Transcriptional Activation of the Human Immunodeficiency Virus Long Terminal Repeat Sequences by Tumor Necrosis Factor

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Abstract. The recombinant plasmid pBHIV-1 carrying the long terminal repeat (LTR) of the human immunodeficiency virus 1 (HIV-1), linked to the reporter chloramphenicol acetyl transferase (CAT) gene, was introduced into human and rat fibroblasts. Stable transfectants were obtained which were resistant to geneticin and expressed CAT-activity from the HIV-1 LTR. The response to TNFα was studied. It was found that, at the optimum concentration of 100 IU/ml in human and 1000 IU/ml in rat fibroblasts, the expression of CAT was stimulated by 2.1 and 2.5-fold respectively. Our findings suggest that TNF-α in physiological concentrations can transcriptionally activate the HIV-1 LTR sequences and this may play an important role in the pathogenesis of HIV infection.

TNF-α is a polypeptide cytokine which is produced by activated monocytes or macrophages and was originally described as a mediator of hemorrhagic necrosis of certain murine tumors (1,2,3). TNF-α alters the levels of various surface components of the blood vessel wall, such as blood coagulation enzyme receptors, leukocyte-adhesive receptors and class I major histocompatibility complex antigens, which may have relevance to its effects in septic shock, angiogenesis and tumor growth (4). The action of TNF-α peptide hormone occurs through membrane receptors and leads to the transcription of specific genes within relevant target cells (5). The mechanism of transduction of these signals which alter gene expression is largely unknown, but probably involves a sequence of enzymatic steps that activate factors in the nucleus that modulate transcription (5).

The human immunodeficiency virus (HIV) is the etiologic agent of the acquired immune deficiency syndrome (AIDS) (6, 7, 8). The LTR of HIV shows a complex organization with several sites recognized to be cis and trans-acting control elements through which cellular factors can regulate gene expression (9, 10). In a previous study, it has been shown that activation of infected T-cells increases the pool of the active transcription factor known as NF-κB. This factor binds to a 11 base pair motif that is repeated twice in the enhancer region of the HIV-1 LTR known as the NF-κB site (11, 12). TNF-α (5), phorbol esters (13), mitogenic lectins (12) and interleukin 1 (5) have all been shown to increase the production of HIV-1 by acting through NF-κB in T-cells.

In the present study we have examined the response to TNF-α from the HIV-1 LTR in two different model systems, those of human and rat fibroblasts. Our findings suggest that TNF-α at low concentrations can provide potent inductive signals for the expression of HIV in chronically infected cells and has important implications for understanding the pathogenic mechanism of HIV infection in humans.

Materials and Methods

Recombinant plasmids and cell lines. Plasmid pBHIV1 carrying a 728 bp (XhoI)-Hind III DNA fragment containing the HIV-1 LTR sequences was constructed by inserting a 1.9 kb BamHI fragment carrying the aminoglycoside phosphotransferase aph gene as a selectable marker for geneticin into the single Bam HI site of plasmid pBC12/HIV/CAT (14). Plasmid pBC12/HIV/CAT was obtained from B.R. Cullen (14).

The spontaneously immortalized rat 208F and the SV40 immortalized human MRCV40TGR fibroblasts were used as recipients to obtain the RFBHIV1-1 and SVTGHIV1-1 stable geneticin resistant transfectant cell lines with plasmid pBHIV1 DNA (Figure 1). Transfections were carried out using the calcium phosphate technique (16) as modified (17).

Treatment of cells and CAT assays. Cells were plated at 1.5x10^6/75cm^2 flask in Ham's F12 medium containing (10%) FCS at (37°C). 48 hours later the medium was replaced with Ham's F12 medium containing 10% FCS and the various concentrations of TNF (Amersham). 1 IU of TNF-α corresponding to 10^3 μg of protein is designated as the amount of TNF-α that is required to mediate half-maximal cytotoxicity with L929 and/or WEHI 164 cells in the presence of Actinomycin D (18). Cells were harvested 24h later and tested for CAT activity as previously described (19).

In brief, the protein content was estimated using the Biorad Protein Assay. 100 μg of protein extract were mixed with 10 μl of 4M Acetyl CoA
TNF-α enhances transcription from the HIV LTR sequences. The recipient rat 208F and human MRCV40TGR and their derivative RFBHIV1-1 and SVTGHIV1-1 transfectant cell lines respectively were treated with TNF-α at concentrations between 10-5×10^3 IU/ml. A representative CAT-assay is shown in Figure 2 (a) and the corresponding histogram in Figure 2 (b). Optimal stimulation was obtained at 100 IU/ml in SVTGHIV1-1 and 1000 IU/ml in RFBHIV1-1 cells. CAT activity increases 2.1 and 2.5-fold respectively.

The transfectant SVTGHIV1-1 cells were treated with TNF-α (100 IU/ml) at various time intervals. A representative CAT assay is shown in Figure 3 (a, b). Optimal stimulation was obtained within 24 hours, where CAT activity increased 2.6-fold.

Discussion

The human immunodeficiency virus LTR contains several defined regulatory elements (9). Among them are a trans-acting responsive element (20), an enhancer region including binding sites for NF-xB and Sp1 transcription factors, a phorbol ester inducible element and a negative regulatory element (9). In a previous study we have investigated the effect of growth factors, i.e. insulin and EGF, and hormones, i.e. Hydrocortizone and Dexamethasone, on the transcription activity of HIV LTR (10). In the present study we have found that TNF-α at the optimum concentrations stimulated the expression of the reporter CAT gene from the HIV LTR in human and rat fibroblasts by 2.1 and 2.6-fold respectively.

Although the action of TNF-α in HIV activation is not clear, several lines of evidence suggest that this cytokine may play a role in the mechanism of viral infection (13). Given also the fact that HIV-infected individuals are frequently infected with a variety of microbes that can lead to the secretion of TNFs by macrophages and T-cells, understanding the mode of action of TNF-α in expression of HIV is of great importance. This cytokine believed to act on HIV LTR through the NF-xB induction. Several reports support the finding that TNF-α induces expression of NF-xB (5, 12, 13). TNF-α induces expression of NF-xB-specific DNA - binding proteins, leading to enhancement of transcription from LTR in infected T-cell clones. Our findings are consistent with the proposed mechanism, as we have observed a significant increase in HIV LTR expression in transfectants treated with TNF-α. Furthermore we showed that TNF-α may exert the above effect in fibroblasts of human and rat origin.

References

Figure 2. a. Chromatogram of representative CAT assays with extracts from recipients' and transfectants' RFBHIV1-1 and SVTGHIV1-1 cells respectively, with and without treatment with TNF-α.
b. Induction of CAT activity by TNF-α, RFBHIV1-1 or SVTGHIV1-1 cells were plated at 1.5x10^4/75cm^2 flask in Ham's F12 medium containing 10% FCS at 37°C. 48 hours later the medium was replaced with Ham's F12 containing 10% FCS and the various concentrations of TNF-α. Cells were harvested 24 hours later and tested for CAT activity as described in Materials and Methods. Relative values of CAT activity in RFBHIV1-1 and SVTGHIV1-1 cells were 1.2 and 35 nmole acetylated chloramphenicol/µg protein per hour of incubation respectively. The average from three experiments is given. Standard deviation was less than 3% of the average values.
Figure 3. Induction of CAT activity in SVTGHIV1-1 cells by TNF-α at various times post-treatment.

a. Chromatogram for representative CAT assays with extracts from SVTGHIV1-1 cells treated with 100 IU/ml TNF-α at various times.

b. CAT values were computed and are presented in histograms as described in Figure 2b.


