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Advances in Brief

Human Immunodeficiency Virus Type 1 *tat* Gene Up-regulates Interleukin 4 Receptors on a Human B-Lymphoblastoid Cell Line

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Abstract

The human immunodeficiency virus type I (HIV-1) regulatory gene, *tat* III, is a powerful *trans*-activator of gene expression from the viral long terminal repeat and is essential for HIV replication. In addition, tat III protein has been shown to be immunosuppressive as indicated by the inhibition of antigen mediated T-cell proliferation. To further test whether *tat* III might play a direct role in the immunosuppressive effects of HIV-1 in addition to its role in virus replication, we examined the regulation of interleukin 4 (IL-4) receptors on a human B-lymphoblastoid cell line (Raji) transfected with HIV-1 *tat* gene (Raji-*tat* III). We used radioligand receptor binding analysis for cell surface expression and Northern blot analysis for the expression of human IL-4 receptor gene in Raji-*tat* III cells. Control Raji cells expressed 1383 ± 361 (SE; $n = 3$) IL-4 binding sites/cell with a dissociation constant (K_d) of 144 ± 27 pM ($n = 3$). However, Raji-*tat* III cells expressed about three times higher IL-4 receptors (4000 ± 633 IL-4 binding sites/cell; $P < 0.03$ compared to Raji cells) with a similar K_d of 273 ± 90 pM ($n = 3$; $P > 0.05$ compared to Raji cells). Whereas both Raji and Raji-*tat* III cells exhibited a single mRNA species (approximately 4 kilobases) of IL-4 receptors by Northern blot analysis, the mRNA level was about 3-fold higher in Raji-*tat* III cells compared to Raji cells. Cycloheximide inhibited the expression of IL-4 receptors by 50% in about 2 h in both cell types indicating both the half-life of IL-4 receptors and the requirement for protein synthesis for the *tat* III up-regulation of IL-4 receptors. Since IL-4 under certain circumstances has been shown to be immunosuppressant, our observation that the HIV-1 *tat* gene up-regulates IL-4 receptors suggests the possibility that the immunosuppressive effects of HIV-1 are mediated at least in part through IL-4 receptors.

Introduction

HIV-1[2] has been shown to be a causative agent of AIDS in humans (1, 2). AIDS is characterized by generalized immunosuppression including a decrease in number and function of T-cells and significant abnormalities of natural killer and B-cells as well as monocytes and macrophages (for a review see Ref. 3). HIV-1 codes for several structural and regulatory genes responsible for viral infectivity and replication (for a review see Ref. 4). Among the regulatory genes, *tat* and *rev* have been shown to be necessary for virus replication *in vitro* (5). HIV-1 *tat* III gene encodes for an 86 amino acid protein which is a strong *trans*-activator of gene expression from the viral long terminal repeat (6).

Cytokines have been shown to regulate replication and expression of HIV-1 in infected cells. IL-1, TNF-α, TNF-β, IL-6, and granulocyte-macrophage colony stimulating factor have all been shown to induce the expression of HIV-1 in infected T-cells and promonocytic cell lines (7–9). Recently, Sastry *et al.* (10) have demonstrated the induction of TNF-β by the transfection of Raji cells by HIV-1 *tat* III gene suggesting that the induction of virus replication by *tat* gene is probably mediated through the induced expression of TNF-β.

Since a hallmark of HIV infection is marked immunosuppression, it is possible that cytokines such as IL-4 play an important role in this process. IL-4, predominantly produced by Th2 lymphocytes and mast cells, has been shown to have diverse biological activities in many cell types including B-cells, T-cells, mast cells, monocytes, and other cells (for a review see Ref. 11). Besides its immunostimulatory functions, IL-4 can also have immunosuppressive effects on many cell types of the immune system. For example, IL-4 can inhibit synthesis of IL-2, IL-2 receptor expression, and IL-2/CD3 dependent proliferation of human T-cells (12), IL-2 induced proliferation and antigen specific immunoglobulin secretion in B-cells (13, 14), IL-2 induced activation of NK cells (15), the cytotoxic T-lymphocyte response to antigen (16), and the production of IFN-γ in human mixed lymphocyte culture (17). IL-4 can also inhibit the production of IL-1, IL-6, and TNF-α by human monocytes or macrophages (18).

