Enhanced E-cadherin expression and increased calcium-dependent cell-cell adhesion in Human T-cell leukemia virus type I Tax-expressing PC12 cells

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SUMMARY

Human T-cell leukemia virus type I (HTLV-I) Tax protein induces the expression of host cellular genes, some of which are crucial in cell proliferation and differentiation. We examined the mechanisms by which HTLV-I Tax protein induces phenotypic changes in PC12 cells. We demonstrated that the HTLV-I Tax gene induces epithelioid changes and increases cell-cell contact in PC12 cells. No change in the expression of the neural cell adhesion molecule was observed between HTLV-I Tax-expressing PC12 cells and PC12 cells transfected with a control plasmid. However, HTLV-I Tax-expressing PC12 cells demonstrated a marked change in the abundance and distribution of E-cadherin, which was concentrated at regions of cellular contact and accompanied by changes in calcium-dependent cell adhesion. Although E-cadherin is expressed at low levels in PC12 and PC12 transfected with a control plasmid cells, the steady state level of E-cadherin in tax-expressing PC12 cells increases significantly, apparently as a result of regulation at the transcriptional level. Diminished expression of Tax protein in Tax-expressing PC12 cells exposed to antisense oligonucleotides for the Tax gene suppresses E-cadherin expression and decreases cell-cell adhesion. These findings imply that HTLV-I Tax protein enhanced E-cadherin expression modulates calcium-dependent cell-cell adhesion mechanisms.

Key words: E-cadherin, HTLV-I, Tax, PC12, Cell-cell adhesion

INTRODUCTION

Human T-cell leukemia virus (HTLV-I) is a type C retrovirus etiologically associated with adult T-cell lymphoma/leukemia (ATLL) (Hinuma et al., 1981), chronic inflammatory arthropathy (Nishioka et al., 1989; Kitajima et al., 1991) and the demyelinating syndrome, tropical spastic paraparesis called HTLV-I associated myelopathy (HAM/TSP) (Gessain et al., 1985; Osame et al., 1986). While the mechanism by which HTLV-I causes these diseases is unknown, HTLV-I may directly or indirectly alter nerve cells from patients with HAM/TSP (Liberski et al., 1988).

In addition to the standard retroviral genes coding for Gag, Pol, and Env, the pX region of the HTLV-I genome encodes several unique products, including the 40 kDa protein, Tax. Tax is essential for viral gene expression, mediating its effects on transcription through cyclic AMP-responsive elements (CRE) in the viral long terminal repeat (LTR) (Jeang et al., 1988). The higher viral load seen in HAM/TSP patients may be explained by a higher transactivation of the HTLV-I LTR by Tax (Yoshida et al., 1989). Tax is known to induce the expression of many cellular genes, including genes encoding the T-cell growth factor, interleukin-2 (Hoyos et al., 1989), and c-fos proto-oncogene (Fuji et al., 1988). The activation of cellular genes by Tax is mediated by at least three distinct cis-acting DNA sites: CRE (e.g. those present in the HTLV-I LTR and in the c-fos promoter), serum-responsive element (e.g. that present in the c-fos promoter), and kB elements (e.g. those present in the IL-2 and IL-2 receptor α-subunit promoter) (Leung et al., 1988; Kanno et al., 1994). While Tax does not bind to DNA directly on its own, Tax interacts with certain cellular transcription factors which bind the elements through which Tax function is mediated (Beraud et al., 1991; Suzuki et al., 1993a,b). Distinct domains of Tax are important for activation through these elements and suggest a specific mechanism of action (Smith et al., 1990, 1991). A better understanding of the function of Tax protein is necessary to define its precise role in cell activation, transformation, proliferation and differentiation.

Stable expression of Tax protein has been successful in a few adherent cell lines. Transfection experiments with Tax-expressing plasmids have demonstrated oncogenic transformation in NIH/3T3 cells and Rat-1 cells (Tanaka et al., 1990). On the other hand, a model of epithelial cells, HeLa cells, which achieved stable expression of Tax, also displayed marked morphologic alterations. These were ascertained by the
presence of round or spindle-shaped cells which interfered with the cellular architecture and the expression of intermediate filaments (Salvetti et al., 1993).

