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Human T-Cell Leukemia Virus-1 Encoded Tax Protein Transactivates α1→3 Fucosyltransferase Fuc-T VII, Which Synthesizes Sialyl Lewis X, a Selectin Ligand Expressed on Adult T-Cell Leukemia Cells[1]

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Leukemia cells in patients with adult T-cell leukemia (ATL) and related cell lines strongly express the carbohydrate determinant sialyl Lewis X, a ligand for selectins. Its expression is thought to be related closely to the extravascular infiltration of the leukemia cells. Human T-cell leukemia virus type 1 (HTLV-1), the etiological agent of ATL, produces Tax protein, which is implicated in leukemogenesis through its transactivating effect on various cellular genes. In this study we investigated the transactivating effect of HTLV-1 Tax on the α1→3 fucosyltransferase Fuc-T VII, the putative rate-limiting enzyme in the synthesis of sialyl Lewis X in human leukocytes using JPX-9 cells. JPX-9 is a subclone of a non-ATL human lymphocytic leukemia cell line, Jurkat, and was established by introducing a metallothionein promoter-driven Tax expression plasmid. The JPX-9 cells as well as parental Jurkat cells did not express Fuc-T VII mRNA under normal culture conditions. When cultured in the presence of 10 μM $CdCl_2$, Tax was induced and a significant amount of the Fuc-T VII message was ascertained by Northern blotting. The amount of the message was 24.5 times as much as was detected in non-treated cells, and was comparable to that which appeared by TPA stimulation of the cells, which is supposed to simulate the sequence of events occurring in normal activation of T lymphocytes activated by more physiological stimuli. Sialyl Lewis X determinant was expressed at the surface of $CdCl_2$-treated cells, while the determinant was not detectable on either unstimulated JPX-9 or parental Jurkat cells. These results indicate that expression of sialyl Lewis X on leukemic cells in patients with ATL is at least partly due to the transactivation of the Fuc-T VII gene induced by the HTLV-1 Tax, and suggest that this leads to the accelerated extravascular infiltration of ATL cells.

Adult T-cell leukemia (ATL) is an aggressive and fatal malignancy of T-helper cells (1,2). We have previously shown that leukemia cells in patients with ATL, and cell lines derived therefrom, strongly express the carbohydrate determinant sialyl Lewis X compared to other types of human lymphocytic leukemia cells (3). We also showed that human lymphocytic leukemia cell lines established from the patients with ATL undergo a clear E-selectin-dependent cell adhesion to IL-1-activated human umbilical vein endothelial cells (3). ATL is characterized by the vigorous extravascular infiltrative activity of the leukemia cells, which adds distinct clinical features to this disease, such as the infiltration of the skin by leukemia cells. The degree of expression of sialyl Lewis X on leukemia cells in ATL significantly correlates with the degree of extravascular infiltration of the leukemia cells (4). The sialyl Lewis X determinant expressed on leukemia cells was suggested to be involved in the infiltration of leukemia cells from blood vessels to tissues and organs, since the binding of sialyl Lewis X on leukocytes and E-, or P-selectin on endothelial cells is known to trigger the extravasation of leukocytes.

Several α1→3 fucosyltransferases have an acknowledged ability to synthesize sialyl Lewis X in human cells and tissues (5). We and other researchers recently

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Abbreviations used; ATL, adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; Fuc-T, fucosyltransferase; sialyl Lewis X, a carbohydrate structure having the structure NeuAcα2→3Galβ1→4[Fucα1→3]GlcNAcβ1→R.

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cloned a new $\alpha 1 \rightarrow 3$ fucosyltransferase, Fuc-T VII (6,7), and showed that this enzyme plays an important role in the synthesis of sialyl Lewis X determinants in human leukocytes including ATL cells using sense- and antisense transfection (7,8).

Adult T-cell leukemia (ATL) is known to be caused by infection of human T-cell leukemia virus type 1 (HTLV-1) virus (9-12). Among the genes encoded by the HTLV-1 virus, the pX gene attracted special attention since its product, the Tax protein, has a profound transactivating effect on a wide variety of important cellular genes encoding such as IL-2, IL-2 receptor α, c-Fos and GCSF, through its binding to a set of enhancer-binding proteins, NF-κB, SRF, CREM or CREB (13-15). In this study, we attempted to clarify the relationship between the action of the Tax protein on the synthesis of sialyl Lewis X determinant, using a transfectant clone, which inducibly expresses the transactivator protein, Tax.

