HIV-1 Tat and heparan sulfate proteoglycan interaction: a novel mechanism of lymphocyte adhesion and migration across the endothelium

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Introduction

The HIV-1 transactivating factor Tat accumulates on the surface of endothelium by interacting with heparan sulfate proteoglycans (HSPGs). Tat also interacts with B-lymphoid Namalwa cells but only when these overexpress HSPGs after syndecan-1 cDNA transfection (SYN-NCs). Accordingly, SYN-NCs, but not mock-transfected cells, adhere to endothelial cells (ECs) when Tat is bound to the surface of either one of the 2 cell types or when SYN-NCs are transfected with a Tat cDNA. Moreover, endogenously produced Tat bound to cell-surface HSPGs mediates cell adhesion of HIV+ ACH-2 lymphocytes to the endothelium. This heterotypic lymphocyte-EC interaction is prevented by HSPG antagonist or heparinase treatment, but not by integrin antagonists and requires the homodimerization of Tat protein. Tat tethered to the surface of SYN-NCs or of peripheral blood monocytes from healthy donors promotes their transendothelial migration in vitro in response to CXCL12 or CCL5, respectively, and SYN-NC extravasation in vivo in a zebrafish embryo model of inflammation. In conclusion, Tat homodimers bind simultaneously to HSPGs expressed on lymphoid and EC surfaces, leading to HSPG/Tat-Tat/HSPG quaternary complexes that physically link HSPG-bearing lymphoid cells to the endothelium, promoting their extravasation. These data provide new insights about how lymphoid cells extravasate during HIV infection.

Reagents

A total of 86 amino acid HIV-1 Tat was expressed and purified by Escherichia coli as glutathione-S-transferase (GST-Tat) or green fluorescent protein (Tat-GFP)22,23 fusion proteins. GST and GFP moieties do not interfere with the heparin-binding, transactivating, and cell-adhesive capacity of Tat.24 Different recombinant mutant forms of GST-Tat were purified as described22 and used: GST-TatC (cysteine 22, 25, and 27 within the cysteine string) and GST-TatC−A (cysteine 22, 25, and 27 within the cysteine string) fusion proteins. GST and GFP moieties do not interfere with the heparin-binding, transactivating, and cell-adhesive capacity of Tat.24 Different recombinant mutant forms of GST-Tat were purified as described22 and used: GST-TatC−A (cysteine 22, 25, and 27 within the cysteine string) and GST-TatC−A (cysteine 22, 25, and 27 within the cysteine string). The online version of this article contains a data supplement.

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cysteine-rich domain mutated to alanine), GST-Tat-Δ8 (arginine 49, 52, 53, 55, 56, and 57 within the basic domain mutated to alanine), and GST-Tat-1e (deletion of the C-terminal amino acid sequence containing the RGD integrin recognition motif). Synthetic peptides representing the amino acid sequences 1 to 20, 41 to 60, and 71 to 85 of Tat were from Medical Research Council AIDS Reagents Project (Potters Bar, United Kingdom); heparan sulfate (HS) from M. Del Rosso (Florence University); anti-VEGFR-2/kinase insert domain receptor (KDR) antibody from H. A. Weich (Max Planck Institute); pCEP-Tat expression vector harboring the HIV-1 Tat cDNA from A. Gualandris (Turin University, Italy); synthetic, LPS-free 86-amino-acid form of Tat (s-Tat) and its biotinylated form (b-Tat) were from Tectogene; heparinase II and III, hyaluronidase, and cycloheximide from Sigma-Aldrich; heparin and Escherichia coli unsulfated K5 polysaccharide from Glycoves Co; suramin from Bayer AG; integrin antagonist linear peptide GRGDSPK and its inactive analog GRADSPK from Neostem Laboratoire; high molecular weight tetramethylrhodamine dextran (R-HMW dextran, 2 × 10⁶ Da) from Invitrogen; CXCL12 and CCL5 from PeproTech EC; tumor necrosis factor α (TNF-α) from R&D Systems; and polyclonal anti-Tat–FITC antibody from Diatheva.