Rat pheochromocytoma (PC12) cells, which have been used as a model for differentiation studies, are induced by nerve growth factor and basic fibroblast growth factor to reversibly differentiate into cells that morphologically and biochemically resemble sympathetic neurons (Greene and Tischler, 1976). In contrast, epidermal growth factor (EGF) does not induce differentiation of neurons but rather functions as a promoter of the epithelioid appearance (Huff et al., 1981). Similar epithelial changes were reported in PC12 cells expressing the Wnt-1 gene, which is important in cellular differentiation and organization in both invertebrates and vertebrates (Bradley et al., 1993). Wnt-1 expression resulted in enhanced E-cadherin expression which increased cellular adhesion without neural differentiation.

Recently, PC12 cells expressing a human immunodeficiency virus type I (HIV-1) Tat were demonstrated to partially differentiate into sympathetic-like neurons when seeded in low-density cultures (Milani et al., 1993). In this study, we transfected the HTLV-I Tax-expressing plasmid into the PC12 cells to define the consequences of Tax protein function. We found that HTLV-I Tax-expressing PC12 cells demonstrated extensive cell-cell contacts accompanied by an epithelioid appearance. Concomitant with these changes were elevated levels of E-cadherin and an increase in calcium-dependent cell-cell adhesion. Members of the cadherin family generally are abundant in epithelial and neural tissues, where they play important roles in morphogenesis and in the maintenance of tissue integrity (Takeichi, 1988, 1991). The phenotypic effects of HTLV-I Tax expression in PC12 cells may be epithelioid differentiation and the regulation of cadherin-based cell adhesion mechanisms.

MATERIALS AND METHODS

Cell culture, transfection and selection

Three PC12 cell lines were purchased from RIKEN Cell Bank (Ibaraki, Japan) and Dainippon Pharmaceutical Co. Ltd (Osaka, Japan), and obtained as a gift from Dr S. Nakai (Otsuka Pharmaceutical Co. Ltd, Tokushima, Japan). These lines were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 5% horse serum and 1% penicillin and streptomycin. The cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂ and 95% air. The PC12 cells were transfected with an HTLV-I Tax-cells were cultured at 37°C in a humidified atmosphere containing 5% calf serum, 5% horse serum and 1% penicillin and streptomycin. The Dulbecco’s modified Eagle’s medium supplemented with 10% fetal virus type I (HIV-1) Tat were demonstrated to partially differentiate into cells that morphologically and biochemically resemble sympathetic neurons (Greene and Tischler, 1976). In contrast, epidermal growth factor (EGF) does not induce differentiation of neurons but rather functions as a promoter of the epithelioid appearance (Huff et al., 1981). Similar epithelial changes were reported in PC12 cells expressing the Wnt-1 gene, which is important in cellular differentiation and organization in both invertebrates and vertebrates (Bradley et al., 1993). Wnt-1 expression resulted in enhanced E-cadherin expression which increased cellular adhesion without neural differentiation.

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Antibodies

The monoclonal HTLV-I Tax antibody was kindly provided by Dr T. Nosaka (Kyoto University, Kyoto, Japan). The polyclonal antibody to the neural cell adhesion molecule (N-CAM) was purchased from Affiniti Research Products Limited (Nottingham, UK). The polyclonal anti-E-cadherin antibody was a gift from Dr R. Kemler (Max-Plank Institute für Immunbiologie, Germany). The blocking antibody against E-cadherin was purchased from Takara Shuzo Co. Ltd (Otsu, Japan). The monoclonal anti-N-cadherin antibody, which reacts with the N-terminal half of anti-A cell adhesion molecule (A-CAM) from chicken, rat, mouse and rabbit, and inhibits adhesion junction formation was purchased from Sigma Immuno Chemical (St Louis, MO). Fluorescein isothiocyanate (FITC)-labeled anti-rabbit IgG and anti-mouse IgG antibodies were purchased from Sigma Corp (St Louis, MO). The peroxidase-conjugated goat anti-rabbit IgG antibody was purchased from Cappel (Organon Teknika Corp., Westchester, PA).

