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Case Report

Localization of retrovirus in the central nervous system of a patient co-infected with HTLV-1 and HIV with HAM/TSP and HIV-associated dementia

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Persons co-infected with HTLV-1 and HIV are at increased risk for neurologic disease. These patients may develop HAM/TSP and/or HIV-associated dementia. In this study, we localized cells infected with retrovirus in the central nervous system (CNS) of a patient with both HAM/TSP and HIV-associated dementia. HTLV-1 was localized to astrocytes and HIV to macrophage/microglia. There was no co-infection of a single cell phenotype in this patient. These data suggest that mechanisms other than co-infection of the same CNS cell may play a role in the development of neurologic disease in patients dual infected with HTLV-1 and HIV. *Journal of Neuro Virology* (2001) **7,** 61–65.

Keywords: HIV; HTLV-1; HIV-associated dementia; HAM/TSP

Introduction

Neurologic complications of HTLV-1 (human T-lymphotropic virus type 1) and HIV (human immunodeficiency virus) infection include HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) and HIV-associated dementia, respectively (Levin *et al*, 1996; Gessain *et al*, 1985; Osame *et al*, 1986; Johnson, 1998; Takahashi *et al*, 1996). In HAM/TSP, central nervous system (CNS) damage is associated with a $CD8^+$ cytotoxic T-lymphocyte (CTL) response to HTLV-1 and infiltration of the CNS by lymphocytes (Levin *et al*, 1996). In the CNS, HTLV-1 has been localized to $CD4^+$ T-lymphocytes and astrocytes (Moritoyo *et al*, 1996; Lehky *et al*, 1995). HIV is associated with several CNS complications, most commonly HIV-associated dementia, an AIDS defining illness. In the CNS of patients with HIV-associated dementia, HIV has been localized to macrophages/microglia, multinucleated giant cells and rarely to astrocytes (Johnson, 1998; Epstein and Gendelman, 1993; Takahashi *et al*, 1996). To better understand the role of dual infection with HTLV-1 and HIV in the expression of neurologic disease, we used immunohistochemistry (IHC) in combination with highly sensitive *in situ* hybridization (ISH) hybridization and PCR/*in situ* hybridization (PCR/ISH) to identify cells infected with retrovirus in a patient with HAM/TSP and HIV-associated dementia (Levin *et al*, 1996; Lehky *et al*, 1995).

Case report

Briefly, a 40-year-old man developed progressive spastic paraparesis (Rosenblum *et al*, 1992). Two years later he was diagnosed with Kaposi's sarcoma and HIV. He then developed progressive dementia. Brain computed tomography showed cerebral atrophy. Retrospectively, the patient's serum was found to be HTLV-1 positive and a clinicopathologic diagnosis of AIDS dementia complex (now, HIV-associated dementia) and HAM/TSP was made

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(Rosenblum *et al*, 1992). Autopsy showed brain atrophy and HIV-1 encephalitis confirmed by positive HIV-1 p24 cells. The spinal cord showed nonvacuolar myelopathy with axonal loss and demyelination. Initial retroviral analysis for HIV and HTLV-1 by ISH was negative. Solution phase PCR of brain showed HTLV-1 specific signal (Rosenblum *et al*, 1992).

In this study, a combination of immunocytochemistry, ISH and PCR/ISH were used to detect HIV and HTLV-1 and co-localize it to specific cell phenotypes in formalin fixed, paraffin embedded CNS and control tissues. For those experiments requiring immunocytochemistry, it was performed first under RNASE free conditions. Primary antibodies included: HAM-56 (Enzo Biochemical Co., New York, NY, USA) for macrophage/microglia and glial fibrillary acidic protein (GFAP) (Dako, Carpinteria, CA, USA) for astrocytes. Biotinylated secondary antibodies (Vector, Burlingame, CA, USA) were used to detect primary antibodies. The antigen-antibody-biotin complex was conjugated to avidin (ABC solution, Vector) and detected with a diaminobenzidine (DAB) peroxide system in which positive staining is brown or by new fuchsin (Dako) in which positive staining is red.

For HIV ISH, a full length HIV-1_{LA1} cRNA probe (^{35}S-dCTP, 2×10^6 d.p.m./ul, alkaline hydrolyzed) was used in the anti-sense configuration to detect HIV-RNA. A HIV infected cell line (H-9) was used as positive control. For HTLV-1-RNA ISH, an antisense 2.1 kb cRNA probe (^{35}S-dCTP, 2×10^6 d.p.m./ul, alkaline hydrolyzed) of HTLV-1-tax was used. To detect HTLV-1-DNA, PCR/ISH with HTLV-1-tax specific primers were used to amplify HTLV-1 tax (Levin *et al*, 1996). Following PCR, ISH was performed using the sense HTLV-1-tax probe to detect amplified DNA. An HTLV-1 infected cell line (HUT-102) was used as a positive control. Uninfected peripheral blood lymphocytes (PBL) and normal CNS tissues were used for negative controls. Following ISH, autoradiograms were prepared with photographic emulsion (Kodak). Slides were developed after five days and counterstained with hematoxylin and/or eosin.

