involvement in the acquired immunodeficiency syndrome. Chest 1985; 87: 104–12.
5. McSherry GD. Human immunodeficiency-virus-related pulmonary infections in children. Semin Respir Infect 1996; 11: 173–83.
6. Klotman PE. HIV-associated nephropathy. Kidney Int 1999; 56: 1161–76.
7. Terada LS, Gu Y, Flores SC. AIDS vasculopathy. Am J Med Sci 2000; 320: 379–87.
8. Patel RC, Frishman WH. Cardiac involvement in HIV infection. Med Clin North Am 1996; 80: 1493–512.
9. Caputo A, Betti M, Boarini C et al. Multiple functions of human immunodeficiency virus type 1 Tat protein in the pathogenesis of AIDS. Recent Res Devel Virol 1999; I: 753–71.
10. Pohlmann S, Soilleux EJ, Baribaud F et al. DC-SIGNR, a DC-SIGN homologue expressed in ECs, binds to human and simian immunodeficiency viruses and activates infection in trans. Proc Natl Acad Sci USA 2001; 98: 2670–5.
11. Gujuluva C, Burns AR, Pushkarsky T et al. HIV-1 penetrates coronary artery ECs by transcytosis. Mol Med 2001; 7: 169–76.
12. Glasgow BJ. Evidence for breaches of the retinal vasculature in acquired immune deficiency syndrome angiopathy. A fluorescent microsphere study. Ophthalmology 1997; 104: 753–60.
13. Newsome DA, Green WR, Miller ED et al. Microvascular aspects of acquired immune deficiency syndrome retinopathy. Am J Ophthalmol 1984; 98: 590–601.
14. Zietz C, Hotz B, Sturzl M et al. Aortic endothelium in HIV-1 infection: Chronic injury, activation, and increased leukocyte adherence. Am J Pathol 1996; 149: 1887–98.
15. Chi D, Henry J, Kelley J et al. The effects of HIV infection on endothelial function. Endothelium 2000; 7: 223–42.

16. Bussolino F, Mitola S, Serini G et al. Interactions between ECs and HIV-1. Int J Biochem Cell Biol 2001; 33: 371–90.
17. Moses AV, Nelson JA. HIV infection of human brain capillary ECs-implications for AIDS dementia. Adv Neuroimmunol 1994; 4: 239–47.
18. Kanmogne GD, Grammas P, Kennedy RC. Analysis of human ECs and cortical neurons for susceptibility to HIV-1 infection and co-receptor expression. J Neurovirol 2000; 6: 519–28.
19. Kanmogne GD, Kennedy RC, Grammas P. Analysis of human lung ECs for susceptibility to HIV type 1 infection, coreceptor expression, and cytotoxicity of gp120 protein. AIDS Res Hum Retroviruses 2001; 17: 45–53.
20. Noonan D, Albini A. From the outside in: Extracellular activities of HIV Tat. Adv Pharmacol 2000; 48: 229–50.
21. Zagury D, Lachgar A, Chams V et al. Interferon alpha and Tat involvement in the immunosuppression of uninfected T cells and C–C chemokine decline in AIDS. Proc Natl Acad Sci USA 1998; 95: 3851–6.
22. Gatignol A, Jeang KT. Tat as a transcriptional activator and a potential therapeutic target for HIV-1. Adv Pharmacol 2000; 48: 209–27.
23. Westendorp MO, Frank R, Ochsenbauer C et al. Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature 1995; 375: 497–500.
24. Dewhurst S, Gelbard HA, Fine SM. Neuropathogenesis of AIDS. Mol Med Today 1996; January: 16–23.
25. Rusnati M, Presta M. Interaction of angiogenic basic fibroblast growth factor with endothelial cell heparan sulfate proteoglycans: Biological implications in neo-vascularization. Int J Clin Labor Res 1996; 26: 15–23.
26. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. Cell 1990; 61: 203–12.
27. Rusnati M, Urbinati C, Presta M. Internalization of basic fibroblast growth factor (bFGF) in cultured ECs: Role of the low affinity heparin-like bFGF-receptors. J Cell Physiol 1993; 154: 152–61.
28. Fedarko NS, Conrad E. A unique heparan sulfate in the nuclei of hepatocytes: Structural changes with the growth state of the cells. J Cell Biol 1986; 102: 587–99.
29. Rusnati M, Coltrini D, Oreste P et al. Interaction of HIV-1 Tat protein with heparin: Role of the backbone structure, sulfation, and size. J Biol Chem 1997; 272: 11313–20.