MATERIALS AND METHODS

Human lymphoid leukemia cell lines. MT-1, MT-2, ATL-2 and HUT102 were the human T-cell leukemia cell lines established from patients with adult T-cell leukemia. MOLT-3 and P12/Ichikawa were the human T-cell leukemia cells not related to adult T-cell leukemia. Raji and Nalm-6 were the human lymphoid leukemia cells having B-cell characteristics. MT-1 and MT-2 were the kind gifts of Dr. Isao Miyoshi, the Third Division of Department of Internal Medicine, Kochi Medical University, Kochi, Japan. ATL-2, HUT102, MOLT-3, Raji and Nalm-6 were obtained from the First Division of Department of Internal Medicine, Kyoto University, Faculty of Medicine. P12/Ichikawa was kindly supplied by Dr. Jun Minowada, Fujisaki Cell Center, Hayashibara Biology Research Institute, Okayama Japan. These cells were maintained in RPMI 1640 medium supplemented with 10 % fetal calf serum.

Induction of Tax expression in JPX-9 cells and Northern blotting. JPX-9 is a stable transfectant clone of Jurkat cells that inducibly expresses the Tax protein. The cells were established by introducing an expression plasmid, pMAXRH*neo*-1, into Jurkat cells by the electroporation method (16,17). The plasmid pMAXRH*neo*-1 contained a coding region for Tax protein preceded by the metallothionein promoter, and the *neo*- resistant gene driven by the SV40 early promoter.

JPX-9 cells were stimulated with 10 μM of $CdCl_2$ to induce Tax. Total RNA was isolated by the guanidinium-CsCl method as described by Maniatis *et al.* (18). Poly(A)$^+$ RNA was isolated from total RNA with Oligo-(dT)-Latex (Takara Shuzo, Co. Ltd, Kyoto, Japan) as described in the manual provided by the company. Measured amounts of the poly(A)$^+$ samples were electrophoresed through 1.2% agarose gels containing formaldehyde and were transferred to a nylon membrane (Hybond-N, Amersham, Buckinghamshire, United Kingdom). Northern blots were pre-hybridized overnight at 42°C in 50% formamide, 5 × SSPE, 2 × Denhardt's reagent, 0.1% SDS, and 150 μg/ml of sheared salmon sperm DNA (18). Blots were then hybridized overnight at 42°C in the same hybridization solution containing ^{32}P-labeled probe.

The probe used for detection of Fuc-T VII was the 459 bp *Kpn* I-*Nar* I fragment isolated from the insert in pCDM8-Fuc-T VII (6,7). The probe for the detection of Tax mRNA was excised from the expression vector, L*tax*SN (19). A human β-actin cDNA was used as a control for quality and even loading of the mRNA.

Monoclonal antibodies and flow cytometric analysis. The anti-sialyl Lewis X antibody 2F3 was established by immunizing a BALB/c mouse with a synthetic sialyl Lewis X carbohydrate determinant, and was reactive to the authentic natural as well as synthetic sialyl Lewis X as described previously (3). The antibody 2F3 detects the sialyl Lewis X antigen expressed on helper memory T cells and adult T-cell leukemia cells much more efficiently than other classical anti-sialyl Lewis X antibodies, as reported earlier (3). The anti-Lewis X antibody LeuM1 was obtained from Becton Dickinson Immunocytometry System, San Jose, CA. For flow cytometric analysis, cells were harvested at a semi-confluent stage and stained with respective monoclonal antibody. Cells were then stained with 1:200 dilution of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgM (μ-chain specific, Silenus Laboratories, Australia) and analyzed on a FACScan (Becton Dickinson, Mountain View, CA).

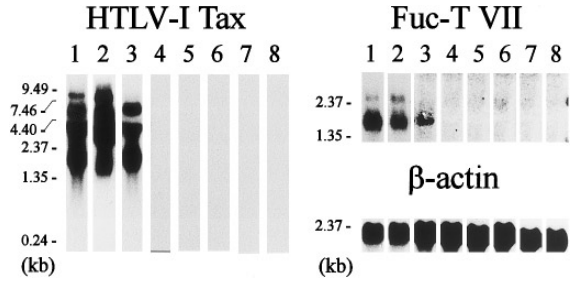

FIG. 1. Expression of HTLV-1 Tax and Fuc-T VII mRNA in human lymphoid cell lines. Left panel, expression of HTLV-1 Tax; right panel, Fuc-T VII and β-actin mRNA. Lane 1, MT-2; lane 2, HUT102; lane 3, ATL-2; lane 4, MT-1; lane 5, MOLT-3; lane 6, Raji; lane 7, Nalm-6; lane 8, P12/Ichikawa.

