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Human T-Cell Leukemia Virus Type I *trans* Activator Induces Class I Major Histocompatibility Complex Antigen Expression in Glial Cells

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Transfection of the *tax* gene encoding the *trans* activator of human T-cell leukemia virus type I into glial line cells induced class I major histocompatibility complex (MHC) antigens on these cells. This occurred through the interaction of *tax* protein with the gene encoding class I MHC antigens but not through any soluble factors, such as interferons, or factors from glial cells. Since neural cells do not usually express MHC antigens, this novel mechanism may be an intermediate event between viral infection and subsequent immune-mediated pathology in the central nervous system.

Human T-cell leukemia virus type I (HTLV-I) contains the *tax* gene, which encodes the *trans* activator of the long terminal repeat (12, 20) and is associated with leukemogenesis in adult T-cell leukemia (28). In particular, HTLV-I-infected T cells synthesize the interleukin-2 receptor (IL-2R) α chain, which may be induced through the *tax* gene product of the virus (9). HTLV-I is also known to be associated with neurological disorders, such as tropical spastic paraparesis or HTLV-I-associated myelopathy (5, 18) and possibly multiple sclerosis (11, 19). However, it is not known how HTLV-I affects central nervous system cells or whether the *tax* gene product is involved in the pathogen-

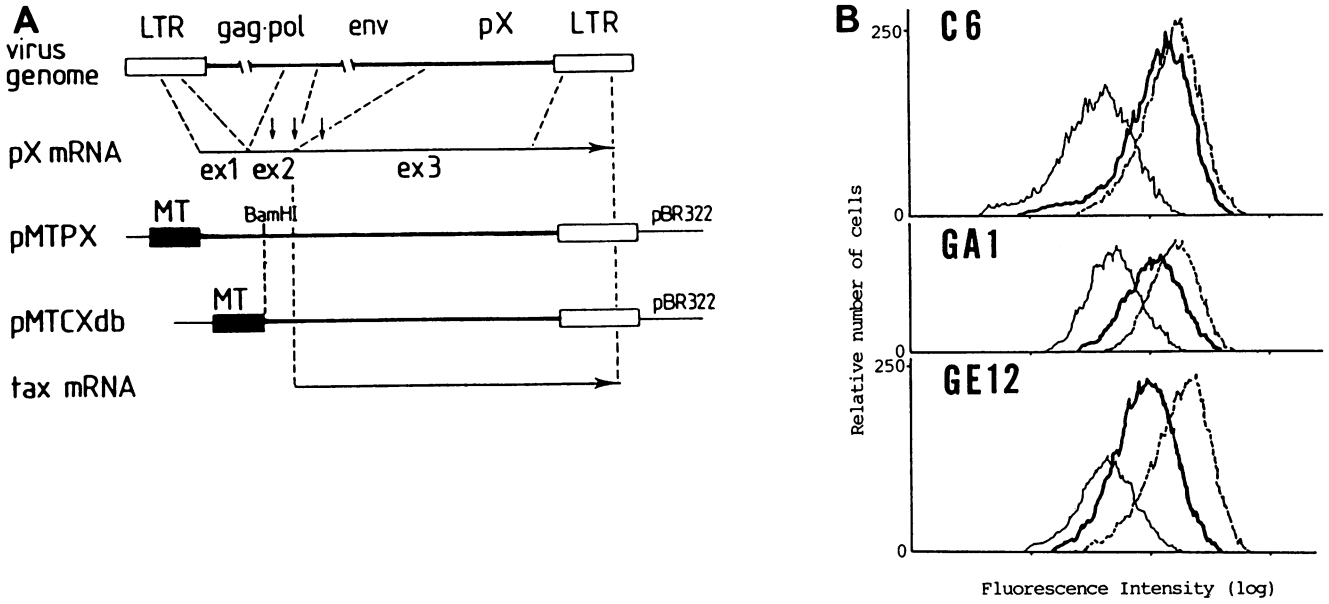

FIG. 1. (A) Expression plasmids pMTPX and pMTCXdb. These plasmids contain the mouse metallothionein promoter (MT, represented by solid rectangles) and the cDNA sequence of the HTLV-I pX gene (solid lines). pMTCXdb is a derivative of pMTPX and has a deletion in the 5′ region upstream of the *Bam*HI site that covers exon 1 and a small 5′ fragment of exon 2 of *p*X cDNA. Arrows represent the AUG codons for initiation of the translation of pX genes. Open rectangles represent the viral long terminal repeat (LTR). (B) Enhanced expression of class I MHC antigens in rat glial cells by pMTCXdb transfection. Cytofluorograms of class I MHC antigens on C6, GA1, and GE12 cells are shown. Thick solid lines indicate the expression of class I MHC antigens enhanced by pMTCXdb transfection. Thin solid lines indicate expression by nontransfected cells. Broken lines represent further enhanced expression by transfected cells after treatment with 1 μg of LPS per ml, which induces the metallothionein promoter. The vertical axes denote the relative cell number, and the horizontal axes show the fluorescence intensity on logarithmic amplification.