30. Rusnati M, Tulipano G, Urbinati C et al. The basic domain in HIV-1 Tat protein as a target for polysulfated heparin-mimicking extracellular Tat antagonists. J Biol Chem 1998; 273: 16027–37.
31. Rusnati M, Tulipano G, Spillmann D et al. Multiple interaction of HIV-1 Tat protein with size-defined heparin oligosaccharides. J Biol Chem 1999; 274: 28198–205.
32. Tyagi M, Rusnati M, Presta M et al. Internalization of HIV-1 Tat requires cell surface heparan sulfate proteoglycan. J Biol Chem 2001; 276: 3254–61.
33. Chang HC, Samaniego F, Nair BC et al. 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 1997; 11: 1421–31.
34. Mitola S, Soldi R, Zanon I et al. Identification of specific molecular structures of human immunodeficiency virus type 1 Tat relevant for its biological effects on vascular ECs. J Virol 2000; 74: 344–53.
35. Rusnati M, Tanghetti E, Dell'Era P et al. Alphavbeta3 integrin mediates the cell-adhesive capacity and biological activity of basic fibroblast growth factor (FGF-2) in cultured endothelial cells. Mol Biol Cell 1997; 8: 2449–61.
36. Barillari G, Sgadari C, Fiorelli V et al. 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 1999; 94: 663–72.
37. Fiorelli V, Barillari G, Toschi E et al. IFN-gamma induces ECs to proliferate and to invade the extracellular matrix in response to the HIV-1 Tat protein: Implications for AIDS-Kaposi's sarcoma pathogenesis. J Immunol 1999; 162: 1165–70.
38. Barillari G, Gendelman R, Gallo R et al. 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 USA 1993; 90: 7941–5.
39. Barillari G, Sgadari C, Palladino C et al. 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 1999; 163: 1929–35.
40. Vogel BE, Lee SJ, Hildebrand A et al. A novel integrin specificity exemplified by binding of the alpha v beta 5 integrin to the basic domain of the HIV Tat protein and vitronectin. J Cell Biol 1993; 121: 461–8.
41. Conforti G, Dominguez-Jimenez A, Zanetti MA et al. Human ECs express integrin receptors on the luminal aspect of their membrane. Blood 1992; 80: 437–46.
42. Albini A, Soldi R, Giunciuglio D et al. The angiogenesis induced by HIV-1 tat protein is mediated by the Flk-1/KDR receptor on vascular ECs. Nat Med 1996; 2: 1371–5.
43. Arese M, Ferrandi C, Primo L et al. HIV-1 Tat protein stimulates *in vivo* vascular permeability and lymphomononuclear cell recruitment. J Immunol 2001; 166: 1380–88.
44. Giraudo E, Primo L, Audero E et al. Tumor necrosis factor-alpha regulates expression of vascular endothelial growth factor receptor-2 and of its co-receptor neuropilin-1 in human vascular ECs. J Biol Chem 1998; 273: 22128–35.
45. Rusnati M, Urbinati C, Caputo A et al. Pentosan polysulfate as an inhibitor of extracellular HIV-1 Tat. J Biol Chem 2001; 276: 22420–5.
46. Mosher DF. Physiology of thrombospondin. Annu Rev Med 1990; 41: 85–97.
47. Schon P, Vischer P, Volker W et al. Cell-associated proteoheparan sulfate mediates binding and uptake of thrombospondin in cultured porcine vascular ECs. Eur J Cell Biol 1992; 59: 329–39.
48. Taraboletti G, Roberts D, Liotta LA et al. Platelet thrombospondin modulates endothelial cell adhesion, motility, and growth: A potential angiogenesis regulatory factor. J Cell Biol 1990; 111: 765–72.
49. Iruela-Arispe ML, Bornstein P, Sage H. Thrombospondin exerts an antiangiogenic effect on cord formation by ECs *in vitro*. Proc Natl Acad Sci USA 1991; 88: 5026–30.
50. Taraboletti G, Belotti D, Borsotti P et al. The 140-kilodalton antiangiogenic fragment of thrombospondin-1 binds to basic fibroblast growth factor. Cell Growth Differ 1997; 8: 471–9.
51. Rusnati M, Taraboletti G, Urbinati C et al. Thrombospondin-1/HIV-1 tat protein interaction: Modulation of the biological activity of extracellular Tat. FASEB J 2000; 14: 1917–30.
