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Induction of HLA Class I and Class II Expression in Human T-Lymphotropic Virus Type I-Infected Neuroblastoma Cells

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Human T-lymphotropic virus type I (HTLV-I) is associated with a neurologic disease, HTLV-I-associated myelopathy–tropical spastic paraparesis, in which both pathological and immunological changes are observed within the central nervous system. The pathogenesis of infection in HTLV-I-associated myopathy–tropical spastic paraparesis is not well understood with respect to the cell tropism of HTLV-I and its relationship to the destruction of neural elements. In this study, neuroblastoma cells were infected with HTLV-I by coculturing with HUT-102 cells to demonstrate that cells of neuronal origin are susceptible to this retroviral infection. HTLV-I infection of the neuroblastoma cells was confirmed by verifying the presence of HTLV-I gp46 surface antigens by flow cytometry and by verifying the presence of HTLV-I *pX* RNA by Northern (RNA) blotting and in situ hybridization techniques. To determine whether HTLV-I infection could potentially lead to changes in cell surface recognition by the immune system, the infected neuroblastoma cells were analyzed for altered HLA expression. The HTLV-I-infected, cocultured neuroblastoma cells were shown, through cell surface antigen expression and RNA transcripts, to express HLA classes I and II. In contrast, cocultured neuroblastoma cells that did not become infected with HTLV-I expressed only HLA class I. HLA class I expression was enhanced by the cytokines tumor necrosis factor α and gamma interferon and in the presence of HUT-102 supernatant. In this system, expression of HLA class I and II molecules appeared to be regulated by different mechanisms. HLA class I expression was probably induced by cytokines present in the HUT-102 supernatant and was not dependent on HTLV-I infection. HLA class II expression required HTLV-I infection of the cells. The observation of HTLV-I infection leading to HLA induction in these neuroblastoma cells provides a possible mechanism for immunologic recognition of infected neuronal cells.

Human T-lymphotropic virus type I (HTLV-I)-associated myelopathy–tropical spastic paraparesis (HAM/TSP) is a neurologic disease associated with HTLV-I infection (2, 10, 11, 13, 17, 35) that occurs in about 1% of all HTLV-I carriers. Clinically, HAM/TSP appears as a progressive spastic paraparesis with minimal symptomatology above the spinal cord level. Pathologically, there is marked degeneration of the spinal cord, particularly the corticospinal tracts, with more diffuse involvement of perivascular mononuclear cellular infiltrates in the spinal cord and brain (15, 31). These mononuclear infiltrates consist of predominantly $CD8^+$ T lymphocytes (31, 42). Despite these pathological changes, electron microscopy studies have failed to localize viral particles to any specific cell within the central nervous system (27). PCR studies have detected the HTLV-I provirus within the spinal cord (3, 20, 32), but it is unclear whether this represents virus within neural elements or the mononuclear infiltrates.

A consistent finding in patients with HAM/TSP is the presence of specific cytotoxic T-lymphocyte activity in response to HTLV-I in circulating peripheral blood lymphocytes and cerebrospinal fluid (16, 18, 19). This activity is greatly diminished or absent in asymptomatic carriers, indicating that immunologic processes may play an important role in the pathogenesis of HAM/TSP. Therefore, an issue in HAM/TSP is the potential relationship between HTLV-I infection and immunopathogenic mechanisms.

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In this regard, it is important to determine whether neural cells can be directly infected with HTLV-I. Earlier studies have demonstrated that astrocytes and microglia could be infected with HTLV-I (14, 41, 43). In the present study, neuroblastoma cell lines derived from sympathetic neuroblast tumors were examined for susceptibility to infection with HTLV-I. Cell lines of neuronal origin were selected, rather than other cell types, because the pathologic observation of significant corticospinal tract involvement compared with other tracts, such as the heavily myelinated posterior columns (15, 31), makes neurons a possible candidate for HTLV-I infection. Furthermore, HLA expression was studied in these neuroblastoma cells to determine whether infection with HTLV-I could lead to altered expression of these molecules. By examining both the susceptibility of neuroblastoma cells to HTLV-I infection and the subsequent effect on HLA expression, the interplay between HTLV-I infection and immunologic mechanisms may be better understood.

MATERIALS AND METHODS

Cell lines. The HLA expression of human neuroblastoma cell lines CHP-126, IMR-5, and NMB has been previously characterized (24–26). The HTLV-I-infected cell line was HUT-102, a human $CD4^+$ T-cell line (34). The cell lines were maintained in RPMI 1640 medium containing 10% fetal calf serum (ICN Flow, Costa Mesa, Calif.), 1% glutamine, and 1% penicillin-streptomycin. The cells were maintained at 37°C and 5% CO_2 in a humidified chamber.

Coculture protocol. Neuroblastoma cells were cocultured with HUT-102 cells at a 1:1 ratio by using two different

protocols. In the first protocol, neuroblastoma cells were cocultured with gamma-irradiated (10,000 rads) HUT-102 cells and analyzed on day 7. The cocultured neuroblastoma cells were restimulated with irradiated HUT-102 cells on a weekly basis. A separate flask containing only irradiated HUT-102 cells was examined on day 7 by trypan blue staining and flow cytometry to determine residual live cells. Both indicated that there was no viable population (data not shown).

In the second protocol, neuroblastoma cells were cocultured with unirradiated HUT-102 cells. Prior to analysis, the HUT-102 cells were removed from the coculture by an initial phosphate-buffered saline (PBS) wash to remove nonadherent cells, followed by immunomagnetic separation with anti-$CD4^+$ magnetic beads (Dynal, Inc., Oslo, Norway). For immunomagnetic separation, the anti-$CD4^+$ magnetic beads were incubated with the remaining adherent cells at a ratio of 10:1 at 4°C for 30 min. The $CD4^+$ cells bound to the magnetic beads were removed by a magnet, leaving a CD4-depleted cell population. This procedure was repeated for a total of two immunomagnetic separations to obtain approximately 99% CD4 depletion, in accordance with the manufacturer's recommendations.

