Original article

HIV-1 Tat protein impairs adipogenesis and induces the expression and secretion of proinflammatory cytokines in human SGBS adipocytes

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Background: HIV-1 Tat protein has been shown to play multiple roles in the pathogenesis of AIDS; however, there is no information currently available on its effects on adipose tissue alterations. We have studied the effects of Tat on SGBS adipocytes to gain insight on its role on the development of lipodystrophy.

Methods: SGBS preadipocytes were exposed to Tat during and after differentiation. Acquisition of adipocyte morphology, expression of gene markers of adipogenesis and inflammation, release of adipokines and cytokines to the medium, and glucose uptake were measured. The action of Tat on tumour necrosis factor (TNF)-α-regulated messenger RNA expression was determined in differentiated adipocytes. The capacity of rosiglitazone, resveratrol and parthenolide to influence the action of Tat was also assessed.

Results: Tat treatment reduced the number of SGBS preadipocytes that acquired adipocyte morphology. It also led to repression of adipogenic gene expression and induced the coordinate expression and release of proinflammatory cytokines in human adipose cells. Moreover, combined treatment with Tat and TNF-α produced an additive effect on the repression of adipocyte genes. The observed effects of Tat on gene transcription in adipocytes were due, in part, to TNF-α that was secreted as a consequence of intracellular exposure to Tat.

Conclusions: Tat impairs adipogenesis in human SGBS preadipocytes and increases the expression and release of proinflammatory cytokines. Positive crosstalk between Tat and TNF-α contributes to the anti-adipogenic and proinflammatory effects. HIV-1 Tat protein may play a role in the adipose tissue alterations that ultimately lead to lipoatrophy and systemic metabolic disturbances observed in HIV-1-infected patients.

Introduction

Lipodystrophy and metabolic disturbances reminiscent of the metabolic syndrome (that is, insulin resistance and dyslipidaemia) are frequently observed in HIV-1-infected patients undergoing antiretroviral treatment. The alterations in adipose tissue in these patients are thought to contribute to the systemic metabolic disturbances. Thus, it has been reported that HIV-1-infected patients who present with lipodystrophy have abnormally low levels of adiponectin and, in some cases, leptin in the blood. Systemic and local increases in the levels of proinflammatory cytokines in adipose tissue have also been reported [1,2].

These alterations appear to develop as a consequence of a complex set of pathogenic insults. Some antiretroviral drugs, such thymidine analogue inhibitors of reverse transcriptase, are especially prone to elicit peripheral adipose tissue atrophy, whereas protease inhibitors appear to be associated primarily with systemic metabolic disturbances [3,4]. However, several observations point to a role for HIV-1 infection per se in the development of lipodystrophy and associated metabolic alterations. Accordingly, mild alterations in adipose tissue distribution [5] and gene expression [6] are observed in HIV-1-infected patients before antiretroviral treatment has been initiated. Some of the adipose tissue alterations in HIV-1-infected patients may be attributable to a proinflammatory environment in
adipose tissue caused by the infection of cells within adipose depots, such as macrophages and, possibly, lymphocytes [2]. Indeed, increased levels of tumour necrosis factor (TNF-α) expression have been observed in adipose tissue from HIV-1-infected treatment-naive patients [6]. The possibility that adipocytes might be infected by HIV-1 has been a matter of debate [7–9], and recent data indicate that such infections may be significant in the context of an environment with high levels of TNF-α [10].