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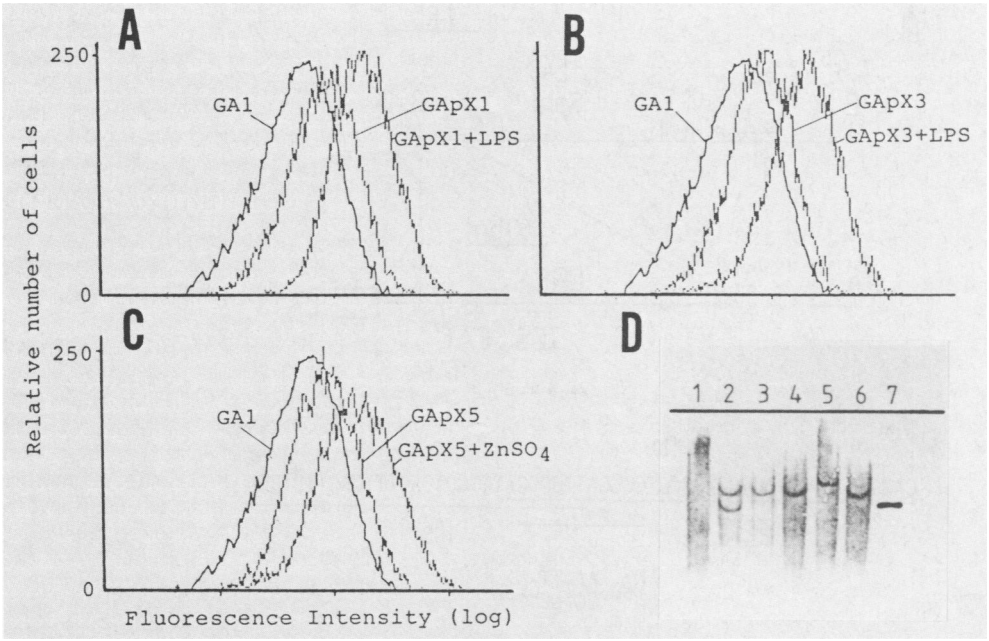
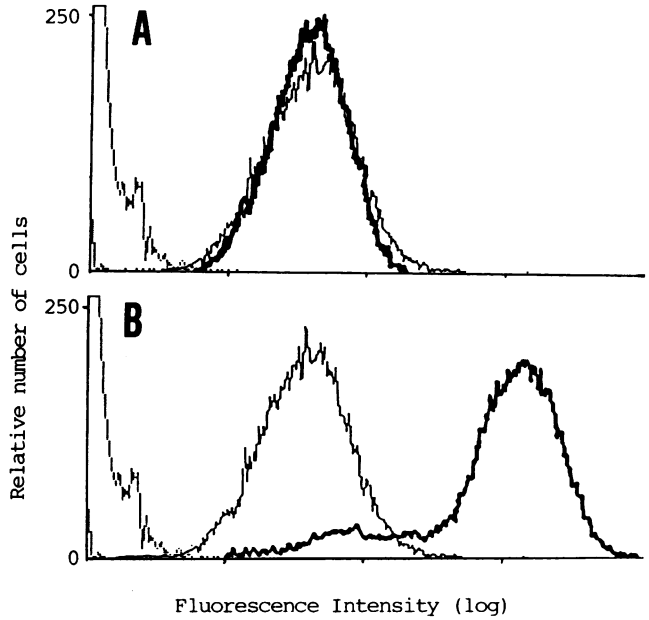


FIG. 2. Class I MHC antigen expression and DNA Southern blotting in stable transformants. GA1 cells were transfected with 10 μg of pMTCXdb and 2 μg of pSV2Neo. After 48 h, the cells were reseeded at 10^5 cells per ml and cultured with 0.4 mg of G418 per ml for 2 weeks. The selected cells, termed GApX1 to -5, were analyzed for surface expression of class I MHC antigens by flow cytometry and for cellular pMTCXdb by Southern blotting. For the DNA blotting assay, 20 μg of cellular DNA was digested with *Eco*RI and applied to a 1% agarose gel. (A to C) Enhanced expression of class I MHC antigens in subclones GApX1, -3, and -5 and further upregulation by LPS or $ZnSO_4$. (D) Detection of pMTCXdb in subclones of GApX. Lane 1, DNA from GA1 cells. Lanes 2 to 6, DNA from subclones GApX1 to -5. Lane 7, 50 ng of pMTCXdb (6.5 kilobases) alone.