The biological effects of IL-4 are mediated through specific cell surface receptors which have been identified on a wide variety of primary cells and established cell lines of both murine and human origin (11, 19). The role of IL-4 in the replication and propagation of HIV-1 or the status of production of IL-4 by the HIV-1 infected CD4 cells is not clear. In the present study, we have investigated the effect of HIV-1 *tat* gene on the expression of IL-4 receptors in a human B-lymphoblastoid cell line. Our data indicate that HIV *tat* gene constitutively expressed in a Raji cell line (20) up-regulated the expression of IL-4 receptors at both the protein and gene levels. Furthermore, we found that protein synthesis is required for this up-regulation.

Materials and Methods

Materials. The Raji cell line transfected with the HIV-1 *tat* gene (Raji-*tat* III) was the kind gift of Dr. Joseph Sodorski (Dana-Farber Cancer Institute, Boston, MA). The control Raji cell line was obtained from the American Type Culture Collection. Previously Sastry *et al.* (10) and Rosen *et al.* (20) have shown that Raji-*tat* III cells express a functional HIV-1 *tat* gene. Raji and Raji-*tat* III cells were cultured in complete media [composed of RPMI 1640 supplemented with 3 mM glutamine, 50 μg/ml gentamicin, and 10% heat inactivated fetal calf serum (Biofluids, Inc., Rockville, MD)]. Recombinant human IL-4 (specific activity, 10^7 units/mg protein) and the cDNA probe for human IL-4R (24) were the generous gifts of Dr. Steven Gillis and Dr. Patricia Beckman of Immunex Corporation, Seattle, WA.

Radioreceptor Binding Assay. Recombinant human IL-4 was enzy-

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[2] The abbreviations used are: HIV-1, human immunodeficiency virus type I; AIDS, acquired immunodeficiency syndrome; TNF, tumor necrosis factor; IL, interleukin.

matically labeled with ^{125}I (Amersham, Arlington Heights, IL) by the Enzymobead method according to the manufacturer's instructions (Bio-Rad, Richmond, CA). The concentration of ^{125}I-IL-4 was estimated by the amount of unlabeled IL-4 required to inhibit 50% of the binding to Ramos.G6.C10 or MLA 144 cells, a human IL-4R$^+$ B-cell or gibbon lymphoma cell line. The specific activity of the preparation ranged from 1.4 to 4.7 × 10^{10} cpm/mg protein.

For IL-4R assays, equilibrium binding studies were performed by the method previously described by Puri *et al.* (19, 21). Briefly, 2.5–5 × 10^6 cells in 126 μl binding buffer (RPMI 1640 containing 0.2% human serum albumin) were incubated with various concentrations of ^{125}I-IL-4 at 4°C in polypropylene tubes. For each concentration of ^{125}I-IL-4, nonspecific binding was determined in a parallel tube by including 100–200 molar excess of unlabeled IL-4. Affinity and number of IL-4 molecules bound/cell were calculated by Scatchard analysis (22) of the equilibrium binding data.

