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HIV-1 Tat Represses Transcription from the Mannose Receptor Promoter

Robert L. Caldwell, 2* Brian S. Egan, 2† and Virginia L. Shepherd 3*‡‡

The mannose receptor is expressed on mature macrophages and immature dendritic cells, and functions to mediate phagocytosis of pathogens and capture of Ags for delivery to MHC class II-containing intracellular compartments. It has been previously reported that HIV-1-infected macrophages have reduced functions associated with the mannose receptor, including impaired Pneumocystis carinii phagocytosis and mannosylated albumin uptake. Several HIV-1-derived proteins including the Tat protein have been shown to transcriptionally repress host cell genes. The present study was undertaken to define the role of the HIV-1-derived protein Tat in HIV-mediated mannose receptor down-regulation. Cotransfection of the human macrophage cell line U937 with a Tat expression vector and a mannose receptor promoter-luciferase reporter construct resulted in down-regulation of mannose receptor promoter activity. This repression was targeted to the basal promoter. Expression of either one- or two-exon Tat resulted in decreased promoter activity. The addition of the transactivation response element (TAR) sequence enhanced the Tat-mediated repression. Down-regulation was also seen when transfected cells were treated with exogenously added Tat protein. These results are consistent with a mechanism whereby Tat reduces mannose receptor promoter activity by interfering with the host transcriptional initiation machinery, potentially resulting in decreased levels of surface mannose receptor available for Ag or pathogen capture. The Journal of Immunology, 2000, 165: 7035–7041.

The mannose receptor is a 175-kDa type I transmembrane protein expressed on the surface of macrophages (1) and dendritic cells (2). It appears to play a key role in host defense against invading pathogens by mediating internalization of these organisms (3) and by delivering foreign Ags to MHC II-containing compartments for subsequent Ag presentation (4). Thus, the mannose receptor is a key effector molecule in initiating an early immune response against invading pathogens. Specific down-regulation of mannose receptor function might then be a mechanism by which pathogens escape immune detection. Recent reports from our laboratory and others have demonstrated that a variety of pathogens are capable of reducing mannose receptor expression, including Candida albicans (5), Leishmania donovani (6), and HIV-1 (7). In the latter study, Koziel et al. reported that mannose receptor function on human alveolar macrophages from HIV-infected individuals was down-regulated, and that in vitro infection of human macrophages reduced the ability of the mannose receptor to mediate ingestion of Pneumocystis organisms.

HIV-1 has evolved a variety of strategies to evade detection and destruction by the host immune system (8). One of these strategies involves the down-modulation of cell surface receptors involved in host defense, including CD4 (9), MHC class I (10), and the mannose receptor (7). Among the HIV-derived proteins implicated in regulating these receptors are gp120, Nef, Vpu, and Tat (reviewed in Ref. 9). These proteins use multiple mechanisms for regulating host protein expression, including interference with normal intracellular trafficking, increased host protein degradation, alterations in host protein biosynthesis, and regulation of transcription of host genes. It has recently been reported that Tat transcriptionally represses expression of both the heavy and light chains of the MHC class I complex (10–14). Since both the mannose receptor and MHC class I are involved in Ag capture and presentation, the current study was designed to investigate the role of Tat in HIV-mediated mannose receptor down-regulation.

The HIV-1 Tat protein is a potent trans-activator of transcription from the viral promoter and is essential for viral replication (reviewed in Refs. 15 and 16). The structure of the Tat protein resembles a typical transcriptional activator containing an activation domain and a nucleic acid-binding domain. Functionally, Tat activates HIV-1 transcription through interaction with the transcriptional machinery of the host cell and also promotes elongation of the viral transcript (16). The full-length tat gene product is encoded on two exons which together result in a product of 86–101 aa depending on the viral isolate (17). In late infection Tat mRNA splicing becomes inefficient giving rise to a 72-aa form of Tat encoded only by exon 1. Both exon 1 and exon 2 Tat activate HIV-1 in cell culture system. No functional differences have been demonstrated between these Tat proteins in their ability to activate transcription from the long terminal repeat (LTR) in vivo.

Tat regulates the synthesis of multiple host cell genes involved in the immune response to HIV infection including cytokines (18–20), cytokine receptors (21, 22), macrophage-inflammatory protein 1α (23), and surface proteins such as HIV coreceptors (24, 25) and MHC class I proteins (10–14). Multiple mechanisms including altered intracellular signaling pathways leading to up- and down-regulation of cellular transcription factors and altered host gene transcription appear to be involved in these regulatory events.