Flow cytometry. Cell surface antigen expression was analyzed by flow cytometry (FACScan; Becton-Dickinson, Mountain View, Calif.) as described previously (26). The primary antibodies used were control mouse immunoglobulin G (IgG; Becton-Dickinson, Sunnyvale, Calif.), anti-HTLV-I gp46 (monoclonal antibody; Cellular Products Inc., Buffalo, N.Y.), anti-HLA class I W6/32 (ATCC HB95; anti-HLA-A, B, C; American Type Culture Collection, Rockville, Md.), anti-HLA-DR L243 (ATCC HB55; anti-human Ia; American Type Tissue Collection), anti-HLA-DQ SPVL3 (monoclonal antibody to a monomorphic HLA-DQ determinant; Serotec, Indianapolis, Ind.), and anti-HLA-DP (monoclonal antibody to a monomorphic HLA-DP determinant; Becton-Dickinson). The secondary antibody was goat anti-mouse IgG conjugated with fluorescein isothiocyanate (Cappel, West Chester, Pa.). The presence of CD4-positive cells was tested with monoclonal antibody Leu3a conjugated with phycoerythrin (Becton-Dickinson) and a mouse IgG-phycoerythrin control (Becton-Dickinson).

In situ hybridization. The protocol followed for in situ hybridization was described previously (8, 9). The cells used in the in situ hybridization were prepared in a thrombin clot (5) with 0.25 ml of A^+ plasma and 0.1 ml of thrombin, fixed in 4% paraformaldehyde, embedded in paraffin blocks, and placed on sialinized slides prepared by American Histolabs, Gaithersburg, Md. Prior to in situ hybridization, the slides were deparaffinized with Hemo-De (Fisher Scientific, Pittsburgh, Pa.). The deparaffinized slides were treated with a succession of steps to improve RNA probe penetration and remove contaminating proteases. This included treatment with 1% hydrochloric acid, proteinase K solution (0.05 mg/ml), acetic anhydride (0.8 ml/liter), and 0.1 M succinic anhydride in 0.1 M triethanolamine buffer. The slides were incubated in a prehybridization cocktail for 2 h at 45°C. The ^{35}S-labeled RNA probe was prepared in a 50% formamide-dextran sulfate hybridization cocktail at a concentration of 2.0×10^6 dpm/μl and pipetted onto the slides. The slides were covered with glass coverslips, sealed with rubber cement, and placed into a 45°C circulating water bath overnight. The coverslips were removed, and the slides were washed serially in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 50% formamide–2× SSC, 2× SSC with 0.1% Triton X-100, and 0.1× SSC and finally digested in an RNase bath containing RNase A (35 mg/liter; Boehringer Mannheim, Indianapolis, Ind.) and RNase T1 (70 μl/liter; Boehringer Mannheim) to remove any residual unbound RNA. After two more washings with 2× SSC, the slides were dehydrated with 0.3 M ammonium chloride in 70 and 95% ethanol, respectively, and allowed to dry in ambient air. The slides were then dipped in NTB-3 emulsion (International Biotechnologies Inc., New Haven, Conn.) and incubated for 3 days at 4°C in a light-tight box. The slides were developed with Kodak D-19 developer and Kodak fixer (Eastman Kodak, Rochester, N.Y.). Hematoxylin and eosin were used as the counterstain.

The sense and antisense RNA probes were derived from a 2.6-kb segment within the *pX* region of the HTLV-I genome (37) and were obtained from Lofstrand Labs Ltd., Gaithersburg, Md. (permission of Scott Koenig, Medimmune, Gaithersburg, Md.). In addition, a β-actin RNA probe (Lofstrand) was used as a positive control to demonstrate the presence of RNA.

Immunohistochemistry. Thrombin clots of cells were prepared as described above, embedded in paraffin, and placed on sialinized slides. Immunohistochemical analysis was done prior to in situ hybridization (39). The specimens were deparaffinized and incubated with 10% fetal calf serum–0.2% Triton in PBS for 1 h at room temperature. They were incubated with a primary antibody at 4°C overnight in a humidified chamber. This was followed by incubation with a biotin-conjugated secondary antibody (4°C, overnight) and an avidin-biotin complex (Vectastain, Burlingame, Calif.) (room temperature, 2.0 h). Biotin-labeled antibodies were detected with diaminobenzidine (Sigma, St. Louis, Mo.). The primary antibody, anti-neurofilament 160 (clone BF10; Boehringer Mannheim), was used at a dilution of 1:5. The secondary antibody, biotin-labeled goat anti-mouse IgG (Boehringer Mannheim), was used at a dilution of 1:300. The slides were counterstained with methyl green (Sigma catalog no. M-8884), a stain specific for nucleoli. Washes between steps were done with PBS containing 0.018% diethylpyrocarbonate (Sigma), an RNase inhibitor. The antibody preparations contained 280 U of RNase inhibitor (from human placenta; Boehringer Mannheim) per ml and were diluted in diethylpyrocarbonate-treated PBS.

RNA isolation and analysis. Total cellular RNA was isolated by the acidified guanidinium phenol-chloroform method (4) and analyzed by Northern (RNA) blotting as previously described (6). The probes used were as follows: pHLA-2a.1, an HLA-A locus-specific fragment (a gift from Harry Orr, University of Minnesota, Minneapolis) (21); HLA-DRA, a 600-bp 3′ *Pst*I fragment of HLA-DRA-10 (a gift from Eric Long, National Institutes of Health, Bethesda, Md.); HTLV-I Tax, a 2.6-kb *Hin*dIII fragment of pCMV-Tax (a gift from John Brady, National Institutes of Health); and actin, a 1.9-kb *Eco*RI-*Hin*dIII fragment of human β-actin (a gift from Herbert Cooper, National Institutes of Health).

Barrier plate cultures. Neuroblastoma cells were plated on 24-well Transwell plates (Costar, Cambridge, Mass.) at a concentration of 10^5 per well. Unirradiated HUT-102 cells were either placed inside a Transwell insert (separating the two cell types with a 0.45-μm-pore-size filter) or directly mixed with neuroblastoma cells. The mixed neuroblastoma and HUT-102 cells underwent immunomagnetic separation of $CD4^+$ cells prior to analysis.

