Expression and Regulation of the Leukemia Inhibitory Factor/D Factor Gene in Human T-Cell Leukemia Virus Type 1 Infected T-Cell Lines

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Abstract

The expression of the leukemia inhibitory factor/D factor (LIF) gene in human T-cell leukemia virus type 1 infected T-cell lines was examined. Human T-cell leukemia virus type 1 infected T-cell lines MT-1, MT-2, H89-59, H89-79, and H109 expressed LIF mRNA, but the T-cell lines MOLT-4 and TALL-1 did not. LIF mRNA expression was enhanced by interleukin 2 or 12-O-tetradecanoylphorbol-13-acetate in MT-2 cells. The biological activity of LIF was detected in culture medium enhanced by interleukin 2 in MT-2 cells. The expression of LIF mRNA was suppressed by 1α,25-dihydroxyvitamin D3 and dexamethasone. These results imply that the expression of the LIF gene is involved in the development of hypercalcemia and abnormalities of the immune system observed in patients with adult T-cell leukemia.

Introduction

ATL2 is an aggressive, usually fatal T-cell neoplasm etiologically associated with HTLV-I infection (1, 2). ATL frequently causes hypercalcemia, which may become an important factor in managing the course of the disease (3, 4). Several investigators have reported that HTLV-I infected leukemic T-cells produce OAF-like activities which can induce bone resorption (5–7). HTLV-I infected T-cells abnormally express IL-1, parathyroid hormone related protein, and tumor necrosis factor β mRNA, which have OAF-like activities (8–11). HTLV-I infected T-cells secrete these factors in the conditioned media of cell lines and ATL patient sera (12, 13). The clinical symptoms, i.e., bone lesions and hypercalcemia, thus might represent a direct consequence of the production of such factors by HTLV-I infected T-cells.

LIF is a pleiotropic cytokine that affects the growth and differentiation of various cell types, including hematopoietic, hepatic, adipogenic, renal, neuronal, and embryonic cells (14). Recently, Abe et al. (15) have reported that LIF produced by osteoblastic cells (MC3T3-E1) stimulated bone resorption by promoting osteoclast formation. LIF is mainly secreted by activated T-cells and monocytes in the immune system (16, 17). Furthermore, it has been reported that HTLV-I infected T-cells have phenotypic similarity to activated T-cells (18). If LIF is secreted by HTLV-I infected T-cells as other OAFs, LIF may contribute to the hypercalcemia and/or the immunological abnormalities of ATL.

We examined the expression of LIF mRNA in HTLV-I infected T-cell lines and analyzed the levels of LIF mRNA regulated by various cytokines and steroid hormones.

Materials and Methods

Cell Lines. The established HTLV-I infected T-cell line MT-2 was provided by Dr. I. Miyoshi (Kochi Medical College, Nangoku-City, Japan), H89-59, H89-79, and H109 established from HTLV-I associated myelopathy patients, were provided by T. Saiida (Department of Neurology, Utano National Hospital). MT-1, MOLT-4, and TALL-1 were gifts from the Japanese Cancer Research Resources Bank (Tokyo, Japan). HL-60 was provided by Dr. R. C. Gallo (NIH, Bethesda, MD). These cell lines were maintained in RPMI 1640 (Flow Laboratories, Rockville, MD) supplemented with 10% fetal bovine serum (M. A. Bioproducts, Rockville, MD) and kanamycin (100 μg/ml). M1 T-22 cells were a gift from Dr. M. Tomida (Saitama Cancer Research Center, Saitama, Japan), and were maintained in Dulbecco’s minimal essential medium (Flow Laboratories) supplemented with 10% fetal bovine serum and kanamycin (100 μg/ml).