Real-time biomolecular interaction assay

A BIACore X apparatus (GE Healthcare) was used. Surface plasmon resonance (SPR) was exploited to measure changes in refractive index caused by the binding of GST-Tat to s-Tat immobilized to a BIACore CM5 sensor chip. Briefly, s-Tat (40 µg/mL) was allowed to react with the sensor chip as described, allowing the immobilization of 6500 resonance units (corresponding to ~0.8 pmol of s-Tat). Similar results were obtained for the immobilization of GST, used as a negative control and for blank subtraction. Next, s-Tat, GST-Tat, or its mutant forms were injected over the s-Tat surfaces for 4 minutes (to allow association with immobilized protein) and then washed until dissociation occurred.

Cell cultures

Burkitt lymphoma–derived Namalwa cells were grown as described. Namalwa clones transfected with the empty vector or with a vector containing syndecan-1 cDNA (EV-NCs and SYN-NCs cells, respectively) were prepared and cultured as described. For Tat transfection, 10⁷ cells were incubated with 30 µg of pCEP-Tat expression vector in 0.5 mL of calcium/magnesium-free phosphate-buffered saline (PBS) at 4°C for 10 minutes. Cells were then electroporated at 322 V and 950 microfarads. Selection was started 48 hours later with 50 µg/mL hygromicin. Stable transfection was achieved after 2 weeks. The selected populations were characterized by Western blot analysis with anti-Tat antibodies.

Fetal bovine aortic GM7373 ECs were grown as described. Fixation of GM7373 cells was achieved by 2 hours of incubation at 4°C with PBS/3% glutaraldehyde, 5 minutes of incubation with 0.1 M glycine and washes with PBS.

Primary cultures of human adrenal gland capillary ECs (HACECs) were grown as described. ACH-2 cells, a chronically HIV infected T-cell line derived from CEM cells, were prepared and cultured as described. For Tat transfection, 10⁷ cells were electroporated at 322 V and 950 µF. Stable transfection, 10⁷ cells were cultured in RPMI 1640, 10% fetal calf serum (FCS), 1% glutamine, and 1% penicillin/streptomycin as described. ACH-2 cells were left untreated or stimulated for 2 days with 10% fetal calf serum (FCS), 1% glutamine, and 1% penicillin/streptomycin. Selection was started 48 hours later with 50 µg/mL hygromicin. SYN-NCs were stained with PKH26 Red Fluorescent Cell Linker Kit (Sigma-Aldrich) and preincubated with s-Tat as described for transmigration assay. Six hours after tail transection, approximately
200 naive or Tat-coated SYN-NCs were injected into the sinus venosus of anesthetized transgenic embryos using borosilicate glass capillary needles and a Picospritzer microinjector. After 24 hours, double-fluorescence images of injected embryos were acquired using an epifluorescence Zeiss Axiovert 200M microscope equipped with a Hal 100 digital camera and AxioVisionLE software.

The extent of SYN-NC extravasation in the injured area was measured by computerized image analysis (Image-Pro Plus; MediaCybernetics).

Results

HSPGs mediate extracellular Tat binding to endothelial and B-lymphoid cells

Cytokines/chemokines accumulate on the EC surface where they recruit leukocytes/lymphocytes. Accordingly, b-Tat binds to an EC monolayer in a dose- and time-dependent but temperature-independent manner (Figure 1A-B). The binding was very rapid (being already appreciable after 1 minute of incubation) and lasted for at least 24 hours (Figure 1B). b-Tat–EC interaction could be inhibited by the sulfated HSPG antagonists suramin and heparin, but not by the unsulfated E coli K5 polysaccharide. In addition, pretreatment of ECs with heparinase II or III, but not with hyaluronidase, prevented the binding of b-Tat in a dose-dependent manner (Figure 1C). Relevant to this point, heparinase III treatment leads to an almost complete removal of HSPGs from the EC surface (supplemental Figure 1, available on the Blood website; see the Supplemental Materials link at the top of the online article). The capacity of Tat to bind to EC monolayers was confirmed in immunofluorescence studies using Tat-GFP (supplemental Table 1). Thus, Tat stably accumulates on the EC surface by binding to the sulfated groups of HSPGs.