52. Taraboletti G, Benelli R, Borsotti P et al. Thrombospondin-1 inhibits Kaposi's sarcoma (KS) cell and HIV-1 Tat-induced angiogenesis and is poorly expressed in KS lesions. J Pathol 1999; 188: 76–81.
53. Cavallaro U, Mariotti M, Wu ZH et al. Fibronectin modulates endothelial response to HIV type 1 Tat. AIDS Res Hum Retroviruses 1997; 13: 1341–8.
54. Taylor JP, Cupp C, Diaz A et al. Activation of expression of genes coding for extracellular matrix proteins in Tat-producing glioblastoma cells. Proc Natl Acad Sci USA 1992; 89: 9617–21.
55. Maroder M, Scarpa S, Screpanti I et al. Human immunodeficiency virus type 1 tat protein modulates fibronectin expression in thymic epithelial cells and impairs *in vitro* thymocyte development. Cell Immunol 1996; 168: 49–58.
56. McArthur CP, Wang Y, Heruth D et al. Amplification of extracellular matrix and oncogenes in tat-transfected human salivary gland cell lines with expression of laminin, fibronectin, collagens I, III, IV, c-myc and p53. Arch Oral Biol 2001; 46: 545–55.

57. Trinh DP, Brown KM, Jeang KT. Epithelin/granulin growth factors: Extracellular cofactors for HIV-1 and HIV-2 Tat proteins. Biochem Biophys Res Commun 1999; 256: 299–306.
58. Shoyab M, McDonald VL, Byles C et al. Epithelins 1 and 2: Isolation and characterization of two cysteine-rich growth-modulating proteins. Proc Natl Acad Sci USA 1990; 87: 7912–16.
59. Tanghetti E, Ria R, Dell'Era P et al. Biological activity of substrate-bound basic fibroblast growth factor (FGF-2): Recruitment of FGF receptor-1 in endothelial cell adhesion contacts Oncogene 2001; 21: 3889–97.
60. Zidovetzki R, Wang JL, Chen P et al. Human immunodeficiency virus Tat protein induces interleukin 6 mRNA expression in human brain ECs via protein kinase C- and cAMP-dependent protein kinase pathways. AIDS Res Hum Retroviruses 1998; 14: 825–33.
61. Park IW, Wang JF, Groopman JE. HIV-1 Tat promotes monocyte chemoattractant protein-1 secretion followed by transmigration of monocytes. Blood 2001; 97: 352–8.
62. Cota-Gomez A, Flores NC, Cruz C et al. The human immunodeficiency virus-1 Tat protein activates endothelial cell E-selectin expression via an NF-kappa -dependent mechanism. J Biol Chem 2002; 277: 14390–9.
63. Dhawan S, Puri RK, Kumar A et al. Human immunodeficiency virus-1-tat protein induces the cell surface expression of endothelial leukocyte adhesion molecule-1, vascular cell adhesion molecule-1, and intercellular adhesionmolecule-1 in human ECs. Blood 1997; 90: 1535–44.
64. Rusnati M, Urbinati C, Musulin B et al. Activation of endothelial cell mitogen activated protein kinase $ERK_{1/2}$ by extracellular HIV-1 Tat protein. Endothelium 2001; 8: 65–74.
65. Scheidegger P, Weiglhofer W, Suarez S et al. Signalling properties of an HIV-encoded angiogenic peptide mimicking vascular endothelial growth factor activity. Biochem J 2001; 353: 569–78.
66. Eliceri BP, Klemke R, Stromblad S et al. Integrin $\alpha_v\beta_3$ requirement for sustained mitogen-activated protein kinase activity during angiogenesis. J Cell Biol 1998; 140: 1255–63.
67. Giuliani R, Bastaki M, Coltrini D et al. Role of endothelial cell extracellular signal-regulated $kinase_{1/2}$ in urokinase-type plasminogen activator upregulation and *in vitro* angiogenesis by fibroblast growth factor-2. J Cell Sci 1999; 112: 2597–606.
68. Oshima T, Flores SC, Vaitaitis G et al. HIV-1 Tat increases endothelial solute permeability through tyrosine kinase and mitogen-activated protein kinase-dependent pathways. AIDS 2000; 14: 475–42.
69. Gu Y, Wu RF, Xu YC et al. HIV Tat activates c-Jun aminoterminal kinase through an oxidant-dependent mechanism. Virology 2001; 286: 62–71.