Cytokine studies. Neuroblastoma cells were cultured in the presence of tumor necrosis factor alpha (TNF-α; Genzyme, Boston, Mass.) or gamma interferon (IFN-γ; Genzyme) for 3 days and analyzed by flow cytometry for expression of cell surface HLA.

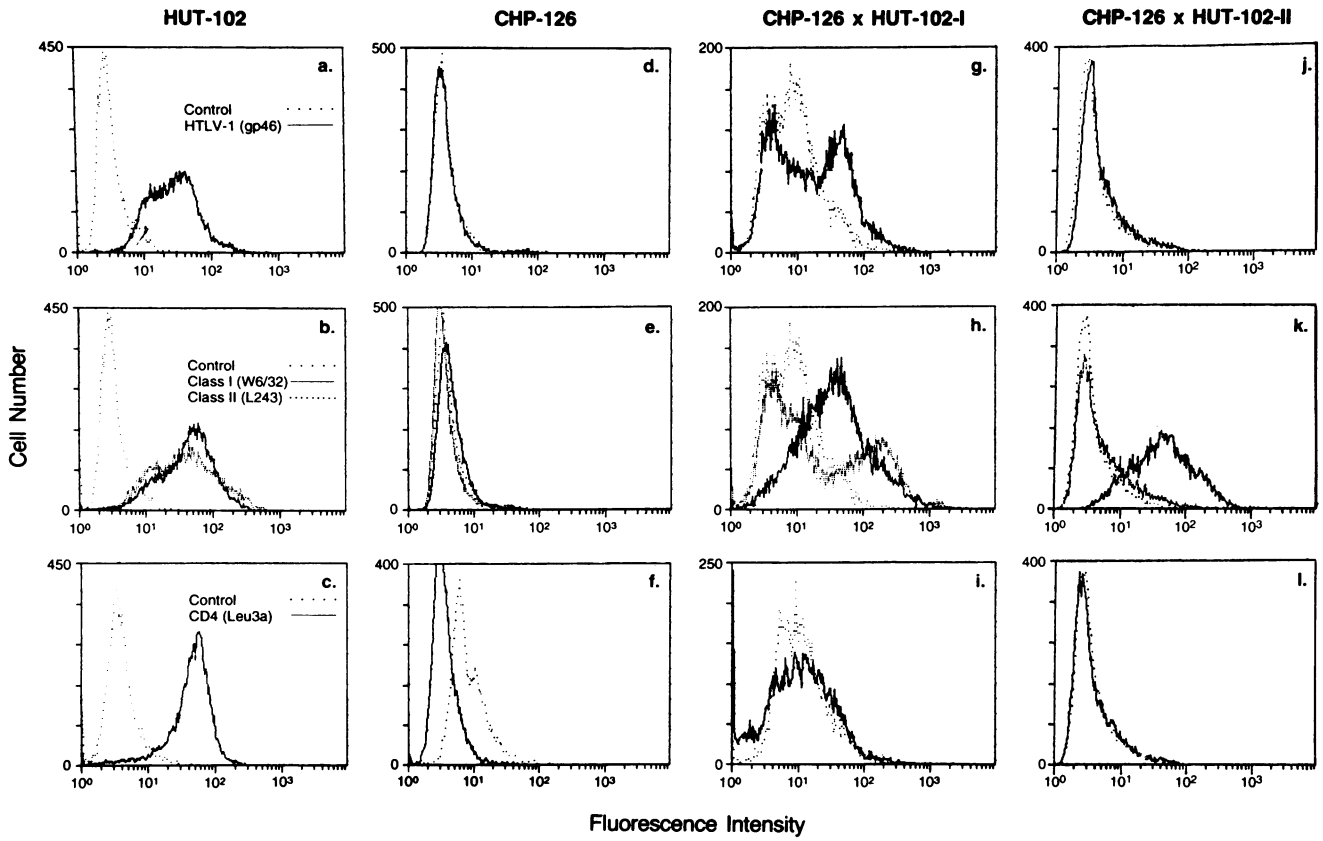


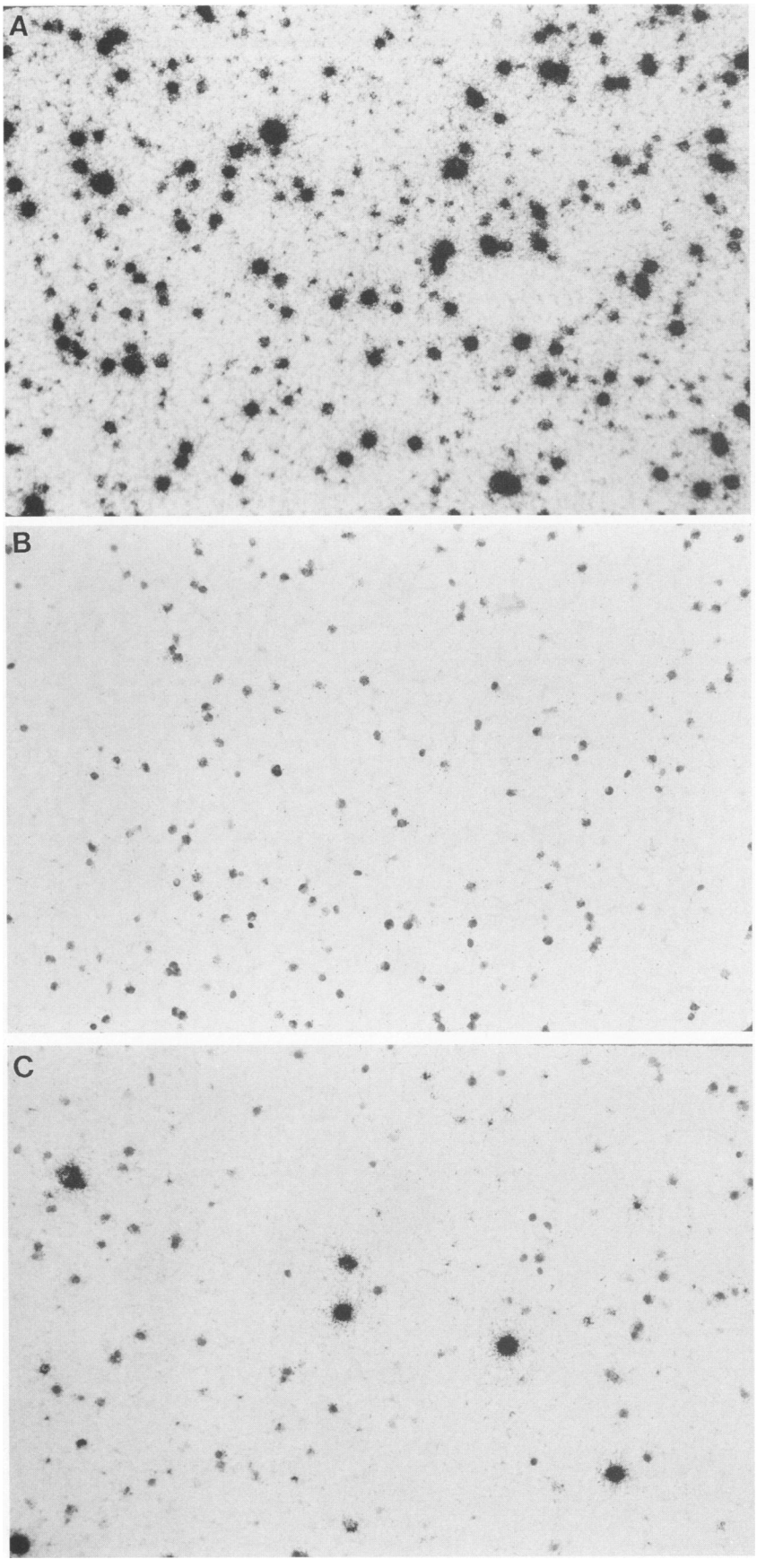
FIG. 1. Demonstration of HTLV-I and HLA expression in cocultured neuroblastoma cells. Cell surface expression of HTLV-I gp46 (a, d, g, and j), HLA class I or II (b, e, h, and k), and CD4 (c, f, i, and l). Fluorescence intensity versus cell number is plotted relative to the background fluorescence of the mouse IgG control (dotted line). CHP-126 × HUT 102-I (g, h, and i) is a coculture of neuroblastoma and irradiated HUT-102 cells with a population of HTLV-I-expressing cells. CHP-126 × HUT-102-II (j, k, and l) is this same coculture at a time when expression of HTLV-I could not be detected.

RESULTS

Demonstration of HTLV-I infection of neuroblastoma cells. (i) Expression of HTLV-I cell surface antigens. Neuroblastoma cells were cocultured with irradiated HUT-102 cells at weekly intervals and analyzed for expression of HTLV-I gp46 and CD4 surface antigens by flow cytometry. The HUT-102 cell line was HTLV-I gp46 and CD4 positive (Fig. 1a and c), while the CHP-126 neuroblastoma cell line was HTLV-I gp46 and CD4 negative (Fig. 1d and f). Cocultures of CHP-126 with irradiated HUT-102 cells led to a population of HTLV-I gp46-positive cells (Fig. 1g) in the absence of CD4 expression (Fig. 1i), indicating that these cells were neuroblastoma cells and not residual