HIV-1 genome-encoded proteins might be among the agents that potentially elicit alterations in adipose tissue. To date, only viral protein R has been reported to be capable of influencing adipocyte function, which it accomplishes via repression of peroxisome proliferator-activated receptor (PPAR)-γ function [11]. In the present study, we have analysed the role of Tat on adipocyte differentiation and function. Tat is an HIV-1 regulatory protein required for efficient viral gene expression [12]. It interacts with the HIV-1 genome-long-terminal repeat to enhance transcription and RNA processing [13]. In addition to its effects on viral replication, Tat is secreted extracellularly by infected cells and can enter uninfected cells and alter multiple cellular functions [14,15]. Moreover, Tat is present at substantial levels in the serum of infected patients [16,17], and may exert paracrine effects on cells in the neighbourhood of infected cells as well as on distant cells and tissues [18]. In addition, Tat modulates host cellular mechanisms, thereby contributing to immune system malfunction. For example, Tat has been shown to affect the production of cytokines, such as interleukin (IL)-10, TNF-α and tumour growth factor-β, in different cell systems [19–21] and to induce apoptosis of T-cells [18]. The extracellular and intracellular HIV Tat has been shown to play multiple roles in the pathogenesis of AIDS [22]; however, there is no information currently available on the effects of HIV Tat protein on the adipocyte environment, which is crucial for the development of lipodystrophy.

In the present study, we analysed the role of the HIV-1 Tat protein in the differentiation and function of SGBS adipocytes. Our results provide the first direct evidence that Tat alters adipogenesis and adipokine release, and promotes cytokine production in adipose cells.

Methods

Materials

Dulbecco’s modified Eagle medium (DMEM)/F12 medium and fetal bovine serum were from Gibco (Life Technologies Corp., Grand Island, NY, USA). All other reagents used for adipocyte cell cultures were from Sigma (St Louis, MO, USA), with the exception of rosiglitazone, which was from Alexis Biochemicals (Enzo Life Sciences Inc, Farmingdale, NY, USA). TNF-α neutralizing antibody was purchased from R&D Systems (Minneapolis, MN, USA).

Cell culture and differentiation

Human SGBS preadipocytes were cultured and differentiated into mature adipocytes as previously described [23]. In brief, SGBS preadipocytes were maintained in DMEM/F12 containing 10% fetal bovine serum and antibiotics at 37°C in a humidified 5% CO₂ environment. After cells had become confluent, differentiation was initiated by first incubating cells for 4 days in DMEM/F12 serum-free containing 20 nM insulin, 0.2 nM triiodothyronine and 100 nM cortisol, supplemented with 25 nM dexamethasone, 500 μM 3-isobutyl-methyl-xanthine and 2 μM rosiglitazone (Quickdiff medium). Subsequently, the cells were switched to adipogenic medium (containing insulin, triiodothyronine and cortisol only) and maintained for up to 16 days.

Cell treatment and adenoviral transduction

Experiments were performed using two experimental settings. For studies on the effects of Tat on adipocyte differentiation, treatments with recombinant Tat (70 nM; Diatheva, Fano, Italy) or TNF-α (1 ng/ml) were initiated on day 0 and continued throughout the entire differentiation process. The levels of Tat in HIV-1-infected patients were reported to be in nM range [16,17]. However, the reliability of in vivo measurements of Tat is a matter of discussion, among other reasons because Tat in vivo might be sequestered by endogenous anti-Tat antibodies [15,17,22,24], and it is possible that Tat concentrations surrounding HIV-1-infected cells are higher than those measured using standard available antibodies [25]. Previous studies on the effects of Tat in a variety of cell types report the induction of proinflammatory pathways at 100 nM [26–29]. Therefore, the present study used similar concentrations of Tat, which are at the lower range of the Tat doses used in comparable studies, and which have been found to be appropriate to model in vitro the effects of Tat in vivo. Previous pilot experiments using 10 nM Tat did not yield substantial effects on acquisition of SGBS adipocyte morphology or expression of marker genes of adipogenesis and inflammation (data not shown).