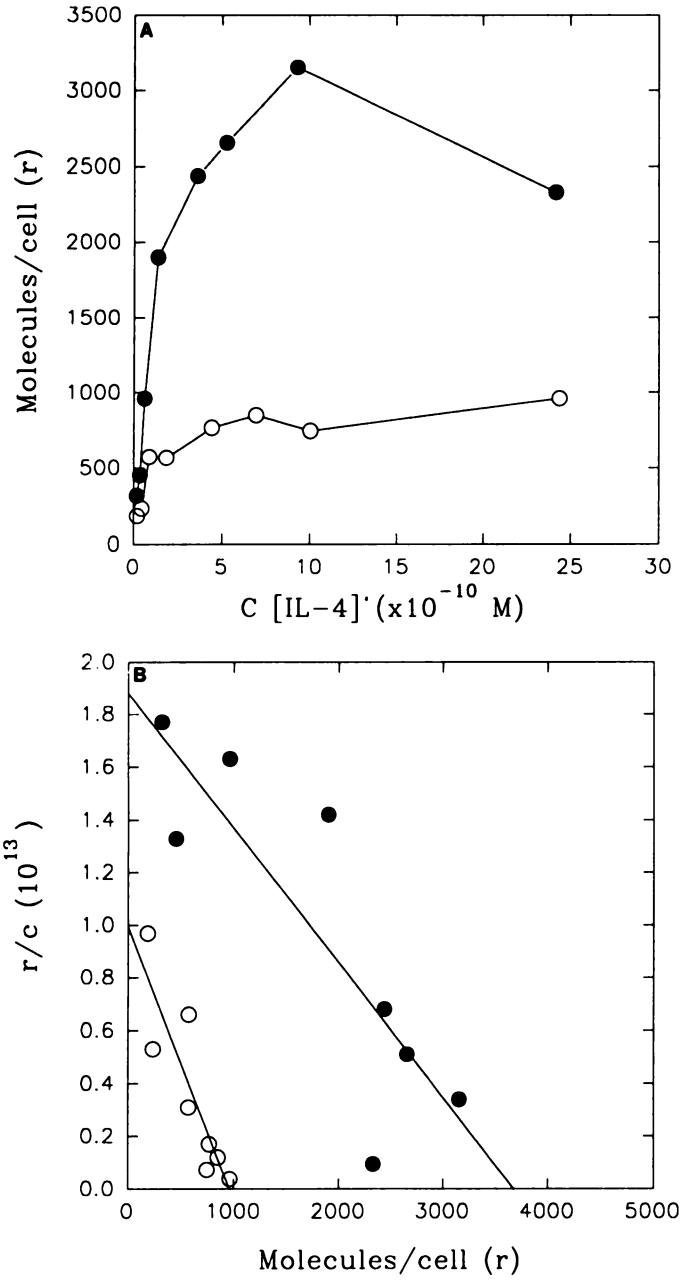

Fig. 1. *A*, equilibrium binding of ^{125}I-rIL-4 to Raji-*tat* III and Raji cells. Cells (2.5 × 10^6) were incubated with ^{125}I-rIL-4 in increasing concentrations with or without 100–200-fold M excess of unlabeled rIL-4. Bound ligand was separated from free by centrifugation of cells through a mixture of phthalate oils. The tips of the tubes containing the cell pellets (bound) and remaining supernatants (free) were counted. The specific binding of ^{125}I-IL-4 is depicted. *B*. Scatchard analysis of the binding data in *A*. ○, Raji cells; ●, Raji-*tat* III cells.

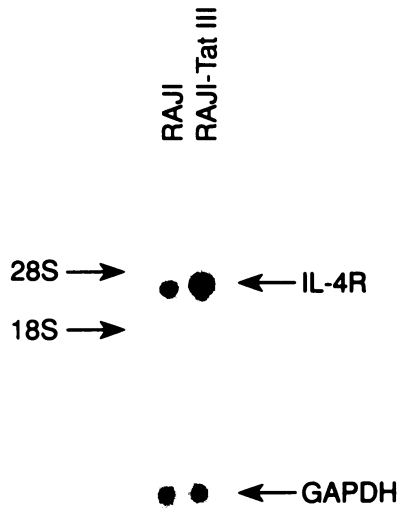

Fig. 2. Northern blot analysis of IL-4 receptor message. Total cellular RNA was isolated as described in "Materials and Methods," and 10 μg RNA were electrophoresed on a 1% agarose gel containing formaldehyde. The RNA blot was probed with IL-4 receptor complementary DNA and autoradiographed for 1–3 days. *Left*, Raji; *right*, Raji-*tat* III cells. After stripping, blots were rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) probe to assess the amount of RNA applied in each lane. *Arrows*, indicate positions of rRNA.

Northern Blot Analysis. Raji-*tat* III and Raji cells were harvested and after several washes, total RNA was extracted with RNAzol (TM Cinna Scientific, Inc., Friendswood, TX) according to the manufacturer's instructions. Ten μg total RNA were electrophoresed through a 1% agarose denaturing gel containing formaldehyde and transferred to a nylon membrane (S and S Nytran; Schleicher and Schuell, Inc., Keene, NH) by capillary action. The nucleic acid was bound to the membrane by UV cross-linking (Stratagene) and allowed to hybridize overnight with a ^{32}P-labeled human IL-4R complementary DNA or glyceraldehyde-3-phosphate dehydrogenase complementary DNA probe following a 4–12-h prehybridization. Filters were exposed to autoradiographic film for 1 to 3 days.