HUT-102 cells. Viral antigen expression was sporadic, being detected as early as day 7 in some cocultures but requiring several weekly restimulations in other cocultures. The infection was transient, and the cocultures eventually reverted to a viral antigen-negative state (Fig. 1j and l). Similar results were obtained with cocultures using two other neuroblastoma cell lines (NMB and IMR-5). More consistent expression of HTLV-I gp46 on cocultured neuroblastoma cells was observed with a coculture protocol that used unirradiated HUT-102 cells which were removed prior to analysis by anti-CD4 immunomagnetic separation. The neuroblastoma cells derived from these cocultures were consistently CD4 negative. The expression of HTLV-I gp46 on these CD4-negative cocultured neuroblastoma cells indicated that these cells were infected with HTLV-I.

In situ hybridization of cocultured neuroblastoma cells. To confirm that neuroblastoma cells were infected with HTLV-I, cocultures of neuroblastoma cells and irradiated HUT-102 cells were examined by in situ hybridization for the presence of HTLV-I-specific RNA transcripts. The CD4-negative cocultured neuroblastoma cells that were HTLV-I gp46 positive specifically hybridized with an antisense HTLV-I *pX* RNA probe (Fig. 2a) but were completely negative with the control sense HTLV-I *pX* RNA probe (data not shown). In contrast, cocultured neuroblastoma cells that did not express HTLV-I (HTLV-I gp46 negative) were also negative by HTLV-I *pX* RNA *in situ* hybridization (data not shown). Uninfected neuroblastoma cell lines (HTLV-I gp46 and CD4 negative) were

