Human T-Cell Leukemia Virus Type 1 Tax Protein Induces the Expression of Lymphocyte Chemoattractant SDF-1/PBSF

Masaaki Arai,*† Takashi Ohashi,* Tomonori Tsukahara,* Tsutomu Murakami,† Toshiyuki Hori,† Takashi Uchiyama,‡ Naoki Yamamoto,† Mari Kannagi,* and Masahiro Fujii*†,1

*Department of Immunotherapeutics and † Department of Molecular Virology, Tokyo Medical and Dental University, Medical Research Division, Yushima, Bunkyo-ku, Tokyo 113; and ‡ Institute for Virus Research, Kyoto University, Kyoto 606, Japan

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We investigated the mechanism of lymphocyte infiltration into tissues infected with human T-cell leukemia virus type 1 (HTLV-1). The cytokine SDF-1/PBSF is a highly efficient chemoattractant for lymphocytes. Reverse transcription–PCR analysis showed that among various human T-cell lines, those infected with HTLV-1 selectively expressed the SDF-1 gene. Expression of the viral protein Tax in a human T-cell line induced the expression of the SDF-1 gene, indicating that the constitutive expression of SDF-1 in virus-infected cell lines is at least in part mediated by Tax. HTLV-1-infected T-cell lines also expressed CXCR-4, a receptor for SDF-1. Moreover, chemotaxis assay showed that a HTLV-1-infected cell line migrated toward synthetic SDF-1. Thus, HTLV-1-infected cells are themselves responders for SDF-1. Our results suggest that SDF-1 induced by Tax may alter the distribution of HTLV-1-infected cells in vivo; hence it may contribute to their infiltration into affected tissues in HTLV-1-associated inflammatory diseases. © 1998 Academic Press

Key Words: lymphocyte infiltration; Tax; SDF-1; HTLV-1.

INTRODUCTION

Human T-cell leukemia virus type 1 (HTLV-1) is an etiologic agent for adult T-cell leukemia and other chronic inflammatory diseases such as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and HTLV-1-associated uveitis (HU) (Hinuma et al., 1980; Poiesz et al., 1980; Gessain et al., 1981; Osame et al., 1986; Mochizuki et al., 1992). A characteristic feature of these HTLV-1-associated diseases is infiltration of lymphocytes including virus-infected cells into affected tissues, such as eyes and central nervous system (Kira et al., 1992). Infiltration of virus-infected cells may, thus, play a role in the development of these diseases.

In addition to the structural genes, HTLV-1 encodes a regulatory gene tax. Interestingly, mice and rats carrying HTLV-1 tax or tax/env transgenes develop various inflammatory diseases such as arthritis, dermatitis, and polyarteritis (Green et al., 1989; Iwakura et al., 1991; Yamazaki et al., 1997). Tax is a transcriptional activator for the viral gene and a number of cellular genes (Uchiyama, 1997; Yoshida, 1995). Thus, dysregulated cellular gene expression by Tax may be involved in the development of HTLV-1-associated inflammatory diseases.

Chemotactic cytokines (chemokines) are thought to play a major role in the migration of cells from one tissue to another, thereby controlling cell migration during inflammation (Barggiolini et al., 1997). Chemokines consist of a large family of cytokines. Each family member has a different activity toward different types of cells. For example, interleukin (IL)-8 is a highly efficient chemoattractant for monocytes and neutrophils, but not for lymphocytes (Barggiolini et al., 1994). SDF-1/PBSF (stromal cell-derived factor-1/pre-B-cell growth-stimulating factor) is a highly efficient chemoattractant for lymphocytes and monocytes but not for neutrophils (Tashiro et al., 1993; Bleul et al., 1996b; Nagasawa et al., 1996).

In the present study, we show that among various human T-cell lines, those infected with HTLV-1 preferentially express SDF-1, and the expression is at least in part mediated by Tax. We also discuss these findings in the context of lymphocyte infiltration into tissues affected in HTLV-1-associated inflammatory diseases.

RESULTS

RNA was prepared from eight human T-cell lines (Table 1), and the expression of the SDF-1 gene in these cells was examined by the reverse transcription (RT)±PCR method. Three (MT-2, HUT102, and TL-Su,) of five HTLV-1-infected T-cell lines expressed SDF-1 RNA, but the latter was not expressed in all three uninfected T-cell lines (Fig. 1). DNA sequence analysis of fragments amplified by SDF-1 primers confirmed that it was derived...
from the SDF-1 RNA. Thus, HTLV-1-transformed T-cell lines preferentially expressed SDF-1.

The HTLV-1 Tax protein activates the expression of a number of cellular genes (Yoshida, 1995). Thus, we next measured the expression of Tax in these T-cell lines by Western blot analysis. The HTLV-1-transformed cell lines, except MT-1 and TL-Oml, expressed 40 kDa protein corresponding to Tax (Fig. 2). Since neither MT-1 nor TL-Oml expressed SDF-1, the expression of Tax correlated with that of SDF-1 in these HTLV-1-infected T-cell lines (Table 1). Anti-Tax antibody also detected several other proteins migrating around 68 kDa (MT-2) and 36 kDa (TL-Su). These proteins are probably derived from chimerical tax genes (Kobayashi et al., 1984).