Flow cytometry

Cells were incubated with saturating concentrations of the first antibodies including anti-HTLV-I Tax antibody, anti-N-CAM antibody, anti-N-cadherin or anti-E-cadherin antibody for 30 minutes at 4°C, and followed by counter-staining with FITC-conjugated anti-mouse or anti-rabbit IgG for 30 minutes at 4°C. After washes, quantitative analysis was performed by flow cytometry using an EPICS Profile (Coulter Electronics Inc., Hialeah, FL). FITC-labeled irrelevant mouse or rabbit antibody was used as a negative control to verify the staining specificity of the experimental antibody.

Western blotting

Cell lysates for western blot analysis of HTLV-I Tax, N-CAM and E-cadherin were prepared by scraping the cell culture into RIPa buffer (25 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1% β-mercaptoethanol, 1% aprotinin, and 1 mM PMSF) at 98°C. Total protein (5 µg per lane) was fractionated in a 10% SDS-polyacrylamide gel and then transferred to Immobilon-P membrane (Nihon Millipore, Ltd, Tokyo, Japan). The membrane was blocked with 5% fat free milk in PBS for 1 hour at 37°C and incubated for 1 hour with anti-HTLV-I Tax antibody (diluted 1/1,000), anti-N-CAM antibody (diluted 1/1,000) or anti-E-cadherin antibody (diluted 1/500). The membrane then was washed four times with PBS containing 0.1% Tween, followed by incubation with peroxidase-conjugated goat anti-rabbit IgG antibody (diluted 1/10,000) for 1 hour at room temperature. The immunoreactive proteins were visualized by enhanced chemiluminescence using the ECL system (Amersham, Buckinghamshire, UK).

Immunofluorescence and confocal laser scanning microscopic analysis

Cells grown on glass cover-slips until confluence were washed twice with PBS containing 1 mM CaCl₂ (PBS), fixed in 10% formaldehyde buffer at room temperature for 1 hour to detect N-CAM or E-cadherin, or fixed with 50% acetic acid/50% methanol for analysis of HTLV-I Tax protein. Autofluorescence was quenched by treatment with 50 mM NH₄Cl. Slides were rinsed in PBS containing 1% bovine serum albumin (BSA) and incubated for 1 hour at 37°C in anti-HTLV-I Tax antibody (diluted 1/300), anti N-CAM antibody (diluted 1/100), anti-E-cadherin (diluted 1/80) or anti-N-cadherin (diluted 1/100). FITC-labeled secondary antibodies were applied at 1:100 dilution and incubated for 30 minutes at room temperature. Confocal laser scanning microscopy (Leica True Confocal Scanner 4D, Leica Lasertech GmbH, Heidelberg, Germany) was utilized for intracellular localization. Each image consisted of 512×512 pixels. Negative control samples were treated similarly but were not exposed to the first antibody.

mRNA isolation and northern blotting

Polyadenylated RNA was extracted from the cells digested with proteinase K and purified by the FastTrack method (Invitrogen, San Diego, CA) (Badley et al., 1988). Samples were denatured and fractionated on formaldehyde/1% agarose gels. They were blotted onto nylon membranes and subjected to ultraviolet fixation. The hybridization probe for E-cadherin was a 520 bp BamHI-SalI fragment derived
from murine E-cadherin cDNA. A rat β-actin cDNA probe was used as a control for the RNA content of the lane. Probes were labeled by the random primer method with [32P]dATP. Specific activities were 6.0 × 10^8 cpm/μg.