70. Zauli G, Previati M, Caramelli E et al. Exogenous human immunodeficiency virus type-1 Tat protein selectively stimulates a phosphatidylinositol-specific phospholipase C nuclear pathway in the Jurkat T cell line. Eur J Immunol 1995; 25: 2695–700.
71. Li X, Multon MC, Henin Y et al. Grb3-3 is up-regulated in HIV-1-infected T-cells and can potentiate cell activation through NFATc. J Biol Chem 2000; 275: 30925–33.
72. Kutsch O, Oh J, Nath A et al. Induction of the chemokines interleukin-8 and IP-10 by human immunodeficiency virus type 1 tat in astrocytes. J Virol 2000; 74: 9214–21.
73. Ganju RK, Munshi N, Nair BC et al. Human immunodeficiency virus tat modulates the Flk-1/KDR receptor, mitogen-activated protein kinases, and components of focal adhesion in Kaposi's sarcoma cells. J Virol 1998; 72: 6131–7.
74. Zauli G, Gibellini D, Celeghini C et al. Pleiotropic effects of immobilized versus soluble recombinant HIV-1 Tat protein on CD3-mediated activation, induction of apoptosis, and HIV-1 long terminal repeat transactivation in purified CD4+ T lymphocytes. J Immunol 1996; 157: 2216–24.
75. Mischiati C, Pironi F, Milani D et al. Extracellular HIV-1 Tat protein differentially activates the JNK and ERK/MAPK pathways in CD4 T cells. AIDS 1999; 13: 1637–45.
76. Fiorelli V, Gendelman R, Samaniego F et al. Cytokines from activated T cells induce normal ECs to acquire the phenotypic and functional features of AIDS-Kaposi's sarcoma spindle cells. J Clin Invest 1995; 95: 1723–34.
77. Albini A, Fontanini G, Masiello L et al. 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 1994; 8: 1237–44.
78. Ensoli B, Gendelman R, Markham P et al. Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. Nature 1994; 371: 674–80.
79. Albini A, Benelli R, Presta M et al. HIV-tat protein is a heparin-binding angiogenic growth factor. Oncogene 1996; 12: 289–97.
80. Iurlaro M, Benelli R, Masiello L et al. Beta Interferon inhibits HIV-1 Tat-induced angiogenesis: Synergism with 13-cis retinoic acid. Eur J Cancer 1998; 34: 570–6.
81. Jia H, Lohr M, Jezequel S et al. Cysteine-rich and basic domain HIV-1 Tat peptides inhibit angiogenesis and induce endothelial cell apoptosis. Biochem Biophys Res Commun 2001; 283: 469–79.
82. Cantaluppi V, Biancone L, Boccellino M et al. HIV type 1 Tat protein is a survival factor for Kaposi's sarcoma and ECs. AIDS Res Hum Retroviruses 2001; 17: 965–76.
83. Park IW, Ullrich CK, Schoenberger E et al. HIV-1 Tat induces microvascular endothelial apoptosis through caspase activation. J Immunol 2001; 167: 2766–71.
84. Liu W, Ahmad SA, Reinmuth N et al. Endothelial cell survival and apoptosis in the tumor vasculature. Apoptosis 2000; 5: 323–8.
85. Gonzalez-Amaro R, Sanchez-Madrid F. Cell adhesion molecules: Selectins and integrins. Crit Rev Immunol 1999; 19: 389–429.
86. McCloskey TW, Ott M, Tribble E et al. Dual role of HIV Tat in regulation of apoptosis in T cells. J Immunol 1997; 158: 1014–9.
87. Del Sorbo L, Arese M, Giraudo E et al. Tat-induced platelet-activating factor synthesis contributes to the angiogenic effect of HIV-1 Tat. Eur J Immunol 2001; 31: 376–83.
88. Albini A, Barillari G, Benelli R et al. Angiogenic properties of human immunodeficiency virus type 1 Tat protein. Proc Natl Acad Sci USA 1995; 92: 4838–42.
89. Toschi E, Barillari G, Sgadari C et al. Activation of matrix-metalloproteinase-2 and membrane-type-1-matrix-metalloproteinase in ECs and induction of vascular permeability *in vivo* by human immunodeficiency virus-1 Tat protein and basic fibroblast growth factor. Mol Biol Cell 2001; 12: 2934–46.
90. Hofman FM, Dohadwala MM, Wright AD et al. Exogenous tat protein activates central nervous system-derived ECs. J Neuroimmunol 1994; 54: 19–28.