Results and Discussion

We examined the expression of IL-4 receptors on Raji-*tat* III and Raji cells by radioligand binding analysis. The number of IL-4 molecules bound/cell and the affinity of the receptor for IL-4 were determined. The equilibrium binding data for a typical experiment is shown in Fig. 1*A*. The data are represented as specific binding; nonspecific binding is 5–20% of the total radioactivity bound to the cell. Specific binding of IL-4 increased with concentrations of ^{125}I-IL-4 up to 400–500 pM at which point saturation of binding occurred in both Raji-*tat* III and Raji cells. Raji-*tat* III cells specifically bound 3 to 5 times higher ^{125}I-IL-4 molecules compared to Raji cells. Scatchard plot analysis of these data (Fig. 1*B*) was consistent with a single class of high affinity IL-4 receptors with a K_d of 144 ± 27 (SE) pM (n = 3) in Raji cells and 273 ± 90 pM (n = 3) in Raji-*tat* III cells. The apparent difference in K_d between Raji-*tat* III and Raji cells was statistically insignificant ($P > 0.05$). The number of IL-4 molecules bound/cell in control cells ranged from 950 to 2100 (1383 ± 361; n = 3). Similar numbers of IL-4 molecules bound/cell on Raji cells have also been previously reported (23). However, in Raji-*tat* III cells IL-4 molecules bound/cell ranged from 3050 to 5200 (4000 ± 633; n = 3). This difference in IL-4 receptor numbers between Raji-*tat* III and control cells was

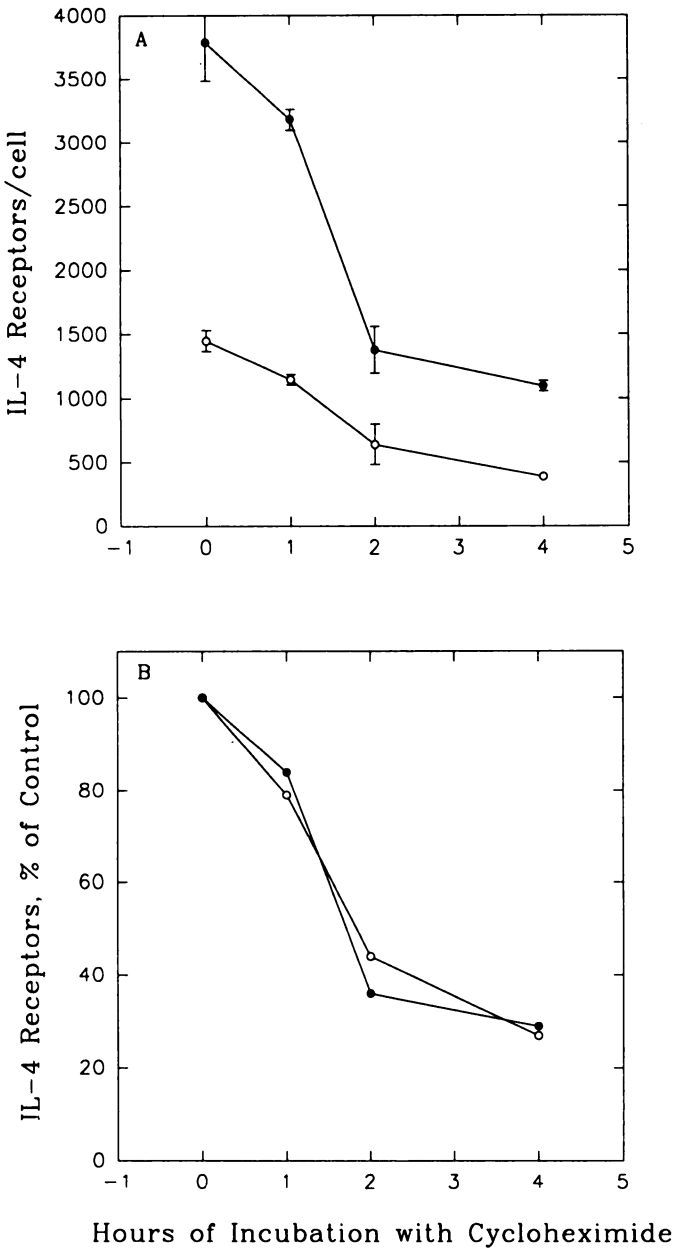

Fig. 3. Effect of cycloheximide on IL-4 receptor up-regulation by HIV-1 *tat*. *A*, Raji and Raji-*tat* III cells were incubated with cycloheximide (10 μg/ml) for the indicated times and cells harvested, washed, and assayed from IL-4 binding as described in "Materials and Methods." Cell viability at the end of the incubation period with cycloheximide was 100%. Data are presented as the mean IL-4 receptors/cell ± SE of quadruplicate samples and representative of two separate experiments. *B*, data from *A* replotted to determine the half-life of IL-4 receptors. ○, Raji cells; ●, Raji-*tat* III cells.

statistically significant ($P < 0.03$).