To assess whether HSPGs play a role also in the interaction of extracellular Tat with B-lymphoid cells, we took advantage of an experimental model in which HSPG-deficient Namalwa cells had been transfected with an expression vector harboring the human syndecan-1 cDNA. The levels of HSPG expression on the surface of SYN-NCs are at least 10 times higher than those expressed by EV-NCs, here used as negative control. On this basis, SYN-NCs and EV-NCs were subjected to fluorescence-activated cell sorter analysis after incubation with Tat-GFP. As shown in Figure 2A, Tat-GFP binds to SYN-NCs but not to EV-NCs. Bound Tat-GFP is completely removed from SYN-NCs by a PBS/NaCl wash (data not shown) indicating its cell surface localization. Indeed, SYN-NCs are unable to internalize the HSPG-bound Tat-GFP (supplemental Figure 2A). To better characterize the binding of extracellular Tat to Namalwa cells, we evaluated the capacity of SYN-NCs and EV-NCs to adhere to GST-Tat–coated plastic. As shown in Figure 2B-C, SYN-NCs, but not EV-NCs, adhere to immobilized GST-Tat in a dose- and time-dependent manner. SYN-NC adhesion was prevented by the sulfated HSPG antagonists heparin and HS, but not by the unsulfated K5 polysaccharide (Figure 2D). Accordingly, peptide Tat (41-60) (corresponding to the basic domain of Tat that mediates heparin/HS interaction) inhibited SYN-NC adhesion to immobilized GST-Tat, whereas peptides Tat (1-20, corresponding to the acidic domain of Tat, here used as a negative control) and Tat (71-85, containing the RGD motif responsible for Tat-integrin interaction) were ineffective (Figure 2D). Taken together, the
results demonstrate that extracellular Tat binds to B-lymphoid Namalwa cells only when these express significant amounts of HSPGs, as it occurs in SYN-NCs. In these cells, Tat interaction is mediated by the sulfated HS chains of the transduced syndecan-1 and is independent of integrin engagement.

Extracellular Tat-HSPG interaction mediates B-lymphoid cell adhesion to the endothelium

To assess the capacity of extracellular Tat to promote the adhesion of B-lymphoid cells to the endothelium, an EC monolayer was incubated for 2 hours at 37°C with GST-Tat, washed with PBS to remove the unbound protein, and evaluated for its ability to sustain EV-NC or SYN-NC adhesion. Pretreatment with GST-Tat caused a dose-dependent increase in the capacity of ECs to mediate the adhesion of SYN-NCs. Similar results were obtained with an s-Tat protein devoid of the GST moiety (Figure 3A-B, supplemental Figure 2C). In contrast, EV-NCs adhered poorly to both naive and GST-Tat–treated endothelium. A significant increase in SYN-NC adhesion was observed also when GST-Tat was added to a fixed EC monolayer (Figure 3C), ruling out the possibility that a de novo synthesis of cell-adhesive molecule(s) may mediate this effect. In keeping with the ability of extracellular Tat to accumulate on the EC surface by binding to HSPGs (see Figure 1C), the adhesion of SYN-NCs to GST-Tat–treated EC monolayers was prevented by removing bound extracellular Tat with a PBS/NaCl wash or by competition with free heparin, but not with the integrin antagonist GRGDSPK peptide or its inactive GRADSPK mutant (Figure 3A). In addition, the capacity of GST-Tat to promote SYN-NC adhesion to ECs was abolished when the EC monolayer was digested with heparinase III (Figure 3D). These observations indicate that extracellular Tat bound to HSPGs expressed on the EC surface mediates the adhesion of HSPG-bearing Namalwa cells, but not of HSPG-deficient cells.