For studies on the effects of Tat on differentiated adipocytes, differentiated SGBS adipocytes were transduced with an adenoviral vector expressing Tat (AdCMV-Tat) or a control adenoviral vector (AdCMV-GFP) at a multiplicity of infection of 100 for 4 h in serum-free DMEM/F12, and cells were incubated for a further 48 h in fresh adipogenic medium. Tat levels were measured in pelleted cells from adenoviral transduction experiments using a Tat ELISA assay (Diatheva) and the concentration attained was 54 ±18 nM. When indicated, after incubating for an additional 24 h in fresh adipogenic
medium, adipocytes expressing Tat or GFP were treated for 24 h with TNF-α (10 ng/ml), rosiglitazone (10 μM), resveratrol (50 μM) or parthenolide (5 μM).

Quantification of adipocyte-secreted adipokines and cytokines
The effects of Tat on the release of adiponectin, IL-8, IL-6 and monocyte chemotactant protein (MCP)-1 during the adipocyte differentiation process was determined using 25 μl of culture medium collected before harvesting cells. The levels of these factors in the medium were quantified using a multiplex analysis system employing fluorescently labelled microsphere beads linked to specific antibodies (Linco Research/Millipore, Saint Charles, MO, USA). Fluorescence was detected using a Luminex100IS v2 system (Luminex Corp., Austin, TX, USA).

Assessment of cytotoxicity
Potential cytotoxic effects of Tat or TNF-α on differentiating SGBS preadipocytes or on adipocytes when already differentiated were determined by the CytoTox96 kit (Promega, Madison, WI, USA) following the manufacturer’s instructions.

Quantitative real-time reverse transcription PCR
RNA was extracted using an RNeasy Mini Kit (Qiagen, Hilden, Germany). Reverse transcription (RT) was performed in a total volume of 20 μl using random hexamer primers (Applied Biosystems, Foster City, CA, USA) and 0.5 μg total RNA. PCR contained 1 μl complementary DNA, 10 μl TaqMan Universal PCR Master Mix (Applied Biosystems), 250 nM probes and 900 nM primers from Assays-on-Demand Gene Expression Assay Mix (TaqMan; Applied Biosystems) in a total volume of 20 μl, and were conducted using an ABI/Prism 7700 Sequence Detector System (Applied Biosystems). Assay-on-Demand probes for the following targets were used: TNF-α (Hs00174131), IL-1β (Hs00605917), MCP-1 (Hs00234140), IL-6 (Hs00174128), PPAR-γ (Hs00174103), adipsin (Hs00174130), GLUT4 (Hs00174132), and 18S ribosomal RNA (Hs99999901). Controls lacking RNA or primers were included in each set of experiments. Each sample was run in duplicate and the mean value of the duplicate was used to calculate the relative amount of individual targets. Each mean value of messenger RNA (mRNA) was normalized to that of 18S ribosomal RNA using the comparative (2^-ACT) method, following the manufacturer’s instructions. Parallel calculations using the RPLP0 reference gene (Hs99999902) were performed and results were essentially the same.

Determination of 3H-labelled 2-deoxyglucose uptake
Prior to assaying for glucose uptake, adipocytes were transduced with GFP or Tat adenoviral vectors, incubated for 24 h, then incubated for another 24 h in the presence or absence of TNF-α. Next, cells were incubated in Krebs-Ringer phosphate buffer (137 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, and 20 mM HEPES pH 7.4) supplemented with 2 mM sodium pyruvate and 0.2% bovine serum albumin. Thereafter, 2-deoxy-D-[3H] glucose (American Radiolabeled Chemicals, Inc., St Louis, MO, USA) was added at a final concentration of 0.1 mM (1 μCi) and assays were stopped after 10 min by placing the cells on ice and adding the stop solution (50 mM glucose in phosphate-buffered saline). After terminating the transport assay with three washes of stop solution, each monolayer was solubilized in 1 M NaOH-0.1% sodium dodecyl sulfate. A 0.2 ml aliquot was removed for determination of radioactivity by liquid scintillation counting. Measurements were made in triplicate and corrected for non-specific diffusion, and normalized to total protein content as determined by Bradford analysis.

Statistics
The distribution of data was controlled for normality. Unpaired Student’s t-test was used to test the level of significance of the differences between means, and to test whether a mean was significantly different from zero (in the case of non-detectable values).