FIG. 2. In situ hybridization of cocultured neuroblastoma cells. (A) HUT-102 cells. (B) Uninfected CHP-126 cells. (C) HTLV-I-expressing cocultured neuroblastoma cells from the same experiment as in Fig. 1g, h, and i (CHP-126 × HUT-102-I). All cells were hybridized with an antisense HTLV-I *pX* RNA probe. Silver grains indicate specific probe binding. Cells were counterstained with hematoxylin and eosin. Light field microscopy (magnification, ×200) was used.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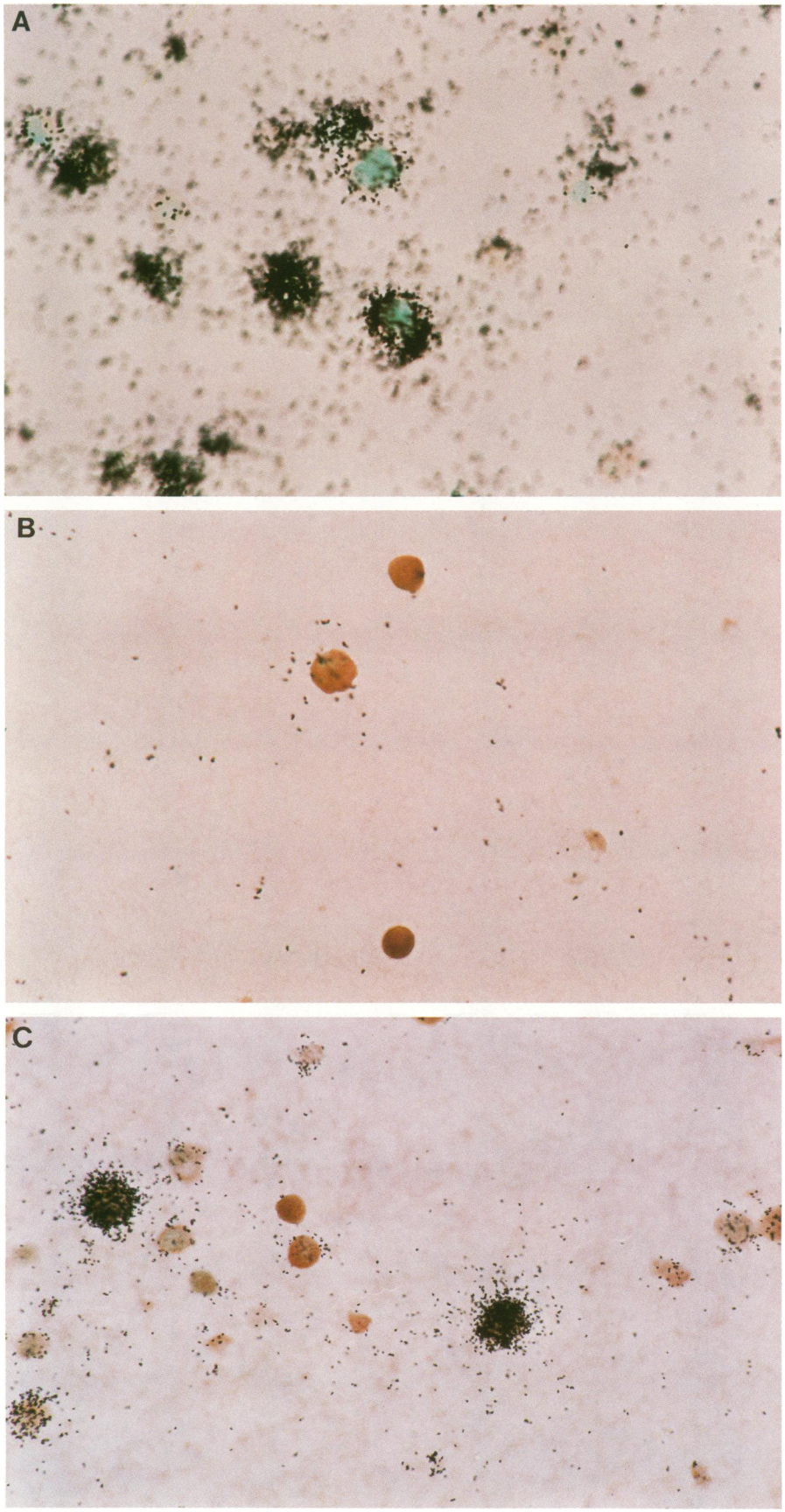
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negative with the antisense HTLV-I *pX* probe by *in situ* hybridization (Fig. 2b). HUT-102 cells were positive with the antisense HTLV-I *pX* RNA probe (Fig. 2c) and negative for the sense HTLV-I *pX* RNA probe (data not shown). All cells were positive with a β-actin antisense RNA probe (data not shown).

To confirm that the neuroblastoma cells were infected with HTLV-I, immunohistochemical analysis with biotin-conjugated neurofilament antibodies was done prior to in situ hybridization. Neurofilament protein is specific for neurons (1) and is not present in astrocytes, oligodendrocytes, microglia, or lymphocytes. Besides allowing distinction between HUT-102 cells and neuroblastoma cells, this antibody was of interest because neuroblastoma cells are capable of differentiating along nonneuronal lineages (23). The cocultured neuroblastoma cells were positive for the presence of neurofilament protein (brown-stained cells) and also expressed HTLV-I RNA (silver grains) (Fig. 3c). In contrast, the HUT-102 cells expressed HTLV-I RNA (silver grains) but were not positive for neurofilament protein (Fig. 3a). The uninfected, noncocultured neuroblastoma cells were positive for neurofilament protein (brown-stained cells) but did not express HTLV-I RNA (Fig. 3b).

These experiments demonstrate that HTLV-I RNA was present in cocultured neuroblastoma cells. The expression of HTLV-I RNA in the cocultured cells by in situ hybridization corresponded to the presence of the HTLV-I gp46 surface antigen detected by flow cytometry. The colocalization of neurofilament protein and HTLV-I RNA in the cocultured cells further confirmed that the neuroblastoma cells were infected with HTLV-I.

Induction of HLA molecules in HTLV-I-infected neuroblastoma cells. (i) Expression of HLA surface antigens. HTLV-I-infected neuroblastoma cells were analyzed for surface expression of HLA. In this experiment, HLA class I and HLA-DR were not expressed on uninfected neuroblastoma cell lines (Fig. 1e) while HUT-102 cells expressed high levels of these cell surface molecules (Fig. 1b). When neuroblastoma cells were cocultured with irradiated HUT-102 cells, two different profiles of HLA expression were evident. HTLV-I gp46-positive, CD4-negative cocultured neuroblastoma cells expressed both HLA class I and HLA-DR (Fig. 1h). In contrast, cocultured neuroblastoma cells that were HTLV-I gp46 and CD4 negative expressed only HLA class I (Fig. 1k). HLA-DR-positive cocultured neuroblastoma cells also expressed HLA-DP and HLA-DQ surface antigens (data not shown), indicating that all HLA class II isotypes could be induced.

Studies investigating the temporal relationship between HTLV-I infection and induction of HLA surface antigens in cocultured neuroblastoma cells indicated that both HTLV-I gp46 and HLA class I appeared by day 2 of the coculture, while the HLA-DR appeared on day 4 (data not shown). These data suggest that HLA class II expression is dependent on HTLV-I infection. In contrast, HTLV-I infection was not a prerequisite for HLA class I expression.