To examine the role of Tax in the expression of the SDF-1 gene, we used JPX-9, which is a derivative of Jurkat and has a tax cDNA under the control of the inducible methalothionein promoter (Fig. 3; Ohtani et al., 1989). The addition of CdCl2 (10 μM/ml) to the culture medium of JPX-9 induced the expression of Tax within 3 days, which persisted until 9 days after treatment. JPX-9 increased the expression of SDF-1 within 6 days of treatment with CdCl2, and it was further enhanced 9 days after the treatment. The induction of SDF-1 was due to Tax but not by CdCl2 treatment, since it was not observed in JPX/M expressing the nonfunctional Tax protein following treatment with CdCl2. These results indicate that Tax is, at least in part, responsible for the constitutive expression of the SDF-1 gene in HTLV-1-transformed cells.

We also examined whether HTLV-1-transformed T-cell lines express CXCR-4 a receptor for SDF-1 (Bleul et al., 1996a; Oberlin et al., 1996). Flow cytometer analysis using an anti-CXCR-4 antibody showed that all examined T-cell lines expressed CXCR-4 (Fig. 4). The expression of CXCR-4 protein on HTLV-1-infected T-cell lines was less than that of three HTLV-1-uninfected cell lines.

Next we examined the SDF-1-induced chemotactic activity of the HTLV-1-infected T-cell line, MT-2. Human peripheral blood mononuclear cells (PBMC) preactivated with PHA and IL-2 were used as control. A Transwell cell migration assay using a 12-μm pore polycarbonate membrane showed that MT-2 cells migrated toward SDF-1 in a dose-dependent manner (Fig. 5A). The migration of MT-2 is specific to SDF-1, since bovine serum albumin used as a control protein did not change the chemotactic activity of MT-2 cells. The migration of MT-2 toward SDF-1 at the concentration of 10 or 100 nM and 1000 nM was less and more than activated PBMC, respectively (Fig. 5A). It is, however, difficult to compare the efficiency, since the cell size of MT-2 and the size of the used membrane pore are different from those in acti-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>HTLV-1 provirus</th>
<th>Tax protein</th>
<th>SDF-1 RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL-Oml</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MT-1</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MT-2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TL-Su</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HUT102</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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</tr>
<tr>
<td>Jurkat</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>MOLT-4</td>
<td>–</td>
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*See Sugamura et al., (1984).*

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**FIG. 1.** SDF-1 gene expression in HTLV-1-infected T-cell lines. RNA was prepared from indicated cell lines, and the expression of SDF-1 in each cell line was measured by RT-PCR analysis. The PCR-amplified fragments separated by a polyacrylamide gel were analyzed by a sequencer, and the gel image is presented. SDF-1/β-actin indicates the amplified SDF-1 fragment relative to that of β-actin. Data are a representative example of three reproducible experiments.

**FIG. 2.** Expression of the Tax protein in HTLV-1-infected T-cell lines. Cell lysate was prepared from the indicated cell lines, and the amount of Tax in each lysate was measured by Western blot analysis using an anti-Tax antibody (Lt-4). Marker (lane 1) is a molecular weight marker.
vated PBMC. Taken together, these results indicated that HTLV-1-infected T-cell lines can efficiently migrate toward SDF-1 at least in vitro.

DISCUSSION
Infiltration of lymphocytes, including virus-infected cells, into affected tissues such as the eyes and central nervous system is commonly observed in HTLV-1-associated inflammatory diseases and may be a critical step in the development of the disease (Uchiyama, 1997). The major findings of the present study were: (i) the expression of SDF-1 in HTLV-1-infected T-cell lines (Fig. 1); (ii) that the viral protein Tax is responsible for the expression of SDF-1 (Fig. 2); and (iii) the expression of CXCR-4 and chemotactic response toward SDF-1 of a HTLV-1-infected T-cell line. SDF-1 is the most efficient chemoattractant for lymphocytes both in vitro and in vivo among the chemokines identified to date (Bleul et al., 1996b). Thus, SDF-1 is a valid candidate for explaining the infiltration of lymphoid cells into HTLV-1-affected tissues.

Chemokines are not the only determinant of lymphocyte accumulation into inflammatory tissues; cell adhesion molecules are also involved in this process (Uchiyama, 1997). Interestingly, Tax also activates the expression of genes encoding several cell adhesion molecules such as E-cadherin and ICAM-1 (Kitajima et al., 1996; Fukudome et al., 1992). Thus, the combined action of multiple cellular genes including SDF-1 may promote lymphocyte infiltration into affected tissues in HTLV-1-associated diseases.