Reagents. Recombinant human IL-1β and IL-6 were purchased from Genzyme (Boston, MA). Recombinant human IL-2 and IFN-α were provided by Takeda Pharmaceutical Co. (Osaka, Japan) and Sumitomo Pharmaceutical Co. (Osaka, Japan), respectively. Recombinant human IFN-γ and TNF-α were gifts from Otsuka Pharmaceutical Co. (Osaka, Japan) and Daiichi Pharmaceutical Co. (Tokyo, Japan), respectively. TPA and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO). 1,25(OH)2D3 was a gift from Teijin Institute for Biomedical Research (Tokyo, Japan).

cDNA Probe. Human LIF cDNA, 0.6 kilobase, in plasmid pIC2OR (19), was provided by Dr. Nick Gough (The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia). The cDNA insert was removed from the plasmid by digestion with BamHI and HindIII followed by agarose gel electrophoresis and electroelution.

Northern Blots. Cell lines were incubated with various reagents for 24 h. Total cellular RNA was extracted from cells using acid guanidinium thiocyanate-phenol-chloroform (20). Twenty μg/lane of total RNA were electrophoresed in a 1% agarose formaldehyde gel, transferred to nitrocellulose filters (Schleicher and Schuell, Dassel, Germany) in 1 m ammonium acetate, and then fixed with 254 nm UV lamp (Stratalinker, Stratagene, CA) to the filters. The filters were hybridized at 42°C in a buffer (40% [w/v] formamide, 5 × SSC [1 × SSC is 0.15 M sodium chloride-0.015 M sodium citrate], pH 7.01 × Denhardt’s solution (0.02% bovine serum albumin-0.02% Ficol1-0.02% polyvinylpyrrolidone)-5 mM NaH2PO4-0.1% SDS-salmon sperm DNA (100 μg/ml)] containing 32P-labeled cDNA probes prepared with the random primer DNA labeling kit (Amersham, Buckinghamshire, United Kingdom; 109 cpm/μg of DNA). After a 16-h hybridization, the filters were washed twice for 20 min in 2 × SSC-0.1% SDS at room temperature and twice for 30 min in 0.1% SDS at 50°C and then autoradiographed.

Determination of LIF Activity (M1 Assay). MT-2 cells (1 × 106) were incubated for 3 days in the presence of IL-2 (100 units/ml). The cell supernatants were harvested, centrifuged, and stored under sterile conditions at −20°C until use. The activity of LIF in 2-fold dilutions of MT-2 cell culture medium was monitored by measuring the induction of phagocytic activity in M1-T22 cells essentially as described by Tomida et al. (21). M1 cells (5 × 105) were incubated for 2 days in 1 ml of...
Eagle's minimal essential medium containing 10% fetal bovine serum and standard LIF activity. The cells were harvested by centrifugation and suspended in 1 ml of serum free Eagle's minimal essential medium containing a 0.2% (v/v) suspension of polystyrene latex particles (average diameter, 1.02 μm; Sekisui Chemicals, Japan). Thereafter, the cells were incubated for 4 h at 37°C. The cells that phagocytized more than 10 particles were counted as being phagocytic. Fifty units of LIF were thereby defined as activity giving 50% maximal response in M1 cells under these conditions.

**Results**

**Expression of LIF mRNA in HTLV-1 Infected T-Cell Lines.** To investigate the LIF mRNA expression in HTLV-1 infected T-cell lines, Northern blots were analyzed using a 0.6-kilobase fragment of the LIF cDNA probe. As shown in Fig. 1, the LIF mRNA was observed in the HTLV-1 infected T-cell lines MT-1, MT-2, H89-59, H89-79, and H109. Normal human peripheral blood mononuclear cells activated by purified phytohemagglutinin, which expressed high levels of LIF mRNA, were used as a positive control (16). HL-60 cells were used as a negative control (16). The LIF transcript was undetectable in the HTLV-1 uninfected T-cell lines, MOLT-4 and TALL-1.

LIF mRNA Enhancement by IL-2. To examine the regulation of LIF mRNA by cytokines, MT-2 cells were cultured in the presence of various cytokines, including IL-1β (250 units/ml), IL-2 (100 units/ml), IL-6 (200 units/ml), IFN-α (200 units/ml), and IFN-γ (1000 units/ml), for 24 h and then Northern blotted as described under “Materials and Methods.” As shown in Fig. 24, IL-2 significantly increased the level of LIF mRNA expression in MT-2 cells. No significant increase in the level of LIF mRNA was detected in the presence of IL-1β, IL-6, IFN-α, and IFN-γ (data not shown). TPA, 10 nm, significantly increased the expression of LIF mRNA.