**Antisense oligodeoxynucleotide synthesis**

Phosphorothioate (PS) modified oligodeoxynucleotides (ODNs) were synthesized on an Applied Biosystems model 380B DNA synthesizer (Foster City, CA) by using phosphoramidite chemistry. The sequences were selected to be complementary to the transcript encoded by the HTLV-I pX cassette, reported previously (Kitajima et al., 1992b). The antisense Tax (TR1) sequence was 5’ACC-CTGGGAAAGTGGG*C*C*3’ (location 7,299-7,315) and another antisense Tax (TR2) sequence was 5’GAAAAGAGATCCTG*T*C*3’ (location 7,319-7,336). Sense ODNs for Tax which has the sequence 5’GGCCCACTTCCCAGG*G*T*3’ (location 7,299-7,315) were used as a control study. Asterisks indicate the sites of PS modification. Following deportation, the ODNs were purified by HPLC, ethanol-precipitated, solubilized in distilled water.

**Cell aggregation assays**

Aggregation assays were performed according to the method of Urushihara et al. (1979). Cultures were dissociated into single cell suspensions by treatment with 0.01% trypsin in PBS or by 0.01% trypsin plus 1 mM EDTA in PBS. Cells were washed twice in PBS or PBS containing 0.01% soybean trypsin inhibitor (Sigma). The cells were resuspended in either PBS or PBS containing 1% BSA and 0.1 mg/ml DNase I (Sigma). Aliquots of 2 × 10^5 cells in a volume of 0.5 ml were added to the wells of a 24-well tissue culture plate and incubated at 37°C on a gyratory shaker at 80 rpm. At various time points (0, 15, 30, 60 and 90 minutes), the total number of particles in suspension was counted. The extent of cell aggregation was calculated as the ratio of the number of particles at each time point (Nt) to the initial number of particles plated (No). Data shown represent the mean values of three similar experiments.

**RESULTS**

**Epithelioid appearance and extensive cell-cell contacts in HTLV-I Tax-expressing PC12 cells**

Expression of HTLV-I Tax in the PC12 cells transfected with the HTLV-I Tax expressing-plasmid (PC12/tax) was detected as a 40 kDa Tax protein by immunoblot analysis (Fig. 1) and also was confirmed by immunofluorescence analysis (data not shown). When grown on a plastic substratum, both PC12 and control plasmid-transfected PC12 (PC12/neo) cells are

![Fig. 1. HTLV-I Tax protein expression in PC12/tax cells. Immunoblot analysis of PC12/neo and PC12/tax cells. Whole-cell lysates normalized for total protein content were analyzed by western blotting using anti-HTLV-I Tax antibody. Positions of molecular mass markers are shown at left (in kDa). The 40 kDa Tax protein was only detected in PC12/tax cells (arrow).](image1)

![Fig. 2. Morphologic effect of Tax expression in PC12 cells. Phase contrast photographs of PC12/neo cells at 3 days (A), 6 days (B) of culture, and PC12/tax cells at 3 days (C), 6 days (D) of culture. PC12/tax cells showed an epithelial appearance (arrow in C). ×100.](image2)
normally round and refractive in appearance at 3 days of culture (Fig. 2A), make very few cell-cell contacts and are poorly adherent after 6 days of culture (Fig. 2B). In contrast, PC12/tax cells demonstrated extensive cell-cell contacts after 12 hours upon seeding in the culture plate. Unlike the control populations, the PC12/tax cells grew as discrete colonies of adherent cells that were epithelioid in appearance at 3 days of culture (Fig. 2C). Moreover, the cells formed extensive cell-cell contacts and compacted after 6 days of culture (Fig. 2D).

**PC12/tax cells express elevated levels of E-cadherin**

Many neuronal tissues including neurons, glial cells, adrenal cells and PC12 cells express a variety of adhesion molecules, including N-CAM (Sporns et al., 1995). However, in a previous study on Wnt-1 expression in PC12 cells (PC12/Wnt-1), the cells which were epithelioid in appearance expressed elevated levels of E-cadherin (Bradley et al., 1993). In the present study, the level of expression of N-CAM was high with no significant difference between the Tax positive and negative PC12 cells (Fig. 3).