Results
Tat inhibits SGBS adipocyte differentiation and induces the expression of proinflammatory genes
The influence of Tat on adipogenesis was examined during conversion of SGBS preadipocytes into adipocytes. SGBS cells, which appear functionally similar to normal human adipocytes, are not immortalized but retain a high capacity for differentiation into mature adipocytes over many generations [23].

To evaluate the effects of Tat on adipocyte differentiation, we exposed cells to 70 nM recombinant Tat or 1 ng/ml TNF-α (for comparative purposes) during the entire differentiation process. These treatments with Tat or TNF-α were not cytotoxic to SGBS cells (data not shown). Representative microscopy images of Tat and TNF-α-treated cells show the morphological changes associated with the acquisition of the adipocyte phenotype during the in vitro differentiation process (Figure 1A). Tat treatment reduced the number of cells that acquired adipocyte morphology. The number of cells that accumulated lipid vacuoles was even more markedly reduced by TNF-α treatment. To determine whether Tat modulated gene expression in SGBS cells, we quantified mRNA levels of adiponectin, PPAR-γ, GLUT4, IL-8, IL-6 and MCP-1 using real-time RT-PCR. Consistent with the observed morphological effects, Tat decreased the expression of gene markers of adipogenic differentiation,
Figure 1. Tat inhibits SGBS adipocyte differentiation and alters the expression and release of genes related to adipogenesis and inflammation

SGBS preadipocytes were cultured and differentiated into mature adipocytes in the presence of 70 nM exogenous Tat or 1 ng/ml tumour necrosis factor (TNF)-α. (A) Representative micrographs. (B) Effects on adiponectin, peroxisome proliferator-activated receptor (PPAR)-γ, glucose transporter type 4 (GLUT4), interleukin (IL)-8, IL-6 and monocyte chemotactic protein (MCP)-1 messenger RNA (mRNA) levels. Total RNA was isolated, and samples were analysed by quantitative reverse transcription PCR. (C) Effects of Tat and TNF-α on the release of adiponectin and proinflammatory cytokines into the cell culture medium by SGBS adipocytes. Results are expressed as protein concentration. Data are presented as mean ±SEM from four independent experiments, and are expressed relative to values from untreated control (Con) cells: *P<0.05, **P<0.001 and ***P<0.01 versus Con.
including adiponectin (2.2-fold), PPAR-γ (1.3-fold) and GLUT4 (1.7-fold; Figure 1B). In addition, Tat increased the expression of mRNAs for genes involved in proinflammatory actions, including IL-8 (4.1-fold), IL-6 (1.6-fold) and MCP-1 (1.8-fold; Figure 1B). Similarly, IL-8, IL-6 and MCP-1 mRNA levels were up-regulated by TNF-α treatment, and adiponectin, PPAR-γ and GLUT4 mRNA levels were down-regulated (Figure 1B). Thus, our data indicate that Tat coordinately represses the expression of genes encoding adipogenic proteins and causes a coordinate induction of proinflammatory proteins in differentiating adipose cells.

Tat and TNF-α similarly modulate the release of adipokines from SGBS adipocytes

The effects of Tat or TNF-α on the release of regulatory proteins by SGBS adipocytes were studied using a multiplex analysis system (Figure 1C). Tat decreased the release of adiponectin (1.4-fold) and caused a significant increase in the levels of IL-8 (1.3-fold), IL-6 (1.9-fold) and MCP-1 (1.4-fold) in the culture medium compared with that in medium conditioned by untreated adipocytes. TNF-α caused similar, albeit more marked, changes in secretion, inducing a 6.7-fold decrease in the level of adiponectin, and increased the levels of IL-8, IL-6 and MCP-1 in the culture medium by 30-fold, 4.2-fold and 2.2-fold, respectively.