### Abbreviations

- LTR, long terminal repeat
- RL, Renilla luciferase
- EIAV, equine infectious anemia virus
- TAR, transactivation response

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Recent work has described in detail the potential mechanisms involved in the regulation of MHC I and β2-microglobulin by Tat. Repression of both was mapped to the basal promoter and appeared to involve the interference by Tat of the interaction of the host cell transcriptional initiation machinery with the basal promoters. Similarities in the MHC class I and β2-microglobulin promoters and the mannose receptor promoter led us to speculate that Tat-mediated repression of transcription of these genes might be occurring through similar mechanisms. In the current study, using mannose receptor-reporter constructs, we have shown that mannose receptor transcription is repressed by HIV Tat, and that this repression is targeted to the basal promoter, similar to the inhibition of the MHC class I and β2-microglobulin promoters. Repression is enhanced by the presence of TAR and is mediated by one- or two-exon Tat. These results demonstrate that Tat-mediated repression of mannose receptor transcription is contributing to HIV-1-induced down-regulation of this receptor, and suggest that this repression may be a general mechanism for regulation of immune receptors including MHC class I, β2-microglobulin, and the mannose receptor.

Materials and Methods

Cells

U937 cells were purchased from the American Type Culture Collection (Manassas, VA). These cells were maintained in RPMI 1640 medium with 10% FBS (Life Technologies, Rockville, MD) plus antibiotics.

Plasmids

The mannose receptor promoter used in these studies was derived from the rat gene. Although a partial sequence for the human promoter has been reported (26), no characterization of regulatory elements has been reported. In contrast, detailed characterization of the rat mannose receptor promoter upstream elements has been reported by our laboratory (27), and was thus used for further analysis of Tat-mediated regulation. The mannose receptor-luciferase reporter gene constructs (MR854, MR656, MR228, MR108, and MR48) were prepared as previously described (27) as follows: The 854-bp mannose receptor promoter was cloned into the pGL2 basic vector (Promega, Madison, WI) containing the luciferase cDNA after BglII/SspI digestion (MR854). The proximal 656-bp promoter fragment (MR656) was generated by HindIII digestion of the 854-bp promoter with subsequent ligation into a HindIII-digested pGEM vector. A construct containing the proximal 228 bp of the mannose receptor promoter (MR228) was generated by digestion of the 854-bp construct with Smal and PvuII, followed by religation. The −108 (MR108) and −48 (MR48) constructs were generated by PCR using the MR656 as a template, the appropriate sense primers, and antisense primers containing the desired mutations were isolated, digested with the appropriate restriction enzymes, and ligated into MR854 following removal of the specific mutated region.

The two-exon Tat proviral construct pNL-ΔΔ and the control vector pNL-A0 that expresses no proviral sequences were obtained from T. Kevin Howcroft and Dina Hall Singer (National Cancer Institute, National Institutes of Health, Bethesda, MD) (11). The following reagents were obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health); the one-exon 72-aa Tat expression vector pSV2Tat72 (Alan Frankel, University of California, San Francisco, CA), HIV-1 Tat protein (John Brady, National Cancer Institute, National Institutes of Health), and antisera to HIV-1 Tat (Bryan Cullen, Duke University, Durham, NC). pGL2 basic and CMV- Renilla luciferase (CMV-RL) vectors were from Promega.

Transfections and reporter gene assays

U937 cells were transfected as follows: 6 × 10^6 U937 cells were suspended in 6 ml of DMEM with antibiotics and 1 ml was added per well in 6-well plates. Mannose receptor-luciferase promoter constructs (3 μg) were mixed with varying amounts of Tat, pNL-A0, or basic vectors (1 μg) plus CMV-RL (1 μg) in 145 μl of DMEM. Superfect (5 μl; Qiagen, Chatsworth, CA) was added to each DNA solution. The mixture was vortexed and then allowed to stand at room temperature for 15 min. DMEM (300 μl) was added to each solution, and the mixture was added to the cells. RPMI (640) with 10% FBS and antibiotics (500 μl) was added to each well, and the mixture was incubated for 24 h before assay. At the end of the incubation period, the cells were collected by centrifugation and washed once with PBS. The cells were suspended in 100 μl of Promega lysis buffer and incubated for 10 min at room temperature. Firefly and RL activities were measured using the dual luciferase reporter assay system (Promega) according to the manufacturer’s instructions. The pGL2 basic vector was used as a negative control in transfection assays. In all transfection experiments, the pRL-CMV vector was used to correct for transfection efficiency. Data are expressed as the luciferase activity for each sample normalized to the RL activity.