RESULTS

Expression of Tax and Fuc-T VII mRNA in cultured human lymphoid leukemia cell lines. The human leukemia cell lines which were established from patients with adult T-cell leukemia frequently expressed Tax mRNA as shown in Fig. 1, while the lymphoid cell lines that did not relate to adult T-cell leukemia did not express Tax mRNA. The expression of Fuc-T VII mRNA in these cells was largely correlated with the expression of Tax mRNA, in that most cell lines that express Tax mRNA tended to contain a large amount of Fuc-T VII mRNA, while the other cell lines contained only a negligible amount of same.

Induction of Tax and Fuc-T VII mRNA in $CdCl_2$-treated JPX-9 cells. Human T- cell leukemia cell line, Jurkat, and the derived transfectant clone JPX-9 barely express Tax message as indicated in the first and second lanes of the left panel of Fig. 2. These cells did not express Fuc-T VII mRNA (Fig. 2, middle panel, first and second lanes). When treated with 10 μM $CdCl_2$, a strong induction of the Tax protein mRNA was observed in JPX-9 cells 48 hours after the treatment (Fig. 2, left panel, third lane). This was accompanied by the induction of a significant amount of Fuc-T VII mRNA as indicated in the third lane of the middle panel of Fig. 2. The increase in the amount of Fuc-T VII mRNA in $CdCl_2$-treated cells was about 24 times as much as that in non-treated JPX-9 cells (Table 1).

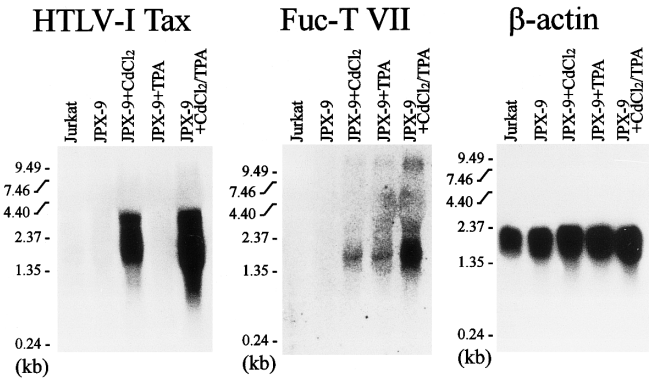

FIG. 2. Induction of HTLV-1 Tax and Fuc-T VII mRNA expression in JPX-9 cells by $CdCl_2$ or TPA treatment. Left panel, expression of HTLV-1 Tax; middle panel, Fuc-T VII, and right panel, β-actin mRNA.

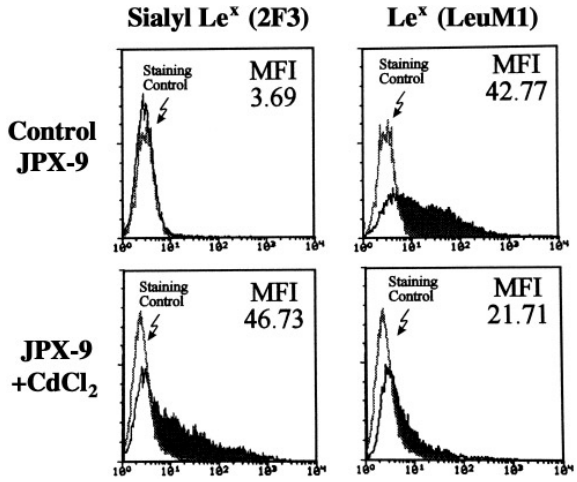

FIG. 3. Flow cytometric analysis of JPX-9 cells treated with $CdCl_2$. MFI, mean fluorescence intensity. Sialyl Lewis X was detected with the 2F3, and Lewis X with the Leu M1 antibodies.

On the other hand, stimulation of JPX-9 cells with 10 ng/ml TPA had no effect on the expression of the Tax protein (Fig. 2, left panel, fourth lane), but induced a comparable amount of Fuc-T VII mRNA in JPX-9 cells (Fig. 2, middle panel, fourth lane). The increase in the amount of Fuc-T VII mRNA in TPA-treated cells was about 21 times as much as that in non-treated JPX-9 cells (Table 1). The effect of $CdCl_2$ and TPA on the mRNA content of Fuc-T VII mRNA was additive, and treatment of JPX-9 cells with $CdCl_2$ and TPA resulted in a marked increase of Fuc-T VII mRNA (Fig. 2, middle panel, fifth lane), which reached about 56 times as much as that in non-treated JPX-9 cells (Table 1).