Tat differentially modulates the expression of genes related to inflammatory actions and adipogenic function in differentiated SGBS adipocytes

We next undertook a parallel study of the short-term effects of Tat on the expression of inflammatory and adipogenesis genes in mature, differentiated SGBS adipocytes. Pilot studies indicated that 48-h treatment with recombinant 70 nM Tat produced very mild effects, for example a near to twofold induction of MCP-1 expression and no significant effect on marker genes of adipogenesis such as adiponectin or PPAR-γ (data not shown).

To improve efficacy, we created an experimental setting that favoured intracellular Tat exposure, employing a gene transfer protocol using an adenoviral vector to drive Tat expression. Differentiated SGBS adipocytes were transduced with control (AdCMV-GFP) or the Tat (AdCMV-Tat) adenoviral vectors on day 16 after initiating the differentiation protocol, at which time >95% of the cells contained multiple lipid droplets. More than 90% of mature adipocytes exhibited GFP fluorescence, indicating efficient transduction. This experimental setting resulted in Tat concentrations in the intracellular fraction in the 50 nM range (see Methods section).

The mRNA levels of IL-8 and MCP-1 were significantly increased in cells endogenously expressing Tat (Figure 2A). Although basal levels of TNF-α mRNA were very low in SGBS adipocytes in the control condition (approximately 35–36 cycle threshold [CT] in the quantitative RT-PCR assay), Tat increased TNF-α mRNA expression significantly (Figure 2A). To study the action of Tat on the expression of adipogenic markers, we analysed the levels of adiponectin, PPAR-γ and GLUT4 mRNA.

In contrast to its variable effects on proinflammatory cytokine genes, Tat caused a significant down-regulation in the mRNA levels of all three adipogenic marker genes (Figure 2A). Consistent with the gene expression results, overexpression of Tat significantly increased secretion of IL-8 and MCP-1 into the medium (Figure 2B) and adiponectin levels in the medium were not significantly altered by Tat.

Collectively, these data indicate that Tat represses the overall expression of adipogenic genes and induces a coordinate change in the expression and release of proinflammatory cytokines in human adipose cells.

Combined treatment with Tat and TNF-α has generally additive effects on cytokine and adipokine expression in differentiated SGBS adipocytes

In addition to studying the effects of Tat alone on mature adipocytes, we also analysed the action of Tat on TNF-α-regulated mRNA expression. Treatment of SGBS adipocytes with TNF-α alone caused a coordinate up-regulation of proinflammatory cytokines, increasing the mRNA levels of IL-8 and MCP-1 by 1,400-fold and 50-fold, respectively, and it also induced dramatically the expression of TNF-α mRNA itself (Figure 3). This is in agreement with previous reports on SGBS adipocytes [30]. The addition of Tat caused a further significant increase in IL-8 and TNF-α mRNA levels beyond that induced by TNF-α alone (Figure 3A). Like Tat, TNF-α alone down-regulated adipogenic genes, reducing adiponectin, PPAR-γ and GLUT4 mRNA to levels that were 0.37, 0.39 and 0.19, respectively, of those in untreated controls. Tat over-expression further reduced the expression of adipocyte genes, decreasing their mRNA levels to one-half the already low values produced by TNF-α treatment (Figure 3A).

Tat decreases glucose uptake in SGBS adipocytes

To determine whether Tat affected glucose transport, we transduced mature SGBS adipocytes with AdCMV-Tat or AdCMV-GFP, incubated cells in the absence or presence of TNF-α, and then determined [3H]-deoxyglucose uptake.

As shown in Figure 3B, Tat over-expression (AdCMV-Tat incubated without TNF-α) caused a significant reduction in glucose uptake by adipocytes; similar results were observed in AdCMV-GFP-transduced cells incubated with TNF-α alone. Combined exposure to Tat and TNF-α further decreased glucose uptake (Figure 3B). These data suggest that the effects of Tat in adipocytes impaired adipogenic gene expression, and that induction of proinflammatory genes are...
associated with altered metabolic functions, such as impaired glucose uptake.