Effect of exogenous Tat on mannose receptor promoter-luciferase activity

U937 cells were transfected as above with MR228 plus pRL-CMV. At 12 h posttransfection, 1 μg of Tat protein was added, and cells plus Tat were incubated for an additional 24 h. Cells were then assayed using the dual luciferase reporter assay system.

Immunoblot analysis

To confirm that the Tat protein was expressed in our transient transfection experiments, cells transfected as above were lysed in lysis buffer (20 mM Tris (pH 7.75) containing 1% Triton X-100, 0.5% deoxycholate, 0.15 M NaCl, 0.02% sodium azide, and 0.34 trypsin inhibitory units of aprotinin/ml). Cell lysates were separated by SDS-PAGE and then transferred to nitrocellulose. The nitrocellulose was blocked with TBS-BSA and then probed with primary Ab to HIV-1 Tat. The blot was washed and then incubated with HRP-conjugated goat anti-rabbit IgG. Tat protein was visualized by incubation of the blot in 0.2 M Tris-HCl (pH 8.5), 2.5 mM luminol, 0.4 mM p-coumaric acid, and 0.0002% H_2O_2, followed by exposure of X-OMAT film (Kodak, Rochester, NY). Using this method, Tat protein was detected in U937 cells transfected with pSV2Tat72 (data not shown).

Results

Tat expression specifically inhibits mannose receptor promoter activity

The identification of the mannose receptor as an Ag-capture molecule suggests that this protein is an important participant in basic immunologic responses (4). As such, decreased mannose receptor expression could be advantageous for invading pathogens. Koziel et al. (7) have demonstrated decreased mannose receptor expression in alveolar macrophages isolated from HIV-infected patients. To determine whether HIV-mediated down-regulation of mannose receptor expression occurs at the level of transcription, an 854-bp mannose receptor promoter-luciferase construct and a control CMV-driven RL construct were cotransfected into the human monocyte cell line U937, with or without the Tat expression vector pSV2Tat72. As shown in Fig. 1A, mannose receptor promoter activity was reduced by 60% in the presence of Tat. The specificity of this decrease was demonstrated by the fact that the activity of the CMV promoter was only minimally affected by Tat expression. To determine whether Tat-mediated repression of the mannose receptor promoter occurred in a dose-dependent manner, transfections were performed with increasing quantities of the pSV2Tat72 expression vector. Fig. 1B shows that the degree to which mannose receptor promoter activity is inhibited is dependent on the amount of pSV2Tat72 added; promoter inhibition was >70% at the highest concentration of the Tat expression vector.

Tat-mediated repression of the mannose receptor promoter is localized to the proximal 48 bp

The region of the mannose receptor promoter through which Tat mediates repression was determined using a variety of mannose receptor promoter truncations (27). Tat-mediated transcriptional repression was observed at similar levels for the MR854, MR656,
MR228, MR108, and MR48 mannose receptor promoter constructs (Fig. 2A). Similar results have been found for the MHC class I promoter where repression was mediated through the proximal 68 bp (11) and the β2-microglobulin promoter where repression was mediated through the proximal 94 bp (13). The 48-bp mannose receptor promoter construct contains a few notable features as shown in Fig. 2B. First, there is a functional TATA box at -33 bp (27). Similar to the findings by Carroll et al. (13) for the β2-microglobulin promoter, mutation of the TATA box in the mannose receptor promoter did not block Tat-mediated inhibition of promoter activity (Fig. 3). Second, MR48 contains a consensus PU.1 binding site at -18 bp that has been shown to be required for full mannose receptor promoter activity in a rat macrophage cell line (B. S. Egan and V. L. Shepherd, unpublished results). Willbold et al. (29) have demonstrated that the equine infectious anemia virus (EIAV) Tat protein binds to the EIAV-LTR at a PU.1 binding site (GTTCCTGTTT). To determine whether the inhibitory effect of HIV-1 Tat occurs through the PU.1-binding element, the site at -18 bp of the mannose receptor promoter (GAGGAA) was mutated to GACCAA. In two separate experiments, this mutation reduced the effect of Tat on mannose receptor promoter activity from 63% repression (wild type) to 44% repression (PU.1 mutation) as shown in Fig. 3, suggesting that Tat may interfere with PU.1 binding to this site.