To assess whether extracellular Tat is able to sustain EC-Namalwa cell interaction also when tethered on the B-lymphoid cell surface, EV-NCs and SYN-NCs were preincubated with GST-Tat (550 nM) and washed with PBS or with PBS/NaCl. Then, cells were allowed to adhere to naive EC monolayer and adherent lymphocytes counted. (F) Western blot analysis with anti-Tat antibodies of Tat-transfected Namalwa cells (600 000 cells). Tat indicates s-Tat (17 pmol); n.t., not transfected; and t., transfected.
addition of GST-Tat to the adhesion medium were sufficient to allow Tat association to the surface of SYN-NCs and their consequent adhesion to the endothelium. At variance, no cell-cell interaction was observed after stimulation with GST-Tat for 2 hours at 37°C followed by its complete removal by PBS/NaCl wash (supplemental Figure 2B).

In addition, Tat-dependent SYN-NC adhesion to EC monolayers was not affected when the preincubation with GST-Tat was performed at 4°C or in the presence of the protein synthesis inhibitor cycloheximide or when adhesion to the EC monolayer was allowed to occur at 4°C, indicating that the de novo synthesis of adhesive molecules is not involved in the process (data not shown).

In vivo, HIV+ lymphocytes synthesize and secrete Tat that remains associated to the HSPGs of the cell surface. Accordingly, cytofluorimetric analysis demonstrated that Tat was present on the surface of chronically HIV-infected ACH-2 T-lymphocytes both in the absence and in the presence of TNF-α activation, but not on the surface of the uninfected CEM A3.01 cell counterpart. Tat associated to the surface of ACH-2 cells could be completely removed by heparinase III treatment, demonstrating its association to cell-surface HSPGs (Figure 4C). Accordingly, ACH-2 cells adhered to endothelium more efficiently than CEM A3.01 cells, and their adhesion was prevented by a PBS/NaCl wash (Figure 4D).

To further confirm the capacity of endogenously produced Tat protein to mediate cell-cell interaction, EV-NCs and SYN-NCs were permanently transfected with an expression vector encoding Tat and s-Tat. Tat protein expression (Figure 4F), SYN-NC, but not EV-NC transfecants, showed an increase in their ability to adhere to endothelium (Figure 4E). In addition, adhesion was prevented when transfected lymphocytes were washed with PBS/NaCl before being seeded on the endothelium (Figure 4E).

In conclusion, exogenously added and/or endogenously produced Tat protein can bind to B-lymphoid cells and HIV+ T lymphocytes when these express HSPGs on their surface. Once bound to lymphocytes, extracellular Tat mediates lymphocyte adhesion to EC surface HSPGs, eventually promoting a heterotypic cell-cell interaction.