The effects of Tat on adipocytes are partially mediated by TNF-α. As shown above, Tat significantly induced TNF-α gene expression and produced effects on mature SGBS adipocytes that were similar to those observed for TNF-α. We hypothesized that the effects of Tat might be caused, in whole or in part, by the autocrine action of TNF-α released in response to Tat. To test whether the TNF-α secreted by Tat-transduced adipocytes is involved in Tat-induced changes in gene expression, we treated cells with a TNF-α neutralizing antibody.

**Figure 2. Effect of Tat on the expression of genes related to inflammatory actions and adipogenic function in differentiated SGBS adipocytes**

Differentiated SGBS adipocytes (day 16 post-induction of differentiation) were transduced with adenoviral vectors AdCMV-GFP (control) or AdCMV-Tat. (A) Effect of Tat on adiponectin and proinflammatory cytokine messenger RNA (mRNA) levels. Total RNA was isolated, and samples were analysed by quantitative reverse transcription PCR. (B) Effect of Tat on the release of adiponectin and proinflammatory cytokines into the cell culture medium by SGBS adipocytes. Results are expressed as protein concentration. Data are presented as mean ± SEM from six independent experiments, and are expressed relative to values from untreated control cells: *P*<0.01 and *P*<0.05 versus controls. GLUT4, glucose transporter type 4; IL, interleukin; MCP, monocyte chemoattractant protein; PPAR-γ, peroxisome proliferator-activated receptor-γ.
As expected, incubation of TNF-α-treated (0.25 ng/ml) control cells with the TNF-α neutralizing antibody for 24 h dramatically impaired TNF-α-induced expression of IL-8, MCP-1 and TNF-α mRNA (Figure 4), indicating that the concentration of antibody used was sufficient to completely inhibit the biological activity of exogenously added TNF-α (compare TNF-α and TNF-α plus anti-TNF-α in Figure 4). When applied to cells over-expressing Tat, the TNF-α neutralizing antibody inhibited the Tat-induced increase in IL-8 and MCP-1 mRNA expression by approximately 50%. The TNF-α neutralizing antibody also completely abrogated the Tat-induced increase in TNF-α mRNA (Figure 4). Interestingly, the TNF-α neutralizing antibody did not significantly affect Tat-induced down-regulation of the adipogenic genes, adiponectin or GLUT4, although it did effectively block the reduction in adiponectin and GLUT4 mRNA levels produced by treatment with TNF-α (Figure 4). Thus, our data indicate that TNF-α secreted as a consequence of intracellular exposure to Tat partially contributes to the effects of Tat on overall gene transcription in adipocytes.

Rosiglitazone, resveratrol and parthenolide modulate Tat-dependent induction of proinflammatory genes

We analysed the capacity of drugs with known anti-inflammatory action to prevent the Tat-dependent induction of proinflammatory genes. We cultured SGBS cells with 10 ng/ml TNF-α for 24 h in the absence or presence of drugs (Rosiglitazone, resveratrol or parthenolide). The results are shown in Figure 5. Data are presented as mean ± SEM from six independent experiments, and are expressed relative to values from control (Con) cells.

Figure 3. Effects of Tat on TNF-α-regulated gene expression in differentiated SGBS adipocytes

A

G)