Detection of HLA transcripts in cocultured neuroblastoma cells. Total cellular RNA was isolated from CHP-126 and NMB cells cocultured with irradiated HUT-102 cells to determine whether HLA class I and II transcripts could be detected in parallel with the results obtained from cell surface expression experiments. We analyzed RNAs from noncocultured neuroblastoma cells (Fig. 4, lanes 1 and 4) and from cocultured neuroblastoma cells that were either negative (Fig. 1j and Fig. 4, lane 2) or positive (Fig. 1g and Fig. 4, lanes 3 and 5) for HTLV-I gp46 surface antigen expression.

Transcripts that hybridized to the HTLV-I Tax probe were observed only in cocultured cells that expressed the HTLV-I gp46 surface antigen. No signal for the HTLV-I Tax probe was detected in cocultured cells with an HTLV-I gp46-negative surface. In parallel, HLA-DR transcripts were observed only in cocultured cells which were HTLV-I gp46 and HLA class II surface antigen positive. HLA-DR transcripts were not observed in noncocultured neuroblastoma cells. HLA class I transcripts were observed at low levels in noncocultured neuroblastoma cells. Cocultured cells, regardless of their HTLV-I gp46 surface expression, showed enhanced levels of HLA class I transcription.

These results demonstrate that cell surface expression of the HTLV-I gp46 surface antigen and HLA class I and II molecules reflects the presence of their respective RNA transcripts within the cell. Therefore, detection of these surface antigens was not simply the result of adsorbed antigens on the neuroblastoma cell surface.

Coexpression of gp46 and HLA class II on cocultured neuroblastoma cells. To determine whether HTLV-I gp46 and HLA-DR were expressed on the same neuroblastoma cells, double-labeled immunofluorescence experiments were performed on neuroblastoma cells cocultured with unirradiated HUT-102 cells. HTLV-I gp46 and HLA-DR surface antigens were seen simultaneously on the same cell populations (Fig. 5) in these CD4-negative, HTLV-I-infected neuroblastoma cells. These results demonstrate that HTLV-I gp46 and HLA-DR surface antigens were expressed on the same population of cells.

Requirement of cell-cell contact for HTLV-I infection and induction of HLA expression. To determine whether cell-cell contact was required for HTLV-I infection and induction of HLA expression, neuroblastoma cells cocultured with unirradiated HUT-102 cells were placed in a barrier plate culture and analyzed by flow cytometry for expression of cell surface antigens on day 7. In the barrier plate culture, in which HUT-102 and neuroblastoma cells were separated by a 0.45-μm-pore-size filter, the cocultured cells did not express the HTLV-I gp46 surface antigen (Fig. 6a). In addition, only HLA class I, not HLA-DR, was expressed on these cells (Fig. 6c and d). Neuroblastoma cells in direct contact with HUT-102 cells expressed HTLV-I gp46, HLA class I, and HLA-DR surface antigens (Fig. 1g, h, and i). Cell-free HUT-102 supernatant with neuroblastoma cells yielded experimental results similar to those obtained with the barrier plate culture (data not shown).

These data demonstrated that direct cell-cell contact was required for infection of neuroblastoma cells with HTLV-I. Also, it strengthens the earlier observation of the dependence of HLA-DR expression on HTLV-I infection. In contrast, expression of HLA class I on neuroblastoma cells was inde-

FIG. 3. Detection of neurofilament protein and HTLV-I RNA in cocultured neuroblastoma cells. (A) HUT-102 cells. (B) Uninfected CHP-126 cells. (C) HTLV-I-expressing cocultured neuroblastoma cells from the same experiment as in Fig. 1g, h, and i (CHP-126 × HUT-102-I). All cells were immunohistochemically labeled to detect neurofilament protein (brown stain) and then hybridized with an antisense HTLV-I *pX* RNA probe (silver grains). All cells were counterstained with methyl green. Absence of neurofilament protein (no brown staining) allows the nucleolar blue-green counterstain to be visualized. Light field microscopy (magnification, ×1,000) was used.