esis of these neurological disorders. In this study, we showed that glial cell lines transfected with the *tax* gene encoding the HTLV-I *trans* activator are induced to express class I major histocompatibility complex (MHC) antigens. The expression of class I MHC antigens was enhanced directly by *tax* protein but not by the effect of any soluble factors, such as interferons.



For transfection, we used pMTCXdb (Fig. 1A) as an expression vector of *tax* protein (9). This plasmid is derived from pMTPX, which contains the mouse metallothionein promoter as an inducible promoter and the cDNA sequence of the pX gene, which encodes three *trans*-acting proteins, including the *tax* protein (17). pMTCXdb has a deletion in the 5′ region upstream of the *Bam*HI site that covers exon 1 and a small 5′ fragment of exon 2 of pX cDNA. Thus, it encodes exactly the same proteins as pMTPX but with higher efficiency for the expression of *tax* protein (9).

To examine whether the *tax* gene product can activate the cellular genes, we transfected the plasmid pMTCXdb into rat glial cell lines C6, GA1, and GE12. Briefly, 10^6 cells in 1 ml of Eagle minimal essential medium containing 10% fetal calf serum and 1% nonessential amino acids were transfected with 10 μg of pMTCXdb by the method of Chen and Okayama (3). After overnight transfection, the cells were washed twice and cultured in the medium for 48 h with or without 1 μg of lipopolysaccharide (LPS) (Sigma Chemical Co., St. Louis, Mo.) per ml or $ZnSO_4$ (80 μM). The cells were then harvested and stained by indirect immunofluorescence with OX 18 anti-rat class I MHC antibody (Sera-Lab, Sussex, England) for 30 min, followed by exposure to fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin G antibody for 30 min. Stained cells were analyzed

FIG. 3. Effect of supernatant fraction of *tax*-transfected GA1 cells on class I MHC antigen expression in untransfected GA1 cells. Shown are cytofluorograms of class I MHC antigens in untransfected GA1 cells with (thick lines) or without (thin lines) the supernatant fractions obtained from *tax*-transfected GA1 (A) or concanavalin A-stimulated rat spleen (B) cells.