**HSPG-mediated B-lymphoid-EC interaction requires Tat homodimerization**

The data shown in Figures 3 and 4 indicate that extracellular Tat mediates cell-cell interaction via a simultaneous binding to HSPGs expressed on the surface of both cell types. The presence of a single heparin-binding site has been demonstrated in Tat protein.22 On the other hand, Tat has the tendency to form stable homodimers,36,37 a property that depends on a cysteine-rich domain distinct from the basic domain involved in heparin interaction.36 Thus, we investigated the possibility that B-lymphoid–EC interaction requires Tat homodimerization, each Tat monomer being involved in a mutually exclusive binding with one of the 2 cell types. To this purpose, we used various Tat muteins characterized by a different capacity to form homodimers or to interact with heparin. Compared with GST-Tat, the GST-TatC–A mutant did not form homodimers, whereas the GST-TatG–A mutant retained a significant, albeit reduced, ability to undergo homodimerization, as assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis under nonreducing conditions (Figure 5A). Tat homodimers were dissociated by boiling the sample in the presence of β-mercaptoethanol or dithiothreitol (data not shown), thus confirming the role of cysteine bridges in Tat homodimerization. To confirm the capacity of Tat to self-interact, we exploited surface SPR technology. As shown in Figure 5B, s-Tat immobilized to a BiAcore sensor chip was able to bind GST-Tat and, to a lesser extent, GST-TatC–A, but not GST-TatG–A and GST devoid of the Tat moiety (Figure 5B). Interestingly, the kinetic parameters and affinity of s-Tat/GST-Tat, GST-TatC–A binding are comparable with those calculated for s-Tat/GST-Tat and s-Tat/s-Tat interactions (supplemental Table 2). Taken together, these data demonstrate the ability of Tat to form homodimers and that this self-interaction requires the cysteine-rich domain but not the heparin-binding basic domain of the protein. Relevant to this point, in keeping with their differential heparin-binding capacity, immobilized GST-TatC–A, but not immobilized GST-TatG–A, retained the ability to promote SYN-NC adhesion. It should be noticed that the GST-Tat-1e mutant (which is characterized by the deletion of the integrin-binding RGD motif but still binds heparin) promoted SYN-NC adhesion, similarly to GST-Tat. No SYN-NC adhesion was instead observed to immobilized GST devoid of the Tat moiety or to BSA (Figure 5C).

On these bases, Tat muteins were evaluated for their ability to promote the interaction of SYN-NCs with an EC monolayer. As anticipated, because of its incapacity to interact with HSPGs,22 and to induce SYN-NC adhesion when immobilized to plastic, GST-TatG–A mutant did not promote B-lymphoid–EC interaction (Figure 5D). The ability to promote B-lymphoid–EC interaction was also lost in the GST-TatC–A mutant (Figure 5D), despite that it retained its Namalwa cell-adhesive capacity when assayed on plastic (Figure 5C). On the contrary, GST-Tat-1e mutant retained the capacity to promote B-lymphoid–EC interaction (Figure 5D), thus confirming the lack of a role for integrin engagement in this
phenomenon. These data tightly associate the capacity of extracellular Tat to form homodimers to its capacity to promote HSPG-dependent adhesion of B-lymphoid cells to the endothelium.

**Tat induces trans-endothelial migration of B-lymphoid cells**

The adhesion of lymphocytes to the endothelium is a prerequisite for their extravasation during inflammation,\(^4^8\) AIDS progression,\(^3^9\) and lymphomas.\(^4^0\) We investigated whether Tat tethered on the surface of B-lymphoid cells was able to promote their transmigration across a monolayer of HACEC. To this purpose, EV-NCs and SYN-NCs were preincubated with s-Tat, washed with PBS to remove the unbound protein, and evaluated for their migration across an EC monolayer in response to the chemokine CXCL12.\(^4^1\) Preliminary experiments demonstrated that CXCL12 exerts a similar chemotactic response in both cell types when tested in a Boyden chamber assay (data not shown). Preincubation with s-Tat significantly increased the capacity of SYN-NCs, but not of EV-NCs, to trans-migrate across the EC monolayer when stimulated by CXCL12 (Figure 6A). Again, in keeping with the HSGP dependency of this effect, a PBS/NaCl wash, as well as heparinase III pretreatment, inhibited SYN-NC trans-migration. It must be pointed out that Tat retains the capacity to stimulate trans-endothelial migration of SYN-NCs but not of EV-NCs also when tethered to the surface of ECs rather than of lymphoid cells (Figure 6B).

Peripheral blood mononuclear cells (PBMCs) isolated from healthy donors express surface HS (supplemental Figure 3A). Similarly to SYN-NCs, also PBMCs increased their migration across a “naive” HACEC monolayer in response to the chemokine CCL5 when preincubated with s-Tat. In addition, a PBS/NaCl wash of Tat-pretreated PBMCs prevented this effect (supplemental Figure 3B). These data rule out the possibility that the ability of extracellular Tat to mediate EC interaction and trans-migration is limited to syndecan-1 transfectants.