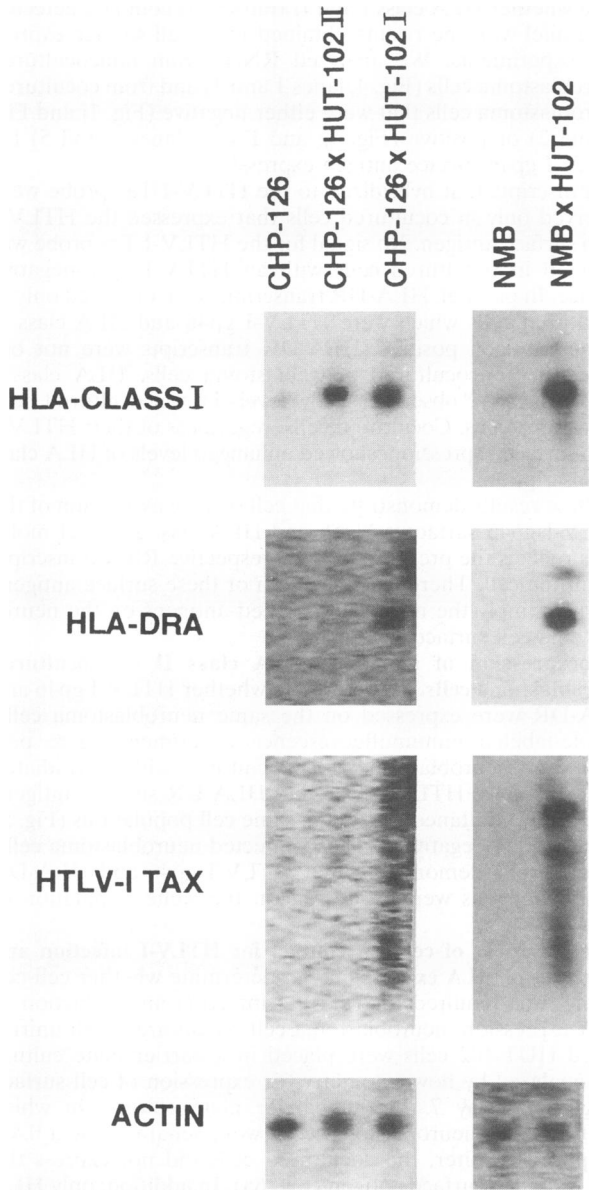

FIG. 4. Detection of HTLV-I and HLA RNA transcripts on cocultured neuroblastoma cells. Northern blot analysis with HLA class I, HLA DRα, HTLV-I Tax, and β-actin probes. Lanes: 1, uninfected CHP-126 neuroblastoma cell line; 2, CHP-126 × HUT-102-II coculture at a time when expression of HTLV-I could not be detected; 3, CHP-126 × HUT-102-I coculture expressing HTLV-I; 4, uninfected NMB neuroblastoma cell line; 5, NMB × HUT-102 coculture expressing HTLV-I. All cocultures used irradiated HUT-102 cells.

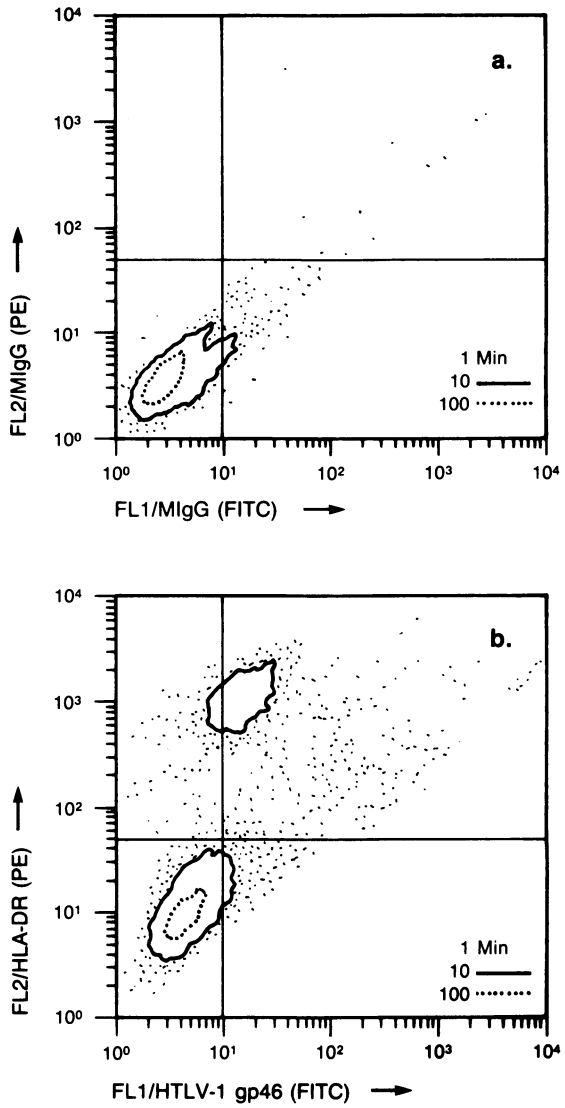

FIG. 5. Coexpression of HTLV-I gp46 and HLA class II on HTLV-I-expressing cocultured cells. Double-labeling immunofluorescence of cocultured neuroblastoma cells (CHP-126 with unirradiated HUT-102 cells) expressing HTLV-I stained with fluorescein isothiocyanate (FITC)-conjugated mouse IgG versus phycoerythrin (PE)-conjugated mouse IgG (control conditions) (a) or fluorescein isothiocyanate-conjugated anti-HTLV-I gp46 versus phycoerythrin-conjugated anti-HLA class II (b).

pendent of HTLV-I infection and did not require cell-cell contact.

Induction of HLA expression on uninfected neuroblastoma cells by cytokines. Since HLA expression could be potentially mediated by cytokines present in the HUT-102 supernatant, as suggested by the barrier plate culture experiment (Fig. 6), neuroblastoma cells were cultured in the presence of TNF-α (100 U/ml) or IFN-γ (100 U/ml) for 3 days and examined for expression of HLA class I and HLA-DR. HLA class I expression was induced in the presence of either TNF-α (Fig. 7a) or IFN-γ (Fig. 7b), whereas HLA-DR expression was not induced by either cytokine. Therefore, these cytokines induced early expression of HLA class I but not HLA class II, confirming previous studies (24–26). This result is consistent with the previous observations suggesting that HTLV-I infection is required for induction of HLA class II in HTLV-I-infected neuroblastoma cells, while HLA class I is cytokine induced.

DISCUSSION

The results of this study demonstrate that neuroblastoma cells can be infected with HTLV-I. This was accomplished by conventional coculturing techniques with HUT-102 cells as the source of HTLV-I. Infection was verified by detection of (i) the

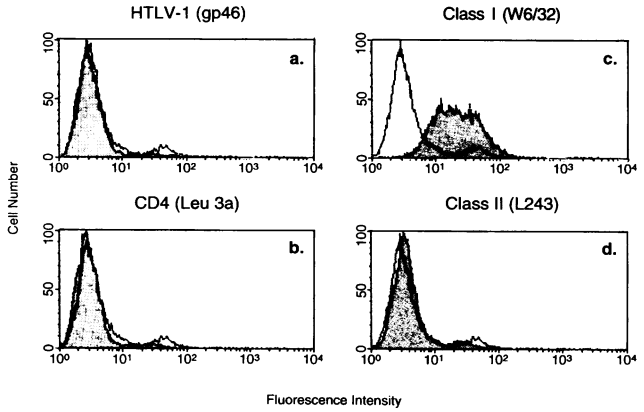

FIG. 6. Requirement of cell-cell contact for HTLV-I and HLA expression. Uninfected neuroblastoma cells were separated from unirradiated HUT-102 cells with a 0.45-μm-pore-size filter in a barrier plate culture for 7 days and examined for HTLV-I gp46 and HLA expression. Fluorescence intensity versus cell number is plotted relative to the background fluorescence of the mouse IgG control (unshaded area). Shaded areas represent staining for HTLV-I gp46 (a), CD4 (b), HLA class I (c), or HLA class II (d).