Effect of Tat on adiponectin and proinflammatory cytokine messenger RNA (mRNA) levels. Data are presented as mean ± SEM from six independent experiments, and are expressed relative to values from control (Con) cells: *P < 0.01 versus Con, #P < 0.05 for comparisons between TNF-α and Tat-treated cells; cP < 0.05 versus Con; dP < 0.001 versus Con. eP < 0.001 for comparisons between TNF-α and Tat-treated cells. GLUT4, glucose transporter type 4; IL, interleukin; MCP, monocyte chemotactic protein; PPAR-γ, peroxisome proliferator-activated receptor-γ.
induction of proinflammatory genes in SGBS adipocytes. First, we tested rosiglitazone, an anti-diabetic drug with anti-inflammatory properties and a known activator of PPAR-γ [31]. Treatment of cells with rosiglitazone for 24 h significantly inhibited Tat-induced expression of IL-8, MCP-1 and TNF-α, although it did not alter the basal levels of these mRNAs (Figure 5). Second we tested resveratrol, a polyphenol with anti-inflammatory properties [32] known to activate the SIRT1 deacetylase [33]. Resveratrol treatment significantly inhibited...
both basal and Tat-induced MCP-1 mRNA expression but, in contrast to rosiglitazone, significantly enhanced the effects of Tat on IL-8 and TNF-α mRNA expression (Figure 5). Third, cells were exposed to parthenolide, an anti-inflammatory agent that inhibits nuclear factor (NF)-κB activity [34]. Parthenolide treatment significantly inhibited Tat-induced expression of IL-8, MCP-1 and TNF-α, and it also lowered the basal levels of IL-8 mRNA expression.

Discussion

In the current study, we provide the first description of the effects of Tat in SGBS adipocytes. Tat caused a marked induction in the expression and secretion of proinflammatory cytokines, including IL-8, TNF-α and MCP-1. Moreover Tat impaired human adipogenesis and reduced adiponectin release. Hypoadiponectinemia is commonly found in HIV-1-infected patients with lipodystrophy, and a proinflammatory local environment has also often been observed in the adipose tissue of HIV-1-infected patients [6]. In related observations, Tat has previously been shown to up-regulate a number of cytokines, the HIV-1 coreceptor CCR5, and the IL-2 receptor (CD25) in HIV-1-infected non-adipose cells [35]. Moreover, several reports have indicated enhanced expression of proinflammatory cytokine genes in adipose tissue from patients with lipodystrophy and even in fat from HIV-infected, treatment-naive patients [6,36]. Thus, the observed in vitro effects of Tat on several aspects of adipocyte function are consistent with the alterations that appear in HIV-1-infected patients who develop lipodystrophy, even among those with mild alterations prior to antiretroviral treatment.

By contrast, Tat reduced the expression of PPAR-γ. PPAR-γ plays an essential role in the development and normal function of white adipocytes and controls the production and secretion of adiponectin [37]. In HIV-1-infected patients with lipodystrophy, there is a decrease in PPAR-γ expression in adipose tissue, as well as increased expression of inflammatory cytokines, such as IL-6 and TNF-α, and decreased expression and circulating levels of adiponectin, which is involved in regulating insulin sensitivity and also plays an anti-inflammatory role [38]. From our results, it is tempting to speculate that impaired PPAR-γ expression may contribute to the anti-adipogenic and proinflammatory effects of Tat, and further analysis would be required to establish it. In any case, even if PPAR-γ levels were reduced by Tat, they are enough to allow for the action of rosiglitazone reducing Tat-dependent activation of proinflammatory genes.

We also explored the potential of the antioxidant resveratrol, a natural polyphenol that has been reported to ameliorate insulin resistance in animal models [39,40], and to protect against protease-inhibitor-induced oxidative stress in cells [41]. Our data indicates that resveratrol enhances the effects of Tat on the expression of proinflammatory cytokines such as IL-8 and TNF-α. This may be due to the action of deacetylase SIRT1, which is activated by resveratrol [33] and has been reported to enhance Tat transcriptional activity via deacetylation [42]. These findings, in addition to reports of the inhibition of PPAR-γ activity by SIRT1 [43,44] may lead to the consideration of resveratrol as an unlikely pharmacological tool for palliating the effects of Tat, in contrast with rosiglitazone. Additionally, the inhibition of Tat induced MCP-1 expression by resveratrol is in

Figure 5. Effects of rosiglitazone, resveratrol and parthenolide on Tat-induced expression of proinflammatory cytokine genes