**Tat tethered to B-lymphoid cells favors their extravasation in the zebrafish embryos**

To investigate whether Tat engaged on the surface of B-lymphoid cells is able to promote cell extravasation in vivo, we used a zebrafish embryo model of tail transection (Figure 7A) already used to investigate inflammation-mediated neutrophil extravasation.\(^4^2\) To better visualize cell extravasation, we used VEGFR2:GRCFP zebrafish embryos, in which GFP expression is driven by the endothelial-specific VEGFR2 promoter,\(^3^1\) and SYN-NCs preloaded with the red fluorescent dye PKH26.\(^4^3\)

In preliminary experiments, PKH26-loaded SYN-NCs were injected into the blood flow of intact zebrafish embryos. Twenty-four hours after injection, SYN-NCs were still viable and circulate inside the GFP-\(^\ast\) vessels (Figure 7B). In addition, microangiography of the zebrafish embryo vasculature performed 6 hours after tail transection did not show any extravasation of injected R-HMW dextran in the injured area, demonstrating the integrity of blood vessels (Figure 7C).

On this basis, untreated or Tat-pretreated SYN-NCs were injected into the blood flow of zebrafish embryos 6 hours after tail transection and evaluated for their extravasation at the site of injury. As assessed by epifluorescence microscopy followed by computerized image analysis of the extravasated cells, Tat pretreatment caused a significant increase of the capacity of SYN-NCs to extravasate, compared with naive cells (Figure 7D-E).

**Discussion**

During HIV infection, the abnormal extravasation of HIV-\(^\ast\) lymphocytes contributes to the dissemination of the virus\(^3^9\) and to the progression of AIDS-associated leukemia/lymphomas.\(^4^0\) Here we describe, for the first time, the capacity of extracellular Tat protein to promote B-lymphoid cell adhesion to the endothelium and extravasation via heterotypic mechanism of cell-cell interaction. This is the result of the ability of Tat homodimers to engage the HS on the cell surface of both cell types, leading to the formation of a HS/Tat-HS quaternary complex that physically links HS-bearing lymphoid cell to the endothelium (supplemental Figure 4). This mechanism is independent of de novo synthesis of adhesive molecules by ECs and/or by lymphocytes, being observed also when Tat is added to a fixed EC monolayer or when B-lymphoid cells are treated with Tat at 4°C or in the presence of cycloheximide.
Extracellular Tat binds various cell surface receptors (integrins, KDR, and HSPGs) via different domains. Here, much evidence demonstrates that the B-lymphoid–EC interaction is selectively mediated by the interaction of Tat with HSPGs expressed by the 2 cells: (1) B-lymphoid SYN-NCs, but not EV-NCs, adhere to ECs after Tat incubation, although both clones retain the capacity to up-regulate the expression of cytokines (TNF-β and CCL3) when stimulated by extracellular Tat (data not shown). (2) A PBS/NaCl wash, known to remove cationic proteins (including Tat) from cell surface HSPGs, prevents Tat-mediated lymphocyte adhesion to EC as well as trans-endothelial migration. Notably, the PBS/NaCl wash per se does not affect trans-endothelial lymphocyte migration (Figure 6A). (3) The HSPG-antagonists heparin, HS, and suramin, as well as HSPG digestion by heparinases, inhibit Tat binding to the EC surface and/or SYN-NC adhesion to immobilized Tat. (4) Experiments performed with recombinant mutated forms or synthetic peptides of Tat or with the integrin-antagonist peptide GRGDSPK demonstrate that the adhesion of SYN-NCs to immobilized Tat requires the basic, heparin-binding domain of Tat but not its integrin-recognition RGD motif. Similarly, neutralizing anti-KDR antibodies do not affect the binding of b-Tat to ECs (data not shown). Noticeably, previous observations had shown the capacity of extracellular Tat to increase EC adhesion and trans-endothelial migration of Burkitt lymphoma cells from AIDS patients, even though these cells do not express αvβ3, αvβ1, αvβ3 integrins, or KDR.