Next, the expression of E-cadherin was examined by western blot and flow cytometric analysis using an antibody specific for E-cadherin. A very faint E-cadherin protein band of 125 kDa was detected in the total cell lysate of PC12 and PC12/neo cells (Fig. 4A, lanes 1 and 2). In the PC12/tax cell lysates, however, the E-cadherin protein was approximately tenfold more abundant (Fig. 4A, lane 3). Fig. 4B illustrates the expression of E-cadherin on the cell surface, as revealed by EPICS® analysis. E-cadherin was weakly expressed on PC12/neo cells, but was markedly expressed on PC12/tax cells. Moreover, this analysis was repeated by expressing Tax in PC12 populations obtained from two other laboratories, and comparable changes in E-cadherin levels were obtained in each case (data not shown).

**Abundant E-cadherin protein is associated with cell margins in PC12/tax cells**

We examined the cell-margin distribution of E-cadherin by immunofluorescence of permeabilized cells using confocal laser scanning microscopy. In the majority of PC12 and PC12/neo cells, E-cadherin staining was very weak and minimal in cell margins (Fig. 5A). However, in PC12/tax cells, dense staining concentrated at the borders between neighboring cells was observed (Fig. 5B). These findings suggest that enhanced E-cadherin expression in PC12/tax cells is involved in adhesive interactions between adjacent cells.

**Increased mRNA of E-cadherin in PC12/tax cells**

To determine whether the elevated levels of E-cadherin protein in PC12/tax cells results from modulation at the transcription level, we performed northern analysis using E-cadherin cDNA as a probe. A single mRNA species of 4.8 kb was abundant in PC12/tax cells (Fig. 6, line 4) as well as in the mouse embryonal carcinoma cell line, F9 (Fig. 6, line 1),
which is known to be enriched for E-cadherin (Takeichi, 1991; Ozawa and Kemler, 1992). The size of this RNA corresponds to that of the E-cadherin transcript previously detected in rodent and other mammalian cells (Takeichi, 1991). On the other hand, both PC12 and PC12/neo cells demonstrated very low signals (Fig. 6, lines 2, 3).

PC12/tax cells treated with antisense Tax oligodeoxynucleotides (ODNs) decreased E-cadherin protein expression

Antisense ODN manipulation was used to study whether expression of E-cadherin was down-regulated in the face of decreased Tax protein in PC12/tax cells. We previously demonstrated that marked down-regulation of HTLV-I Tax protein occurred with the PS modified type of antisense ODN for Tax (Kitajima et al., 1992a,b). The expression levels of Tax and E-cadherin were analyzed quantitatively by EPICS®. When both antisense ODNs TR1 (Fig. 7b) or TR2 (Fig. 7c) for the Tax sequence were administered to PC12/tax cells, Tax protein was down-regulated and E-cadherin protein also was decreased (Fig. 7e,f). In contrast, sense ODNs for the Tax sequence did not affect Tax or E-cadherin expression (Fig. 7a,d).

PC12/tax cells demonstrate increased calcium-dependent adhesion which decreased with treatment of antisense inhibition of tax protein

To examine whether the elevated E-cadherin levels in PC12/tax cells were accompanied by increased calcium-dependent cell-cell adhesion, we tested the ability of the cells to aggregate following dissociation into the single-cell suspension. As shown in Fig. 8A, control plasmid transfected PC12/neo cells failed to aggregate in this assay, and after 90 minutes 95% remained as single cells. In contrast, PC12/tax cells aggregated in the presence of 1 mM CaCl₂ and the number of particles in suspension was reduced by 50% after 60 minutes and by 45% after 90 minutes. However, the PC12/tax cells treated with 1 mM EDTA failed to aggregate. These findings suggest that the aggregation of PC12/tax cells required the presence of calcium in the buffer.

The effects of the antisense inhibition of Tax protein were studied to confirm that the aggregation was dependent on E-cadherin expression. The PC12/tax cells pretreated with two antisense ODNs (TR1 and TR2) for Tax or sense ODNs for Tax were dissociated by trypsin in the presence of calcium. PC12/tax cells treated with both antisense Tax ODNs demonstrated less aggregation for 90 minutes, while cells treated with sense Tax ODNs or without treatment had less than a 60% reduction in the number of particles (Fig. 8B). These findings suggest that the expression level of Tax protein was closely related to E-cadherin expression and calcium-dependent cell-cell adhesion.