SGBS adipocytes were cultured and transduced as in Figure 2, and were further treated with rosiglitazone (Rosi; 10 μM), resveratrol (Resv; 50 μM) or parthenolide (Parth; 5 μM) for 24 h. Data are presented as mean ± sem from three independent experiments, and are expressed relative to values from control (Con) cells: *P<0.01 and **P<0.05 versus Con; †P<0.05 versus Tat-transduced cells. IL, interleukin; MCP, monocyte chemotactic protein; mRNA, messenger RNA; TNF, tumour necrosis factor.
good agreement with a recent study that demonstrated an anti-inflammatory effects of resveratrol on HIV-1 Tat induced MCP-1 production in the hippocampus [45]. However, the action of resveratrol lowering Tat-induced MCP-1 should deserve further research to explore potential beneficial effects on specific inflammatory pathways.

We also examined effects of parthenolide in the HIV-1 Tat-mediated up-regulation of cytokines in SGBS adipocytes. It is well known that NF-kB plays a critical role in the transcriptional regulatory mechanisms of various inflammatory genes [46]. In addition, HIV-1 Tat protein has been reported to activate NF-kB in a variety of cell types, such as endothelial cells, lymphocytes, astrocytes and human breast cancer cells [26,47–49]. In the present study, we observed for the first time that exposure to an NF-kB inhibitor can significantly attenuate Tat-induced up-regulation of proinflammatory cytokines in SGBS adipocytes.

A remarkable finding of the current study is the positive crosstalk between Tat and TNF-α in adipocytes. Tat induced TNF-α gene expression in SGBS adipocytes, similar to what has been reported in other cell types [20,50]. This is consistent with the enhanced levels of TNF-α expression found in adipose tissue from HIV-1-infected patients. Our present data indicate that TNF-α induction by Tat caused paracrine effects in adipocytes that contributed to the anti-adipogenic and proinflammatory effects of Tat. It has been reported that TNF-α promotes susceptibility to HIV infection in adipocytes [10]. Thus, it is likely that adipocytes in patients may experience both intracellular exposure to Tat due to the promotion of HIV-1 infection by TNF-α as well as exposure to Tat released by other cell types within adipose depots (for example, macrophages) that are highly susceptible to HIV-1 infection. Our current results also indicate that the effects of Tat on SGBS adipocytes are mediated, in part, through the induction of TNF-α. These findings indicate a vicious cycle of proinflammatory and anti-adipogenic effects arising from Tat–TNF-α crosstalk. Concerning the occurrence of some TNF-α-independent effects of Tat, it may be speculated that known effects of Tat on stress-associated protein kinases or on transcription factors such as CREB or Sp1 [22] may be involved in such effects, a matter that should deserve further research in the context of the adipose cell.

In summary, our results indicate that exposure of SGBS pre-adipocytes to Tat and over-expression of Tat in SGBS adipocytes reduces adipogenesis and promotes the expression and release of proinflammatory cytokines. These same alterations are found in adipose tissue from HIV-1-infected patients to distinct degrees in relation to the extent of lipodystrophy. Tat may be a novel mediator of adipose tissue alterations, either as a consequence of direct infection of adipocytes or due to the exposure of adipocytes to Tat secreted by surrounding non-adipose cells. However, the present in vitro study has obvious limitations in relation to its direct translation to the analysis of alterations in HIV-1-infected patients. In addition to the intrinsic limitations of the in vitro approaches, it is difficult to directly compare the levels of exposure of cells to Tat in our experimental settings with the actual exposure of patient adipocytes to Tat. Further research will be necessary to improve the technological complexities of Tat measurements in human samples and to establish the actual local concentration of Tat in the adipose tissue microenvironment, in order to fully establish the role of Tat in the adipose tissue derangements occurring in HIV-1-infected patients. If confirmed, attempts to prevent and/or ameliorate metabolic alterations in HIV-1 patients, including lipodystrophy, should take into consideration targeting the deleterious action of Tat on the human adipocyte function.

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Disclosure statement

The authors declare no competing interests.

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