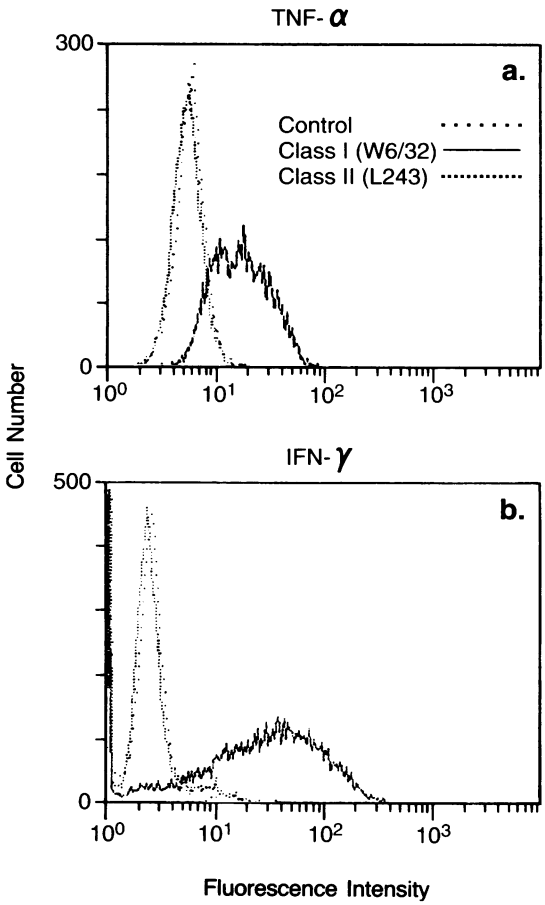

FIG. 7. Effects of TNF-α and IFN-γ on HLA expression. Uninfected CHP-126 neuroblastoma cells were incubated with TNF-α (a) or IFN-γ (b) and examined by flow cytometry for expression of HLA classes I (solid line) and II (small-dotted line). Fluorescence intensity versus cell number is plotted relative to the background fluorescence of the mouse IgG control (large-dotted line).

presence of the HTLV-I envelope gp46 antigen on the cell surface, (ii) HTLV-I transcripts by Northern blotting, and (iii) the presence of HTLV-I RNA signals in individual cells by in situ hybridization techniques. HTLV-I infection of neuroblastoma cells was confirmed by colocalization of neurofilament protein and HTLV-I RNA (Fig. 3). As demonstrated in other systems (7, 22, 28, 30), infection of neuroblastoma cells with HTLV-I required direct contact with an infected cell since neuroblastoma cells could not be infected with HTLV-I when a permeable barrier was placed between these cells and HUT-102 cells (Fig. 6).

An important observation of this study was increased expression of both HLA classes I and II in these HTLV-I-infected neuroblastoma cells. However, expression of these molecules appeared to be regulated by different mechanisms. By use of barrier plate cultures (Fig. 6), it was shown that the HUT-102 supernatant alone could induce HLA class I but not HLA class II. This probably reflects the HLA class I-inducing capacity of a number of cytokines, such as TNF-α and IFN-γ, that are present in HTLV-I-infected cell supernatants (12, 33, 40). This potential has been confirmed by the ability of these two cytokines to induce only HLA class I on uninfected neuroblastoma cells (Fig. 7 and references 24–26).

In contrast, HLA class II expression in neuroblastoma cells could not be induced by these cytokines. Rather, HLA class II expression appeared to be dependent on HTLV-I infection. This conclusion was based on a number of observations. (i) In neuroblastoma cells cocultured with irradiated HUT-102 cells, HLA class II expression was observed only when the HTLV-I surface antigen was also detected (Fig. 1). This correlation was also observed at the level of HLA class II transcripts (Fig. 4), demonstrating that the viral antigens detected at the cell surface are not simply the result of nonspecific adsorption of proteins from HUT-102 cells by these neuroblastoma lines. (ii) Barrier cultures that prevented HTLV-I infection of neuroblastoma cells showed no HLA class II expression (Fig. 6). (iii) Double-labeled cytofluorometric analysis showed that in HTLV-I-infected neuroblastoma cells, there was concomitant expression of the HTLV-I gp46 antigen and HLA class II on the same population of cells (Fig. 5). (iv) The temporal relationship between HTLV-I expression and HLA class II induction demonstrated that HTLV-I gp46 expression precedes HLA class II expression (data not shown). Collectively, these data suggest that HTLV-I infection is a necessary event in the induction of HLA class II antigens in neuroblastoma cells. In addition, preliminary studies indicate that the HLA class II induced on these HTLV-I-infected neuroblastoma cells is functional because they can present antigens to HLA class II-restricted cytotoxic T lymphocytes (5a).

On the basis of HAM/TSP patient studies (16, 18), it has been suggested that CD8 T-lymphocyte-mediated cytotoxicity involving HLA class I is important in the pathogenesis of this disorder. Early induction of HLA class I in cocultured neuroblastoma cells indicates that a similar process of cell-mediated cytotoxicity may be possible in HTLV-I-infected or uninfected cells given an appropriate cytokine-enriched microenvironment. In contrast, induction of HLA class II only in HTLV-I-infected cocultured cells indicates that other immunologic events that are specific to the infected cells may occur. For example, HTLV-I-infected, HLA class II-positive cells may induce cytokine production (36), leading to an enhanced HLA class I-mediated T-lymphocyte response.

In summary, these results demonstrate that cells of neuronal

origin can be infected with HTLV-I. Moreover, these cells showed alterations in their cell surface antigenicity through cytokine-mediated induction of HLA class I and up-regulation of HLA class II in correlation with HTLV-I infection. This is particularly interesting since HLA molecules are not detectable on neurons in situ, even after exposure to IFN-γ (23, 29, 38), and earlier studies of these neuroblastoma cells showed that only HLA class I was induced by cytokines (24–26). These observations may represent a mechanism for immunologically mediated recognition of neuronal cells in which there is HLA induction as a result of HTLV-I infection.

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