91. Barillari G, Ensoli B. Angiogenic effects of extracellular human immunodeficiency virus type 1 tat protein and its role in the pathogenesis of AIDS-associated Kaposi's Sarcoma. Clin Microbiol Rev 2002; 15: 310–26.
92. Corallini A, Campioni D, Rossi C et al. Promotion of tumour metastases and induction of angiogenesis by native HIV-1 Tat protein from BK virus/tat transgenic mice. AIDS 1996; 10: 701–10.
93. Corallini A, Betti M, Rusnati M et al. Characterization of the effects of two polysulfonated distamycin A derivatives, PNU145156E and PNU153429, on HIV type 1 Tat protein. AIDS Res Hum Retroviruses 1998; 14: 1561–71.
94. Possati L, Campioni D, Sola F et al. Antiangiogenic, antitumoural and antimetastatic effects of two distamycin A derivatives with anti-HIV-1 Tat activity in a Kaposi's sarcoma-like murine model. Clin Exp Metastasis 1999; 17: 575–82.
95. Huber BE, Richards CA, Martin JL et al. Alterations in tumor angiogenesis associated with stable expression of the HIV tat gene. Mol Carcinog 1992; 5: 293–300.
96. Samaniego F, Markham PD, Gendelman R et al. Inflammatory cytokines induce ECs to produce and release basic fibroblast growth factor and to promote Kaposi's sarcoma-like lesions in nude mice. J Immunol 1997; 158: 1887–94.
97. Geier SA, Kronawitter U, Bogner JR et al. Impairment of colour contrast sensitivity and neuroretinal dysfunction in patients with

symptomatic HIV infection or AIDS. Br J Ophthalmol 1993; 77: 716–20.

98. Obrams GI, Grufferman S. Epidemiology of HIV-associated non-Hodgkin lymphoma. Cancer Surv 1991; 10: 91–102.
99. Rabkin C and Blattner WA. HIV infection and cancers other than non Hodgkin's lymphoma and Kaposi's sarcoma. Cancer Surv 1991; 10: 151–60.
100. Ramendra KK, Sangiorgi F, Lan-Yng W et al. Expression of the human immunodeficiency virus-Tat gene in lymphoid tissues of transgenic mice is associated with B-cell lymphoma. Blood 1999; 94: 275–82.
101. Corallini A, Altavilla G, Pozzi L et al. Systemic expression of HIV-1 tat gene in transgenic mice induces endothelial proliferation and tumors of different histotypes. Cancer Res 1993; 53: 5569–75.
102. Vogel J, Hinrichs SH, Reynolds RK et al. The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. Nature 1988; 335: 606–11.
103. Prakash O, Tang ZY, He YE et al. Human Kaposi's sarcoma cell-mediated tumorigenesis in human immunodeficiency type 1 tat-expressing transgenic mice. J Natl Cancer Inst 2000; 92: 721–8.
104. Weiss R, Boshoff C. Addressing controversies over Kaposi's sarcoma. J Natl Cancer Inst 2000; 92: 677–9.
105. Barillari G, Buonaguro L, Fiorelli V et al. 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 1992; 149: 3727–34.
106. Regezi JA, MacPhail LA, Daniels TE et al. Human immunodeficiency virus-associated oral Kaposi's sarcoma. A heterogeneous cell population dominated by spindle-shaped ECs. Am J Pathol 1993; 143: 240–9.
107. Uccini S, Sirianni MC, Vincenzi L et al. 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 1997; 150: 929–38.
108. Moses AV, Fish KN, Ruhl R et al. Long-term infection and transformation of dermal microvascular ECs by human herpesvirus 8. J Virol 1999; 73: 6892–902.
109. Maruo N, Morita I, Shirao M et al. IL-6 increases endothelial permeability *in vitro*. Endocrinology 1992; 131: 710–4.
110. Chirivi RG, Taraboletti G, Bani MR et al. Human immunodeficiency virus-1 (HIV-1)-Tat protein promotes migration of acquired immunodeficiency syndrome-related lymphoma cells and enhances their adhesion to ECs. Blood 1999; 94: 1747–54.
111. Hofman FM, Wright AD, Dohadwala MM et al. Exogenous tat protein activates human ECs. Blood 1993; 82: 2774–80.
112. Fiorelli V, Gendelman R, Sirianni MC et al. 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 1998; 91: 956–97.
113. Hofman FM, Chen P, Incardona F et al. HIV-1 tat protein induces the production of interleukin-8 by human brain-derived ECs. J Neuroimmunol 1999; 94: 28–39.