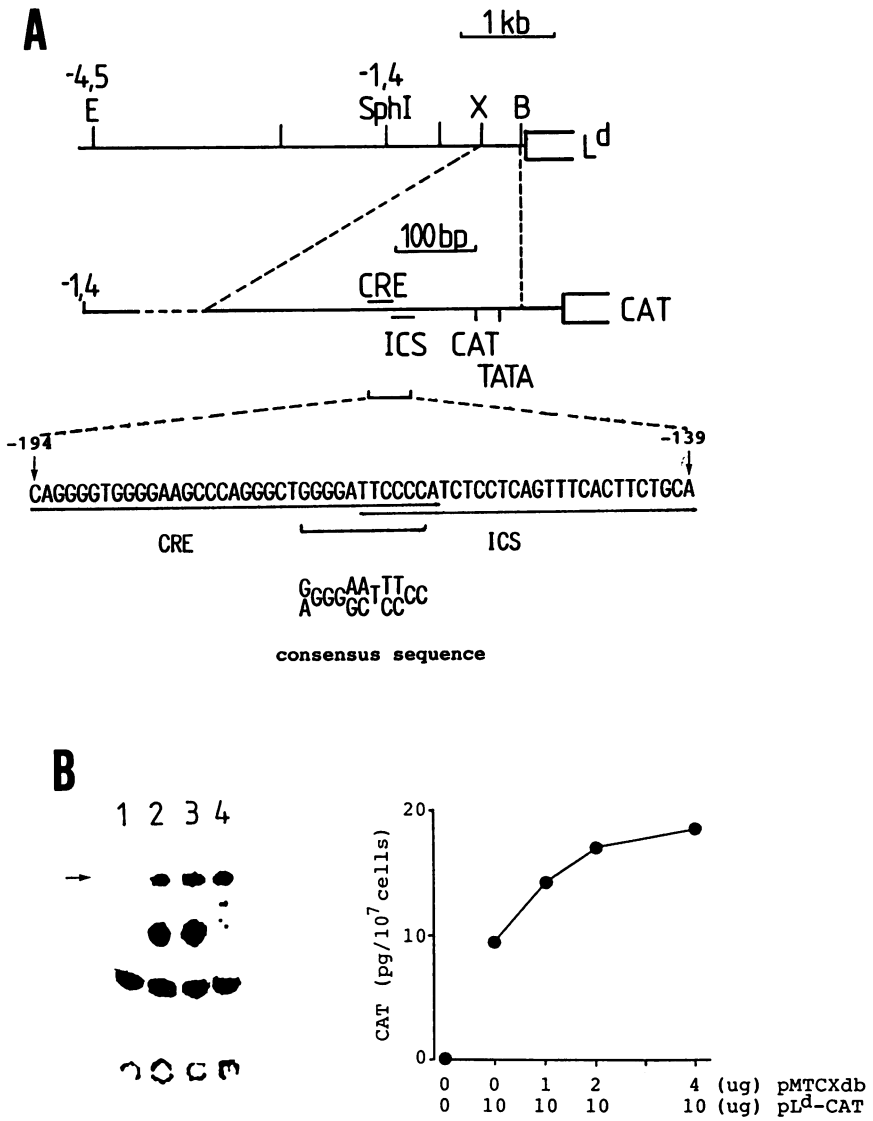


FIG. 4. (A) Schematic representation of the upstream sequence of mouse class I MHC *H-2*^{d} region. A plasmid, pL^d-CAT-1.4K (the second line) used in this study is composed of the *E. coli cat* gene and a 1.4-kilobase upstream sequence of pL^d promoter which contains the CAT box, TATA box, and three consensus enhancer sequences, a class I regulatory element (CRE), an interferon consensus sequence (ICS), and a κB-binding sequence. (B) pL^d-CAT-1.4K expression is stimulated by simultaneous transfection of pMTCXdb. Left: Autoradiogram of CAT assay. Lane 1, GA1 cells transfected with 5 μg of pL^d-CAT-1.4K alone. Lanes 2 to 4, GA1 cells transfected with both pL^d-CAT-1.4K and pMTCXdb weighing 1 (lane 2), 2 (lane 3), and 4 (lane 4) μg. Right: Increase in CAT in pL^d-CAT-1.4K-transfected GA1 cells by cotransfection with pMTCXdb. GA1 cells (5×10^6/ml) were transfected with 10 μg of pL^d-CAT-1.4K with the indicated dose of pMTCXdb. After 48 h, CAT in crude cell extracts was measured by a colorimetric enzyme-linked immunosorbent assay (CAT assay kit; 5 Prime→3 Prime, Inc., West Chester, Pa.).

by flow cytometry on an EPICS V cytometer (Coulter Electronics, Inc., Hialeah, Fla.). Enhanced expression of class I MHC antigens on these transfected cells was observed in all three glial cell lines (Fig. 1B), whereas the peak of fluorescence intensity in mock-transfected cells was almost identical to that of untransfected cells. When the expression of the *tax* gene was induced by the activation of the metallothionein promoter (located 5′ upstream of the gene) with 1 μg of LPS per ml, further enhancement of MHC antigen expression was observed on the three transfected cell lines (Fig. 1B). $ZnSO_4$ also increased class I MHC antigen expression, although less potently than LPS. LPS (1 μg/ml) or $ZnSO_4$ (80 μM) itself did not alter MHC antigen expression on untransfected cells as examined by flow cytometry. The expression of class I MHC antigens was maximal on day 2 after transfection (data not shown); this resembled the transient expression of the *tax* gene product reported earlier (9). On the other hand, expression of IL-2R and class II MHC antigens was not observed (data not shown).

The transient expression of genes, if uncontrolled, sometimes yields unexpected results. However, analysis of MHC antigen expression in stable transformants of glial cells obtained by cotransfection of pMTCXdb and pSV2Neo into GA1 gave similar results. We obtained five subclones by selection with 0.4 mg of G418 sulfate (Genesticine) per ml for

2 weeks. pMTCXdb in each subclone was detected by Southern blot analysis of DNA (Fig. 2D). Enhanced expression of class I MHC antigens and further upregulation by LPS or $ZnSO_4$ were observed in these stable transformant subclones (Fig. 2A to C). The peak of fluorescence intensity in pSV2Neo-transfected GA1 cells was identical to that of untransfected GA1 cells (data not shown). Therefore, it would appear that transfection of the *tax* gene induces class I MHC antigens on transfected glial cells.