Both one-exon and two-exon Tat inhibit mannose receptor promoter activity

The Tat protein is encoded on two exons and the full-length Tat protein is synthesized from a spliced viral transcript giving rise to an 86–101 aa protein depending on the isolate (16). During late HIV infection, splicing of the Tat transcript becomes inefficient, resulting in the expression of a 72-aa Tat protein. Both Tat proteins are capable of activating transcription through the HIV LTR. Conflicting reports have appeared concerning the effectiveness of one-exon vs two-exon Tat in down-regulation of MHC I transcription (11, 14). To determine whether HIV-mediated down-regulation of
This effect is attributed to Tat being released from one cell and entering the nucleus of another cell to directly activate LTR-mediated transcription. Using this general mechanism, HIV can then modulate the expression of cellular genes in uninfected cells. To test the effect of extracellular Tat protein on the activity of the mannose receptor promoter, recombinant Tat protein was added to U937 cells 12 h posttransfection with MR854, and cultures were allowed to incubate for an additional 24 h. As shown in Fig. 5, addition of exogenous Tat resulted in decreased promoter activity. These data suggest that direct infection of cells is not required to down-regulate mannose receptor expression, providing HIV with a mechanism to compromise immune function in uninfected cells.

TAR expression augments Tat-mediated repression of the mannose receptor promoter

To gain insight into the mechanism by which Tat mediates repression of mannose receptor promoter activity, an experiment was designed to examine the possibility that Tat functions as a trans factor in the nucleus. Previous studies have shown that TAR, a sequence found in the nascent HIV-1 transcript, enhances the transactivation properties of Tat (16). It has also been shown that TAR augments repression of the β2-microglobulin promoter (13). We therefore reasoned that if Tat is gaining entry to the nucleus and acting as a repressor of the transcriptional machinery, TAR might enhance this effect. Cells were transfected with MR854 plus pSV2Tat72 in the presence and absence of the pNL-A0 vector which expresses the TAR sequence but no viral protein sequences. Fig. 6 shows that Tat expression alone reduces mannose receptor promoter activity by 60%, and the addition of pNL-A0 further reduces this activity to 20% of control levels. These data suggest that the Tat effects on mannose receptor expression require the Tat protein domain that binds to the TAR element. This same Tat domain is involved in both HIV-1 transactivation and repression of β2-microglobulin promoter activity, since TAR enhances both of these effects. This result also suggests that Tat-mediated down-regulation of mannose receptor promoter activity occurs in the nucleus, since TAR-Tat interactions are localized to the nucleus.

Discussion

The current study is the first description of Tat-mediated transcriptional regulation of the mannose receptor, a host cell surface receptor intimately involved in the innate immune response. It has

FIGURE 3. Mutation of the PU.1 site or TATA box in the mannose receptor promoter does not alter Tat-mediated down-regulation of promoter activity. The MR854 TATA mutant (mut) and MR854 PU.1 mutant constructs were prepared as described in Materials and Methods. Cells were transfected with 3 μg of the wild-type MR854 or the mutant constructs plus 3 μg of pSV2Tat72 or basic vector as described in Fig. 1. Results, normalized to RL, are the average ± SD for triplicate determinations and are representative of two separate experiments. *, p < 0.05 for MR854 or MR854 mutants plus pSV2Tat72 (open columns) compared with samples minus pSV2Tat72 (filled columns).

FIGURE 4. Both one-exon and two-exon Tat inhibit mannose receptor promoter activity. U937 cells were transfected with MR854 DNA plus basic vector, pSV2Tat72, pNL-ΔΔ, which expresses only Tat-86 (two-exon Tat), or pNL-A0, which expresses no HIV protein sequences (11). Data were normalized to RL activity and are expressed as the average ± SD of triplicate determinations. This experiment is representative of two separate experiments. *, p < 0.03.