Expression of sialyl Lewis X determinant on the $CdCl_2$-treated JPX-9 cells. Significant expression of sialyl Lewis X was detected on JPX-9 cells by flow cytometric analysis after the $CdCl_2$ treatment (Fig. 3). In terms of fluorescence intensity, expression of sialyl Lewis X was about 12.7 times increased when compared to the non-treated JPX-9 cells, which was essentially negative for the antigen.

Interestingly, expression of Lewis X antigen, which was expressed on Jurkat cells and JPX-9 cells under usual culture conditions, was significantly reduced in $CdCl_2$-treated JPX-9 cells (Fig. 3). This finding is in line with our previous observation that Fuc-T VII contributes to the synthesis of sialyl Lewis X, but not to the synthesis of Lewis X (8). We recently showed that the expression of sialyl Lewis X and Lewis X is independently regulated in human lymphoid cells, the former by Fuc-T VII, and the latter probably by Fuc-T IV (8).

TABLE 1

Fuc-T VII mRNA Expression in JPX-9 Cells by Treatment with $CdCl_2$ or TPA

Cells and treatment	Fuc-T VII/β-actin $\times$ 100[a]
Jurkat	1.2
JPX-9	2.0
JPX − 9 + $CdCl_2$	49.0
JPX − 9 + TPA	42.5
JPX − 9 + $CdCl_2$/TPA	112.0

[a] The amount of Fuc-T VII mRNA relative to that of β-actin was quantitated with a FUJIX Bio-imaging analyzer (BAS 2000, Fuji Photo Film Co. Ltd., Japan).

DISCUSSION

The present study indicates that the viral protein Tax encoded by the HTLV-1 virus transactivates an endogenous $\alpha 1 \rightarrow 3$ fucosyltransferase Fuc-T VII, and this induces expression of sialyl Lewis X determinant at the cell surface. These results strongly suggest that the enhanced expression of sialyl Lewis X in adult T-cell leukemia cells we reported previously (3,4) is due to the transactivation of the endogenous Fuc-T VII by Tax protein encoded by HTLV-1 virus, the etiological agent of adult T-cell leukemia. Fuc-T VII is assumed to be a rate-limiting enzyme in the synthesis of sialyl Lewis X in human leukocytes (8,20), and its message is induced by physiological stimulation, which is mimicked *in vitro* by treatments with various reagents such as TPA [(21) and this paper]. The present results indicated that the strength of induction of Fuc-T VII mRNA by Tax is comparable to that obtained by more physiological stimuli including TPA, and also that the Tax-induced pathway for the transactivation of Fuc-T VII gene is at least partly independent from that induced by TPA.

We have previously shown that leukemic T cells in patients with adult T-cell leukemia and cell lines established therefrom strongly express sialyl Lewis X, and

this mediates adhesion of the leukemia cells to E-selectin expressed on endothelial cells (3). We also showed that its expression correlates with the degree of tissue infiltration by leukemia cells (4). The findings described in the present paper suggest that this extravasation of leukemic cells is triggered by the sialyl Lewis X synthesized by Tax-induced Fuc-T VII, and that the leukemic cells that strongly express Tax would promptly undergo extravasation through the interaction of induced sialyl Lewis X with endothelial cells. This could explain the disproportionately low incidence of Tax-expressing leukemic cells in the circulating blood of patients with ATL, a well-known enigma in the pathophysiology of ATL. It would be interesting to see if the leukemic cells infiltrating these tissues have a strong expression of Tax.

Tax is known to transactivate a variety of important cellular genes through several mechanisms involving transcription-activating factors, including CREB, NF-κB and SRF (13-15). The exact mechanism by which Tax induces Fuc-T VII message in ATL cells remains unclear from the present experiments. We have recently made a reporter construct in which the 5′-untranscribed region of genomic Fuc-T VII gene of about 1 kb in length was ligated to the luciferase gene. By the co-transfection of this construct to Jurkat cells with a Tax expression vector, an increase of about 30-fold luciferase activity was triggered (Hiraiwa, N. and Kannagi, R., manuscript in preparation). The 5′-untranscribed region of Fuc-T VII contained authentic binding sequences for CREB, SP-1 and AP-2, and three putative NF-κB binding sites were also detected. Study on the exact transactivating mechanism of the Tax protein on the Fuc-T VII gene is now under way in our laboratory.

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