Since the expression of class I MHC antigens on astrocytes is enhanced by a soluble factor from virus-infected astrocytes (23, 24) and by interferons (25, 26), we examined the possibility that some soluble factors were involved in the enhancement of class I MHC antigen expression on *tax*-transfected glial cells. The supernatant fraction of *tax*-transfected GA1 cells (5×10^5 cells per ml) stimulated with 1 μg of LPS was applied to untransfected GA1 cells in a final volume of 1 to 25% to see whether it could induce MHC antigen expression on these cells. After 48 h of incubation, the cells were harvested, stained by indirect immunofluorescence with anti-rat class I MHC (OX 18) antibody, and assayed by flow cytometry as described above. The supernatant fraction of *tax*-transfected GA1 cells did not enhance the expression of class I MHC antigens on untransfected GA1 (Fig. 3A), while the expression of class I MHC antigens on a similar preparation treated with crude lymphokine was enhanced (Fig. 3B). Therefore, it appears that enhanced expression of class I MHC antigens on the transfected cells was not mediated by any soluble factors released from the transfected cells themselves but was most likely mediated via intracellular mechanisms, such as the interaction between *tax* or other cellular factors and the genes encoding class I MHC antigens.

To investigate whether the enhanced expression of class I MHC antigens depends on the interaction of *tax* with the gene encoding class I MHC antigens, we transfected a plasmid, pL^d-CAT-1.4K (16), together with pMTCXdb into GA1 cells and assayed chloramphenicol acetyltransferase (CAT) activity on day 2 after transfection using [^{14}C]chloramphenicol (6). This plasmid is a hybrid of the mouse *H-2L*^{d} promoter and the *cat* gene of *Escherichia coli* and has a 1.4-kilobase upstream sequence of the cap site in the 5′-flanking region of mouse *H-2L*^{d} (Fig. 4A). CAT activity was increased by cotransfection of pMTCXdb, as indicated by autoradiography (Fig. 4B). The actual amount of CAT in pL^d-CAT-1.4K-transfected GA1 cells also increased in a dose-dependent manner (Fig. 4C). This indicates that *tax* expressed in GA1 cells activates a mouse *H-2L*^{d} promoter. Therefore, it is possible that augmentation of class I MHC antigen expression in GA1 cells occurs through interaction between *tax* protein and the gene encoding the class I MHC promoter.

Recently, HTLV-I isolated from mononuclear cells from cerebrospinal fluid of patients with HTLV-I-associated myelopathy and viruses detected in patients with this disease were shown to be identical to those from patients with adult T-cell leukemia in DNA blot analyses (8, 27). The presence of antibodies that react with the HTLV-I *gag* (p24) protein in serum and cerebrospinal fluid of multiple sclerosis patients has also been reported (11, 19). HTLV-I can infect human astrocytes in culture (7). In this study, we demonstrated that the *tax* protein of HTLV-I can induce the expression of class I MHC antigens on glial cells (Fig. 1) by affecting the promoter of class I MHC genes (Fig. 4). A similar enhancement of class I MHC antigen expression has been reported in cultured T cells infected with HTLV-I (15).

The *tax* gene encodes a *trans* activator of the viral long terminal repeat (12, 20) and the IL-2R α chain of the cellular gene of T cells (9). The effects of the *tax* product on IL-2R expression are mediated by the induction of a nuclear factor, NF-κB, which activates IL-2R α-chain promoter containing the κB-binding sequence (2, 14). The κB-binding sequence is also present in the class I MHC promoter of mouse (1) and human (22) genes. The class I MHC promoter used in this study also contains the κB-binding sequence (Fig. 4A). Therefore, it is possible that enhanced expression of class I MHC antigens is mediated by a κB-like factor(s). However, precise mechanisms are unclear.

The pathological role of *tax*-enhanced class I MHC antigen expression awaits elucidation. It is possible that class I MHC antigen functions as a receptor that is recognized by cytotoxic T cells in association with foreign or new antigens. Normal central nervous system tissue does not usually express MHC antigens and has been considered immunologically privileged. However, since astrocytes expressing class I MHC antigens are reportedly susceptible to antigen-specific lysis by cytotoxic T cells (21), induction of class I MHC antigens by the *tax* gene may trigger the interaction between glial and effector T cells in retrovirus infection. Another possible role of class I MHC antigens is that of viral receptor. It has been considered that HTLV-I infects cells via its binding to IL-2Rs or class I MHC antigens (13), both of which have a sequence homologous to that of HTLV-I envelope protein and can bind it (4, 10). According to this hypothesis, the *tax* gene product enhances viral spread.

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