FIGURE 5. Exogenous Tat down-regulates mannose receptor promoter activity. U937 cells were transfected with MR228 plus basic vector and CMV-RL as described in Fig. 1. At 12 h posttransfection, 1 μg of Tat protein was added, and the cells plus Tat were incubated for an additional 24 h. For comparison, cells were transfected with the Tat expression plasmid (pSV2Tat72) as described in Fig. 1. Results were normalized to RL and are the average ± SD of triplicate determinations. This experiment is representative of three separate experiments.
Tat regulation of both the MHC class I gene and the $\beta_2$-microglobulin gene has been studied in some detail (10–14). Howcroft et al. (10, 11) first reported that Tat down-regulated transcription of the MHC class I gene. The same group has recently described a similar repression of transcription of the $\beta_2$-microglobulin light chain, an associated component of MHC class I (13). Repression of both of these genes as with the mannose receptor occurs through the basal promoter region. The MHC class I basal promoter lacks a consensus TATA box, and activity appears to be dependent on the presence of two overlapping CCACC(C) (S box) motifs which bind an Sp1-like factor. Two other Sp1-dependent promoters, the MDR1 promoter and the minimal SV40 early promoter, were also found to be repressed by Tat. Although the exact mechanism involved in Tat-mediated repression of Sp1-containing promoters is not known, it has been suggested that Tat binds to an Sp1-related factor, resulting in interference with the assembly of the transcription complexes (11). Jeang et al. (38) reported that Tat can bind to Sp1, and a more recent report suggests that Tat modulates Sp1 activity through enhanced phosphorylation (39). Sp1 is known to bind the TFIID complex and can also interact with the TATA-binding protein directly (40). Pugh and Tjian (41) have reported that Sp1 plays a critical role in transcriptional activation in TATA-less promoters. Therefore, the physical interaction of Tat and Sp1 or an Sp1-like factor might block the interaction of Sp1 with the appropriate transcription initiation factors and/or the S box element in the promoter itself.

Comparison of the mannose receptor promoter and the MHC class I promoter reveals several important differences that might suggest that Tat is repressing activity of these promoters through different elements (Fig. 7). First, the mannose receptor promoter contains an Sp1 binding site, and this site has been implicated in myeloid-specific expression in the murine system (42). However, this site is not present in the basal, Tat-inhibitable promoter. Second, the mannose receptor promoter contains a functional TATA box (TTTAAAA), whereas the MHC class I promoter contains the sequence TCTAAA which does not conform to any of the sequences previously reported to function as a TATA box (43). Careful examination of the mannose receptor promoter and the $\beta_2$-microglobulin promoter reveals many shared features (Fig. 7) which suggest that Tat may be mediating repression of these promoters through a similar mechanism. Both the mannose receptor and the $\beta_2$-microglobulin promoters contain a triad composed of a CAATT box or inverted CAATT box, a TATA box, and an Inr-like element. Carroll et al. (13) reported that mutation of any of these elements in the $\beta_2$-microglobulin promoter did not reduce Tat-mediated repression, and they suggested that regulation might

FIGURE 6. TAR expression enhances Tat-mediated repression of mannose receptor promoter activity. U937 cells were transfected with 3 $\mu$g of MR854 plus 3 $\mu$g of the basic vector or pSV2Tat72 in the presence and absence of 2 $\mu$g of pNL-A0. Results were normalized to RL and are expressed as the average ± SD of triplicate determinations. These results are representative of two separate experiments. For MR854 plus Tat compared with MR854, *, $p < 0.01$; for MR854 plus Tat plus pNL-A0 compared with MR854 plus Tat, **, $p < 0.0003$.

FIGURE 7. Comparison of the basal promoters for the mannose receptor, MHC class I, and $\beta_2$-microglobulin genes. rMR, rat mannose receptor; $\beta_2$-murine $\beta_2$-microglobulin; hMR, human $\beta_2$-microglobulin; pMHC class I, porcine MHC class I; hMHC class I, human MHC class I.
interfere with Tat through the interaction of the initiation machinery and the host gene promoter. Supporting this model are the findings from several groups that Tat can bind to components of the basal transcription machinery (9). Why does Tat repress transcription from specific host promoters using host cell transcriptional initiation factors, while activating HIV transcription using the same basal machinery? The answer to this question may be that other trans factors in the nucleus control whether Tat acts as a repressor or activator. In the HIV LTR, in addition to the TAR domain, there is a modular region and a core region (8). The core region contains a basal promoter including three copies of Sp1 elements and a Tat box. The modular region contains numerous cis-acting sequences for the binding of host nuclear transcription factors including NF-kB, NFAT, and AP-1. Although HIV replication can occur in an NF-kB-independent fashion, the presence of Tat and NF-kB dramatically enhance viral replication. In addition, Tat has been reported to dramatically up-regulate NFAT-driven transcription (54). The finding that Tat increases SV40 promoter activity in the presence of upstream enhancer elements and represses promoter activity when these elements are removed further supports the role of additional cis-elements in the promoter in Tat-mediated repression (11). Studies are currently underway to further define the mechanisms involved in Tat-mediated down-regulation of mammalian transcription.

References