We next examined the expression of IL-4 receptor mRNA in Raji and Raji-*tat* III cells. Total cellular RNA was isolated from these cells and analyzed by the Northern blot technique using a cDNA probe derived from CTLL-2 cells (24). The RNA from Raji-*tat* III and Raji cells exhibited a single mRNA species of approximately 4 kilobases (Fig. 2). The level of IL-4 receptor gene expression after normalizing for apparent differences in glyceraldehyde-3-phosphate dehydrogenase mRNA level (as determined by densitometric scan) in Raji-*tat* III cells was about 3-fold higher compared to Raji cells.

To understand the mechanism, we investigated whether protein synthesis is required for HIV-1 *tat* induced up-regulation of IL-4 receptors on Raji cells. Cycloheximide (10 μg/ml) was added to control and *tat* III transfected Raji cells and then cells cultured for various times at 37°C. As depicted in Fig. 3*A*, cycloheximide inhibited the expression of IL-4 receptors in a time dependent manner in both cell types. After about 2 h of incubation with cycloheximide there was a 74% decrease in IL-4 receptors/cell in Raji-*tat* III cells and thus receptor numbers became equivalent to nontransfected Raji cells. These data indicate that protein synthesis is required for HIV-1 *tat* up-regulation of IL-4 receptors. Cycloheximide also decreased IL-4 receptors on Raji cells in about 2 h to a 50% level compared to untreated cells. These data indicate that the half-life of IL-4 receptors on both control and *tat*-transfected Raji cells is about 2 h (Fig. 3*B*).

Thus, we demonstrate that HIV-1 *tat* gene up-regulates the expression of receptors for IL-4, an immunomodulatory and growth regulatory lymphokine. To our knowledge, this is the first report showing that HIV-1 *tat* can up-regulate the expression of IL-4 receptors in any cell type. Because B-cells are a primary target for IL-4, we used a human B-lymphoblastoid cell line transfected with HIV-1 *tat* gene for our study. Whether tat also up-regulates IL-4 receptors in T-cells, in which HIV-1 undergoes replication or monocytes which serve as a reservoir for HIV-1, is not clear.

The mechanism of action of *tat* III is not clearly understood. HIV-1 *tat* gene product can easily be taken up by other cells (25) and localized in the nucleus (26), thus suggesting its biological significance. The addition of exogenous *tat* III protein can inhibit the antigen mediated T-cell proliferative response (27). Transfer of *tat* III gene into mouse germ-line cells results in lesions that appear to be similar to human AIDS associated Kaposi's sarcoma (28) and liver cancer (29). Furthermore, *tat* III protein can function as a growth factor for Kaposi's sarcoma cells *in vitro* (30).

Since *tat* protein and IL-4 can inhibit antigen mediated T-cell proliferation (27) and cytotoxic T-lymphocyte development (16), respectively, it is possible that the inhibitory effects of *tat* are mediated through IL-4 receptors. IL-4 can also inhibit IL-2 induced proliferation of human B-cells (13), antigen specific immunoglobulin secretion (14), and proliferation of natural killer/lymphokine activated killer cells (15). The effects of HIV-*tat* III gene on these functions have not been evaluated, but it is plausible that *tat* protein can also inhibit these functions. Recently, IL-4 has also been shown to enhance HIV-1 replication and lead to multinucleated giant cell formation in HIV-1 infected monocyte derived macrophages (31). Since *tat* is essential for the replication of virus, it is plausible that *tat* induced replication of HIV-1 is mediated through enhancement of the IL-4 response by the up-regulation of IL-4 receptors. Furthermore, since IL-4 under certain conditions has been shown to be immunosuppressive, it is possible that HIV-1 *tat* gene mediated immunosuppression is also mediated through regulation of IL-4 receptors.

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