Conventional ISH with the antisense ^{35}S-labeled HTLV-1-tax probe was used to detect HTLV-1-tax RNA (Table 1). In HUT 102 cells (an HTLV-1 infected cell line that expresses multiple copies of HTLV-1-RNA), there was intense silver grain localization (Figure 1a). There was no specific signal in uninfected PBL (Figure 1b) or in normal

Table 1 Detection of HTLV-1 and HIV in normal and retrovirus-infected tissues

	HTLV-1	*HIV*
Uninfected PBL	(—)	(—)
HTLV-1 (+) HUT 102 cells	(+) RNA and DNA	nd
HIV (+) H9 cells	nd	(+)
Normal, uninfected CNS	(—)	(—)
Dual (HTLV-1/HIV) infected CNS	(+)	(+)
GFAP positive astrocytes	(+) RNA and DNA	(—)
Macrophage/microglia	(—)	(+)

nd, not done.

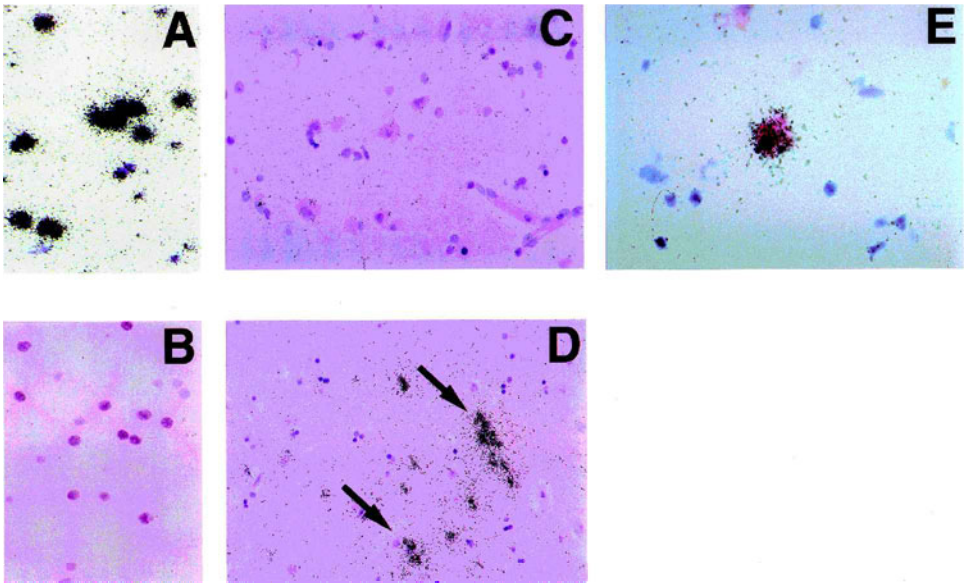

Figure 1 ISH for HTLV-1-tax-RNA with the antisense ^{35}-S HTLV-1-tax probe (magnification 400 ×, hematoxylin and eosin counterstain except where noted). (**a**) positive control, HUT-102 cells. Intense silver grain localization in all cells; (**b**) negative control, uninfected PBL. No signal present within cells; (**c**) normal brain. No specific signal present; (**d**) dual infected brain. Multiple HTLV-1-tax-RNA positive cells are present; (**e**) dual infected brain stained with GFAP (pink) and counterstained with hematoxylin only. HTLV-1-tax-RNA (silver grains) co-localized to a GFAP positive astrocyte (pink).

CNS (Figure 1c). In the dual infected case, there were multiple positive HTLV-1-RNA infected cells (Figure 1d). These cells were located in white matter and there were no inflammatory cells. Furthermore, HTLV-1-RNA was co-localized to GFAP-positive astrocytes (Figure 1e). This data is consistent with data seen in patients with HAM/TSP alone, in which HTLV-1 RNA was localized to astrocytes in the CNS in areas devoid of inflammation (Lehky *et al*, 1995). HTLV-1 RNA did not localize to macrophage/microglial cells (Table 1).

PCR/ISH was used to detect HTLV-1-DNA (Table 1). Using the sense ^{35}S-HTLV-1-tax probe, there was no specific signal detected in HUT 102 cells (Figure 2a). This is consistent with conventional ISH not being sensitive enough to detect HTLV-1-DNA in low copy numbers (Levin *et al*, 1996). Following PCR/ISH, there was intense silver grain localization in HUT 102 cells (Figure 2b), but not in uninfected PBL (Figure 2c). Also, there was no signal if Taq polymerase or tax specific primers were eliminated from the PCR cocktail (not shown) (Levin *et al*, 1996). PCR/ISH of normal CNS for HTLV-1-tax DNA was negative (Figure 2d). In contrast, PCR/ISH of dual infected CNS showed multiple positive cells in regions similar to that of conventional ISH for HTLV-1-RNA (Figure 2e), and some of these cells co-localized to GFAP-positive astrocytes (Figure 2f).