PC12/tax cells are more directly linked to the increased expression of E-cadherin than N-cadherin

N-cadherin has been reported to appear in neural tissues and in neural development (Takeichi, 1988). We examined whether the expression of N-cadherin was also elevated in PC12/tax cells. The increase in calcium-dependent cell-cell adhesion was also due to the expression of N-cadherin. The expression levels of N-cadherin and E-cadherin between PC12/neo and PC12/tax cells were...
were analyzed quantitatively by EPICS® analysis. The N-cadherin was detected in both PC12/neo and PC12/tax cells, which had the same expression levels. However, E-cadherin was very little expressed in PC12/neo cells, while E-cadherin was significantly increased in PC12/tax cells, the expression level of which was higher than that of N-cadherin (data not shown).

Next, we performed cell aggregation assays in PC12/tax cells in the presence of blocking antibodies against E- and N-cadherin. The effects of blocking antibody against E-cadherin (100 µg/ml) were significant and the number of aggregated particles was only reduced by 89% after 30 minutes, 85% after 60 minutes and retained at 83% up to 90 minutes after treatment, while the N-cadherin blocking antibody (100 µg/ml) was less effective than the E-cadherin antibody and the cells were gradually aggregated and particle number was reduced by 78%, 64% and 58% at 30, 60 and 90 minutes after treatment, respectively (Fig. 9).

DISCUSSION

The mechanisms by which HTLV-I Tax protein induces phenotypic changes in PC12 cells were examined in this study. In response to the expression of Tax, PC12 cells adopt a morphology reminiscent of epithelial cells and form extensive cell-cell contacts. In PC12 cells, N-CAM is generally known to be very important for adhesive interactions (Lahr et al., 1993). Moreover, increased expression of N-CAM has been observed in addition to the neural differentiation of PC12 cells treated with nerve growth factor (Prentice et al., 1987). However, no alteration in N-CAM expression was observed between PC12/neo and PC12/tax cells. When PC12/tax cells expressed an epithelial phenotype, no change in neural differentiation occurred.

We demonstrated marked changes in the abundance of E-cadherin in PC12/tax cells. Intense E-cadherin signals are detected from not only the cell membrane but also cytoplasm. Several explanations of the cytoplasmic staining are possible. Fine immunolocalization of several cadherins indicated that these molecules are associated with the microfilament system involving actin and vinculin (Geiger et al., 1990). The anti-E-cadherin antibody we used might react with the counterpart of the highly activated E-cadherin protein on PC12/tax cells. Another possibility is that some E-cadherin protein overpro-
E-cadherin expression in PC12 by HTLV-I Tax

molecules are less adhesive. However, when cadherin-deficient cells are transfected with complementary DNA that encodes for cadherin, they acquire the calcium-dependent adhesive activity. In addition, cell morphology is altered from the fibroblastic cell type to the epithelial cell type (Takeichi, 1988). Moreover, the presence of E-cadherin and plakoglobin induced by Wnt-1 in PC12 cells has been reported to be indicative of cadherin-based adhesive interactions and results in an epithelial phenotype (Bradley et al., 1993). We obtained similar findings in the present study. Although E-cadherin is expressed at low levels in PC12 cells and PC12/neo cells, the steady state level of E-cadherin in PC12/tax cells increased significantly and the cell became epithelioid in appearance.