114. Camussi G, Montrucchio G, Lupia E et al. Angiogenesis induced *in vivo* by hepatocyte growth factor is mediated by platelet-activating factor synthesis from macrophages. J Immunol 1997; 158: 1302–9.
115. Montrucchio G, Lupia E, Battaglia E et al. Platelet-activating factor enhances vascular endothelial growth factor-induced endothelial cell motility and neoangiogenesis in a murine matrigel model. Arterioscler Thromb Vasc Biol 2000; 20: 80–8.
116. Fan J, Bass HZ, Fahey JL. Elevated IFN-gamma and decreased IL-2 gene expression are associated with HIV infection. J Immunol 1993; 151: 5031–40.
117. Maruyama K, Mori Y, Murasawa S et al. Interleukin-1 beta upregulates cardiac expression of vascular endothelial growth factor and its receptor KDR/flk-1 via activation of protein tyrosine kinases. J Mol Cell Cardiol 1999; 31: 607–17.
118. Lafrenie RM, Podor TJ, Buchanan MR et al. Up-regulated biosynthesis and expression of endothelial cell vitronectin receptor enhances cancer cell adhesion. Cancer Res 1992; 52: 2202–8.
119. Marchisone C, Benelli R, Albini A et al. Inhibition of angiogenesis by type I interferons in models of Kaposi's sarcoma. Int J Biol Markers 1999; 14: 257–62.
120. Minami T, Rosenberg RD, Aird WC. Transforming growth factor-beta 1-mediated inhibition of the flk-1/KDR gene is mediated by a 5′-untranslated region palindromic GATA site. J Biol Chem 2001; 276: 5395–402.
121. Borghi MO, Panzeri P, Shattock R et al. Interaction between chronically HIV-infected promonocytic cells and human umbilical vein ECs: Role of proinflammatory cytokines and chemokines in viral expression modulation. Clin Exp Immunol 2000; 120: 93–100.
122. Fan ST, Hsia K, Edgington TS. Upregulation of human immunodeficiency virus-1 in chronically infected monocytic cell line by both contact with ECs and cytokines. Blood 1994; 84: 1567–72.
123. Chen P, Mayne M, Power C et al. The Tat protein of HIV-1 induces tumor necrosis factor-alpha production. Implications for HIV-1-associated neurological diseases. J Biol Chem 1997; 272: 22385–8.
124. Sastry KJ, Reddy HR, Pandita R et al. HIV-1 tat gene induces tumor necrosis factor-beta (lymphotoxin) in a human B-lymphoblastoid cell line. J Biol Chem 1990; 265: 20091–3.
125. Nath A, Conant K, Chen P et al. Transient exposure to HIV-1 Tat protein results in cytokine production in macrophages and astrocytes. A hit and run phenomenon. J Biol Chem 1999; 274: 17098–102.
126. Rusnati M, Urbinati C, Tanghetti E et al. Cell membrane GM1 ganglioside is a functional coreceptor for fibroblast growth factor 2. Proc Natl Acad Sci USA 2002; 99: 4367–72.
127. Soldi R, Mitola S, Strasly M et al. Role of alphavbeta3 integrin in the activation of vascular endothelial growth factor receptor-2. EMBO J 1999; 18: 882–92.
128. Rusnati M, Coltrini D, Campioni D et al. Upregulation of urokinase-type plasminogen activator by endogenous and exogenous HIV-1 Tat protein in tumour cell lines derived from BK virus/tat-transgenic mice. AIDS 1997; 11: 727–36.
129. Morini M, Benelli R, Giunciuglio D et al. Kaposi's sarcoma cells of different etiologic origins respond to HIV-Tat through the Flk-1/KDR (VEGFR-2): Relevance in AIDS-KS pathology. Biochem Biophys Res Commun 2000; 273: 267–71.
130. Deregibus MC, Cantaluppi V, Doublier S et al. HIV-1-Tat protein activates phosphatidylinositol 3-kinase/AKT-dependent survival pathways in Kaposi's sarcoma cells. J Biol Chem 2002; 277: 25195–202.
131. Kelly GD, Ensoli B, Gunthel CJ et al. Purified Tat induces inflammatory response genes in Kaposi's sarcoma cells. AIDS 1998; 12: 1753–61.