Tat forms stable homodimers, a property that depends on a cysteine-rich domain from the basic domain involved in heparin interaction. Here we have shown that site-directed mutagenesis of Tat protein in either one of the 2 regions destroys the ability of Tat to mediate B-lymphoid–EC interaction. Thus, the cysteine-rich and the basic domains of Tat appear to cooperate in mediating the HSPG-dependent lymphocyte adhesion to the endothelium, the former region promoting the assembly of Tat homodimers that will engage the 2 facing cells by binding cell surface HSPGs via the basic domain 2 Tat protomers (supplemental Figure 4). Interestingly, Tat-Tat complexes dissociate very slowly (supplemental Table 2), indicating a very stable interaction between the 2 Tat molecules. Further studies are required to formally demonstrate the formation of HSPG+Tat-Tat, HSPG quaternary complexes during EC-B-lymphoid cell interaction.

Previous observations had shown that Tat activates HIV+ lymphocytes by an autocrine mechanism of action, leading to overexpression of various cell adhesive molecules. In addition, Tat released by HIV+ cells acts paracrinally on ECs, causing the damage of capillaries, the increase of their permeability, the activation of a proinflammatory program, and the overexpression of cell-adhesive molecules on the EC surface. These effects will eventually promote lymphocyte/leukocyte adhesion to the endothelium and extravasation. Our data extend these observations by demonstrating the existence of an additional, direct mechanism of action of extracellular Tat that physically links the 2 cell types in a metabolically independent manner, the only prerequisite being the presence of cell surface HSPGs on both cell types. All these mechanisms may concur to the observed increase in the ability of Tat-pretreated SYN-NCs to extravasate in a zebrafish embryo model of tail transection.

The Tat-dependent, HSPG-mediated EC interaction may contribute to the abnormal extravasation of HIV+ lymphocytes/leukocytes that occurs during HIV infection and that promotes HIV dissemination and progression of leukemia/lymphomas in AIDS patients. Here we show that significant levels of HSPG-associated Tat protein are present in chronically HIV-infected ACH-2 T lymphocytes also in the absence of TNF-α activation. Accordingly, low but significant levels of viral transcription are detectable in nonactivated cells (data not shown). Thus, extracellular Tat may contribute to EC adhesion and extravasation of latently HIV-infected lymphocytes. On the other hand, once released by HIV+ cells in the lymph nodes, Tat may remain entrapped in the HSPG-rich EC surface of high endothelial venules where it may promote extravasation of uninfected lymphocytes in HIV+ tissue, a process that may contribute to CD4+ cell depletion during AIDS progression (Chen et al and references therein). Indeed, we have observed that extracellular Tat mediates EC-lymphoid cell interaction either when produced endogenously by Tat-transfected SYN-NCs or when added exogenously as a recombinant protein.

When produced and released by HIV+ cells, extracellular Tat readily associates to the surface of producing cells and retains the capacity to bind HIV-1 gp120 protein, enhancing virus attachment and entry into the cell. In addition, the expression of syndecan-1 has been proposed as a marker for the diagnosis of AIDS-related lymphomas. Thus, the Tat-dependent, HSPG-mediated B-lymphoid–EC interaction may represent a target for pharmacologic interventions in AIDS and AIDS-associated pathologies. Preliminary observations showed that heparin-mimicking, synthetic sulfonic acid polymers act as multitarget compounds by acting as extracellular Tat and gp120 antagonists and by inhibiting Tat-mediated EC/lymphocyte interaction (data not shown). Further studies are required to explore this hypothesis.

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Authorship

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