The steady state levels of E-cadherin in PC12/tax cells increased as a result of regulation at the transcriptional level. The promoter of the E-cadherin gene has been studied to elucidate the mechanisms that regulate tissue-specific expression (Behrens et al., 1991). Two regions have been reported to contribute to the tissue-specific activity of the promoter: firstly G+C-rich regions, which generate basic epithelial promoter activity, most likely in combination with an initiator element present at the single transcription start site of the gene; and secondly, a palindromic sequence (named E-pal) that potentiates the activity of the proximal E-cadherin promoter and whose sequence is homologous to cis regulatory elements active in the keratin gene promoter. Moreover, the potential transcription factor binding sites are AP-2, SP-1, variant SP-1 and CAAT box. Although the promoter of E-cadherin does not contain any specific tax-related elements (TRE) involving CRE and NF-κB, Tax is able to activate the transcriptional factor-binding motifs such as AP-2 (Muchardt et al., 1992) and SP-1 (Gegonne et al., 1993), suggesting up-regulation of E-cadherin mRNA expression. Furthermore, several other explanations are possible. Additional factors, which increase the expression of E-cadherin mRNA, may be induced by Tax protein. On the other hand, inactivation of a factor suppressing E-cadherin transcription may be created by the Tax protein. Previously, the human E-cadherin gene promoter was demonstrated to be inactive in non-expressing cells, which may have resulted from binding of the repressor protein to the promoter (Bussemakers et al., 1994).

To study the function of Tax in vivo, an HTLV-I Tax transgenic model, which was established by inserting the Tax gene downstream from the U3 and R regions of the viral long terminal repeat was established (Nerenberg et al., 1987). Tax expression in the transgenic mouse is associated with proliferation of ductal cells of the salivary glands and the development of tumors in the iris, ear, tail and adrenal medulla (Nerenberg et al., 1987). Prominent features of the tumors included a spindle-shape cell morphology characteristic of tumors of mesenchymal derivation in which expression of the Tax protein was higher than that in any other tissue but without evidence for metastasis or lymphatic spread (Nerenberg et al., 1987). E-cadherin generally is not ubiquitously expressed by mesenchymal cells, and participates in the formation of a junctional complex, cell polarization or tumor invasion. E-cadherin is present in well-differentiated non-invasive carcinomas, but its expression is often down-regulated in poorly differentiated invasive tumors (Shimoyama et al., 1989; Shiozaki et al., 1991). These findings imply that HTLV-I Tax induced tumor cells may highly express E-cadherin which may modulate tumor adhesion.
Clinical manifestations suggest involvement of some cell-adhesion molecules, since characteristic features of ATLL commonly include lymphadenopathy and frequent skin infiltration. Previous studies have shown that many adhesion molecules are directly induced by HTLV-I infection. For example, intracellular adhesion molecule-1 (ICAM-1) expression also has been strongly and constitutively induced in HTLV-I-transformed T-cells (Fukudome et al., 1992). The surface expression of LFA-3 (CD58) also is constantly enhanced in HTLV-I-positive T-cell lines (Tanaka et al., 1995). Thus, Tax protein enhances cell-cell association with endothelial cells and lymphocytes. However, homotypic cell-cell aggregation in HTLV-I-infected cells involving the cadherin family has not yet been studied. E-cadherin among the various adhesion molecules induced by Tax in PC12/tax cells is probably most important, because inactivation of other adhesion systems had a minimal effect on cell-cell adhesion in the presence of functional cadherins (Larjava et al., 1990; Takeichi, 1991). Tang and co-workers (1993) reported that epidermal Langerhans cells expressed E-cadherin, and that the Langerhans cells adhered to keratinocytes through E-cadherin. They suggested that expression of E-cadherin promotes the persistence of these cells in the epidermis, and that E-cadherin is responsible for certain biological properties of these highly specialized bone marrow-derived cells. Furthermore, Cepek and co-workers (1994) demonstrated that heterotypic adhesive interactions between epithelial cells and T lymphocytes were mediated by E-cadherin and $\alpha^3\beta^3$ integrin. Thus, it will be of interest to determine if E-cadherins are involved in interactions between ATL cells and epithelial cells in patients with the cutaneous T-cell lymphoma type of ATLL.

We are grateful to Miss Nobue Uto for technical assistance. This work was supported in part by grants from the Uehara Memorial Foundation of Japan.

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E-cadherin expression in PC12 by HTLV-I Tax

(Received 28 September 1995 - Accepted 15 December 1995)