Effect of IL-2 on LIF mRNA Kinetics. The time dependent effects of IL-2 on LIF mRNA levels were measured by means of Northern blotting (Fig. 2B). Cells were cultured with IL-2 (100 units/ml) for periods ranging from 3 to 24 h. LIF mRNA started to accumulate at 3 h and peaked at 12 h. LIF mRNA levels decreased at 24 h during continuous exposure to IL-2.

LIF Activity in the Culture Medium of MT-2 Cells. To determine whether LIF mRNA expression results in the production of LIF protein, we determined the biological activity of LIF in MT-2 cell supernatants. LIF activity in the culture medium of 1 × 10⁶ MT-2 cells was assayed by means of the differentiating activity of a subline of murine myeloid leukemia, M1-T22 cells (Fig. 3). Culture medium treated with IL-2 (100 units/ml) for 3 days induced M1-T22 differentiation. The medium of MT-2 cells cultured with IL-2 contained 40 units/ml of LIF activity.

Down-Regulation of the Expressed LIF mRNA by Steroid Hormones. We then determined whether the expression of LIF mRNA was modulated by 1,25(OH)₂D₃ and dexamethasone (Fig. 4). Dexamethasone and 1,25(OH)₂D₃ suppressed the expression of LIF mRNA. This effect of 1,25(OH)₂D₃ was more potent than that of dexamethasone. TPA in combination with 1,25(OH)₂D₃ decreased the accumulation of LIF mRNA, compared to TPA alone.

**Discussion**

In this study, we demonstrated that HTLV-1 infected T-cell lines produce LIF and express the LIF gene. Various human tumor cells, e.g., melanoma, lung adenocarcinoma, bladder carcinoma, and the T-cell lymphoma, express the LIF gene and secrete LIF into the culture medium (22–24). Five of five HTLV-1 infected T-cell lines expressed LIF mRNA, but uninfected T-cell lines did not express detectable levels of LIF mRNA (Fig. 1). LIF has been purified as human interleukin DA produced by HTLV-1 infected T-cells, C10-MJ2 (25). Therefore, our results suggest that HTLV-1 infection is closely associated with LIF gene expression in T-cells.

The Tax-1 product, which is encoded by the pX region of HTLV-1, activates various cytokine genes, IL-2, IL-3, and granulocyte-macrophage colony stimulating factor, and the IL-2 receptor α-chain, mediated to the NF-κB-like site (26). However, the 5'-flanking region of LIF does not contain the NF-κB-like sequence (27). Further studies are required to investigate the role of Tax-1 on LIF gene activation.

A key role of IL-2 in LIF production has been reported in allogeneic human T-clone (28). In our study, IL-2 increased the level of LIF mRNA on MT-2 cells, which peaked at 12 h, and secreted LIF protein. The IL-2 induced LIF mRNA level was increased upon treatment with cycloheximide, suggesting that LIF mRNA stimulated by IL-2 did not require new protein.
Fig. 2. A, effect of IL-2 and 12-O-tetradecanoylphorbol-13-acetate (TPA) on LIF mRNA expression in MT-2 cells. MT-2 cells were incubated (12 h, 37°C) with recombinant human IL-2 (100 units/ml) and 12-O-tetradecanoylphorbol-13-acetate (10 nM). Total RNA was isolated and analyzed by Northern blot (top). Bottom, ethidium bromide stained formaldehyde gel with bands shown as a loading control. Data are representative of two independent experiments. B, kinetics of LIF mRNA accumulation. MT-2 cells were incubated with recombinant human IL-2 (100 units/ml). Total RNA was isolated at the indicated times and analyzed by Northern blot. Densitometry readings are shown of the blot in B, normalized for the amount of β-actin in each lane.