In addition to its action as a chemoattractant, SDF-1/PBSF acts as a growth-stimulating factor for pre-B-cells (Nagasawa et al., 1996). Tax-induced SDF-1 may, thus, affect the proliferation and/or differentiation of B-cells in vivo. In this regard, anti-DNA or anti-immunoglobulin antibodies have been detected in the sera of patients with HTLV-1-associated diseases (Osame et al., 1987; Usuku et al., 1988), and elevated levels of secreted immunoglobulin and abnormal B-cell expansion are present in transgenic mice carrying tax (Peebles et al., 1995). Thus, SDF-1 may be a factor responsible for these B-cell abnormalities in HTLV-1-infected individuals.
The expression of the genes of several chemokines, including MIP-1α, MIP-1β, and IL-8, is low or undetectable in many tissues including PBMC. These genes are induced by stimulation with mitogens in PBMC (Baggionini et al., 1997). In contrast, SDF-1 is constitutively expressed in several tissues (Tashiro et al., 1993). However, we observed that PBMC expressed SDF-1 only after stimulation with mitogen (data not shown). Thus, inducible SDF-1 in normal PBMC may play a role in lymphocyte migration during immune reaction.

**MATERIALS AND METHODS**

**Cell lines**

Human T-cell lines used in the present study have been characterized previously (Sugamura et al., 1984). TL-Su, MT-2, MT-1, TL-Oml, and HUT102 are HTLV-1-transformed T-cell lines. Jurkat, MOLT-4, and CCRF-CEM are HTLV-1-negative human T-cell lines. JPX-9 and JPX-9/M cells are derivatives of Jurkat and have a stably integrated tax and tax-mutant gene under the control of a methallothionein promoter, respectively (Ohtani et al., 1989). To determine the expression of Tax, the cells were cultured in the presence of CdCl₂ (10 μM) at 37°C for 0–9 days. They were cultured in RPMI 1640 medium supplemented with 10% FCS (RPMI/FCS).

**Western blotting analysis**

Cell lysate was prepared from T-cell lines, and the proteins in these lysates were resolved by electrophoresis on discontinuous 10% polyacrylamide gels with sodium dodecyl sulfate. They were then transferred to PVDF membranes. The blots were incubated with anti-Tax antibody, then rinsed and incubated with anti-mouse immunoglobulin conjugated with horseradish peroxidase. Sites of antibody binding were visualized using the ECL Western blotting detection system (Amersham). Anti-Tax antibody (Lt-4) is a mouse monoclonal antibody.
which was kindly provided by Dr. Y. Tanaka of Kitasato University (Lee et al., 1989).

RT-PCR analysis

RNA was extracted from T-cell lines according to the Isogen method described previously by Chomczynski (1993). For RT-PCR analysis, rTth DNA polymerase with reverse transcriptase activity was used for reverse transcription and sequential PCR in the presence of sense and antisense primer (Perkin–Elmer). Primers used for the amplification of SDF-1 and β-actin were CCGCGCTCGTCTAGCGAGGGAAG (sense primer), CTTGTTTAAGCTCTTTCCAGGTACT (antisense primer), and AAGAGGCATCCTCACCCT (sense primer), TAGATGGCTGGGGTGTTGAA (antisense primer), respectively. The sense primer for SDF-1 and β-actin was labeled with 6-carboxyfluorescein (6-FAM) and 4,7,2',7'-tetrachloro-6-carboxyfluorescein (TET), respectively. The amount of the expected 218-bp (β-actin) and 227-bp fragments (SDF-1) was quantitated by measuring their absorbency at 532 nm (6-FAM) and at 543 nm (TET) with a sequencer (Prism 377; Applied Biosystems) according to the instructions provided by the manufacturer. The relative amount of SDF-1 RNA represented the amount of SDF-1 fragment relative to that of β-actin after amplification.

Flow cytometry

T-cell lines were incubated with 100 ng/ml of IVR7 antibody against human CXCR-4 for 1 h on ice. After washing, the cells were further incubated with an antihuman immunoglobulin labeled with phycoerythrin (PE). In the next step, the cells were washed and analyzed on a FACScan (Becton Dickinson). IVR7 antibody was provided by Dr. T. Uchiyama (Institute for Virus Research, Kyoto University) (T. Hori et al., submitted for publication).

Chemotaxis assay

SDF-1 was synthesized as described (Bleul et al., 1996a) and kindly provided by N. Fujii and H. Tamamura (Kyoto University, Kyoto, Japan). For the preparation of activated PBMC, human PBMC were isolated from blood of healthy donors by centrifugation on Ficoll-Paque and cultured in RPMI/FCS with 1% phytohemagglutinin for 2 days. After washing PBMC were further cultured in RPMI/FCS with recombinant interleukin 2 (2 ng/ml) for 7 days. The chemotaxis assay was carried out as described (Bleul et al., 1996b). Briefly, 5 × 10^5 MT-2 cells or 1 × 10^6 preactivated PBMC were washed with PBS and suspended in RPMI 1640 containing 0.25% human serum albumin. They were then added to the top chamber of a 12-μm (for MT-2) or 5-μm (for PBMC) pore polycarbonate Transwell culture insert (Costar, Cambridge, MA), the bottom chamber of which had SDF-1 or bovine serum albumin as control protein. The cells were then cultured at 37°C for 3 h, and then the number of cells that transmigrated into the lower chamber was counted by trypan blue exclusion.

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