LIF GENE EXPRESSION IN HTLV-I INFECTED T-CELLS

A.

Fig. 3. LIF/D-factor activity secreted by MT-2 cells. MT-2 cells (1 x 10⁶) were incubated for 3 days in either culture medium alone (cont.) or medium with IL-2 (100 units/ml) as indicated. The culture medium was assayed with M1-T22 cells as described in "Materials and Methods." Data are representative of three independent experiments. Bars, SD.

B.

Fig. 4. Effect of steroid hormones on LIF mRNA expression in MT-2 cells. MT-2 cells were incubated (24 h, 37°C) with control (Lane a), 1,25(OH)₂D₃ (VD₃), 10 nM (Lane b), 1,25(OH)₂D₃, 100 nM (Lane c), 12-O-tetradecanoylphorbol-13-acetate (TPA), 10 nM (Lane d), 1,25(OH)₂D₃, 10 nM + 12-O-tetradecanoylphorbol-13-acetate 10 nM (Lane e), or dexamethasone (Dex), 1 μM (Lane f). LIF mRNA expression was determined by Northern blot analysis (top). Bottom, ethidium bromide stained formaldehyde gel with bands shown as a loading control. Data are representative of two independent experiments.

synthesis. On the other hand, IL-2 and the IL-2 receptor α-chain are trans-activated by Tax-1 and are considered to develop an autocrine loop in the early stage of ATL (29, 30). The induction of LIF by IL-2 may be not important only for reactions of the immune and hematopoietic systems but also for the pathophysiology of ATL patients.

Both 1,25(OH)₂D₃ and glucocorticoid exert multiple immunoregulatory effects (31-33). Activated T-cells are inhibited by 1,25(OH)₂D₃ and dexamethasone (31, 32). Previously, we and others have reported that both 1,25(OH)₂D₃ and dexamethasone inhibit the expression of IL-2 or IFN-γ in activated T-cells (33, 34). Moreover, both 1,25(OH)₂D₃ and dexamethasone suppress proliferation in some HTLV-1 infected T-cell lines (35). We demonstrated that the expression of the LIF gene was suppressed by 1,25(OH)₂D₃ and dexamethasone (Fig. 4). The suppressive mechanisms of 1,25(OH)₂D₃ and dexamethasone on LIF gene expression may be similar to that on IL-2 or IFN-γ in activated T-cells. However, neither 1,25(OH)₂D₃ nor dexamethasone affected MT-2 cell growth (36). The effect on

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the LIF gene suppression of 1,25(OH)2D3 and dexamethasone to MT-2 cells remains unclear.

LIF gene expression is induced by TPA in several T-cell lines (22, 25). TPA also induced high levels of the LIF gene in MT-2 cells (Fig. 2). TPA and 1,25(OH)2D3 synergistically induced LIF mRNA accumulation in human monocytes but not lymphocytes (16, 37). In this study, no synergistic effect of 1,25(OH)2D3 and TPA was observed in HTLV-1 infected T-cells (Fig. 4).

With regard to hypercalcemia in ATL patients, the production of TNF-β, IL-1β, and parathyroid hormone related protein in HTLV-1 infected T-cells has been reported and implicated in accelerated bone resorption (8-11). Abe et al. (15) have shown that LIF as well as IL-1, TNF-α, TNF-β, and PTH-rp have OAFe activity. The bone resorption activity of LIF is synergistically increased by combination with IL-1 and IL-6 (38). We therefore consider that LIF is an additional factor which induces hypercalcemia, in conjunction with other cytokines and abnormalities in the immune systems of ATL patients.

Recently, it has been reported that HTLV-II infected T-cells produce Oncostatin M (39), which is related to LIF in structure, functionally increased by combination with IL-1 and IL-6 (38). We (Fig. 4).

LIF mRNA accumulation in human monocytes but not lymphocytes (40), and receptor systems (41). These cytokines may provide another explanation for the pathophysiology of ATL.

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References


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