Conventional ISH with the antisense ^{35}S-labeled HIV-1 probe was used to detect HIV-RNA (Table 1). In H9 cells (an HIV infected cell line that express HIV RNA), there was intense silver grain localization (Figure 3a). There was no specific signal using uninfected PBL (Figure 3b). ISH for HIV-RNA was also negative in normal CNS (Figure 3c). In the dual infected autopsy case, there were multiple positive HIV-RNA infected cells (Figure 3d). These cells were located in both gray and white matter. Furthermore, HIV-RNA localized to macrophage/microglial cells (Figure 3e) and to multinucleated giant cells (Figure 3f). This is consistent with the localization of HIV in the CNS described previously (Takahashi *et al*, 1996). There was no localization of HIV-RNA to GFAP-positive astrocytes (Figure 3f).

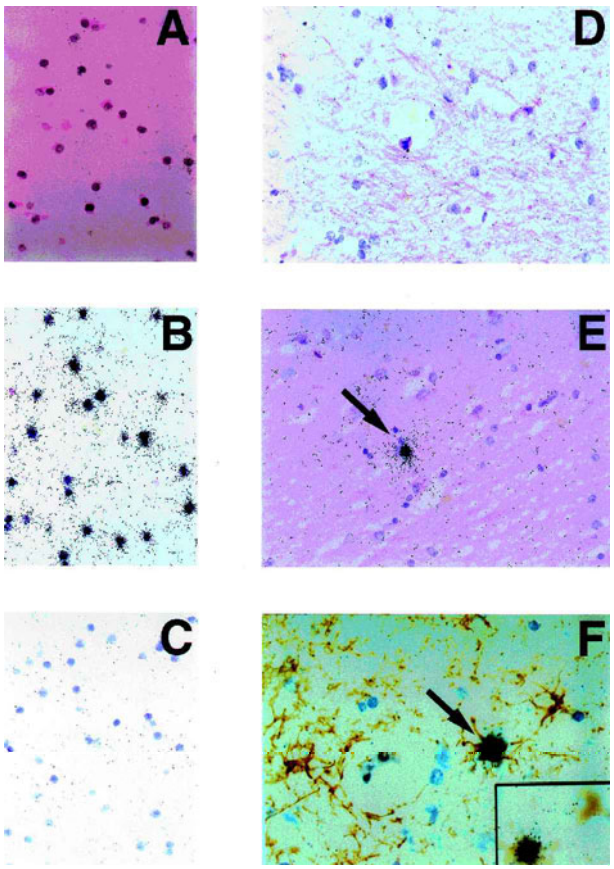

Figure 2 PCR/ISH for HTLV-1-tax DNA with the sense ^{35}-S HTLV-1-tax probe following PCR amplification (except where noted) with HTLV-1-tax specific primers (magnification 400 ×, hematoxylin and eosin counterstain except where noted). (**a**) positive control HUT 102 cells without PCR amplification. No silver grain localization to show that ISH alone is not sensitive enough to detect HTLV-1-tax DNA; (**b**) positive control HUT 102 cells following PCR amplification. Intense silver grain localization in all cells; (**c**) negative control, uninfected PBL. No silver grain localization to cells; (**d**) normal brain. No specific signal present; (**e**) dual infected brain. Positive HTLV-1-tax DNA positive cells present; (**f**) dual infected brain stained with GFAP (brown) and counterstained with hematoxylin only. HTLV-1-tax DNA (silver grains, arrow) co-localized to a GFAP positive astrocyte (brown). The insert shows the identical cell with the silver grains in focus which are within the emulsion layer of the autoradiogram. The full figure shows the focus on the immunocytochemistry.

Discussion

In this co-infected patient, HTLV-1-RNA was localized to astrocytes. Infection was confirmed by showing that HTLV-1-DNA co-localized to astrocytes by PCR/ISH (Figure 1D). Other studies localized HTLV-1 to infiltrating CD4$^+$ T-lymphocytes in the CNS of HAM/TSP patients (Moritoyo *et al*, 1996). These data may not be mutually exclusive. HTLV-1 infected CD4$^+$ lymphocytes (the source HTLV-1 in blood) may enter the CNS and infect an astrocyte. Early in disease, CD4$^+$ T-lymphocytes are plentiful and may act as an antigen presenting cell to stimulate a CD8$^+$ CTL response, and upon the secretion of toxic levels of cytokines, indirectly damage CNS tissue. Later in disease, an HTLV-1 infected astrocyte may act as a target for the CTL, which may result in neurologic damage.

HIV-RNA localized to multinucleated giant cells and to macrophage/microglial cells, but not to astrocytes. Other studies have shown low levels of HIV-RNA expression in astrocytes, particularly in children (Saito *et al*, 1994). In the adult patient we studied, HIV-RNA was not detected in astrocytes (Figure 3f). This is consistent with reports in the literature, in which HIV RNA expression is low or undetectable (Takahashi *et al*, 1996). Current data suggests that HIV infection of macrophages/micro-

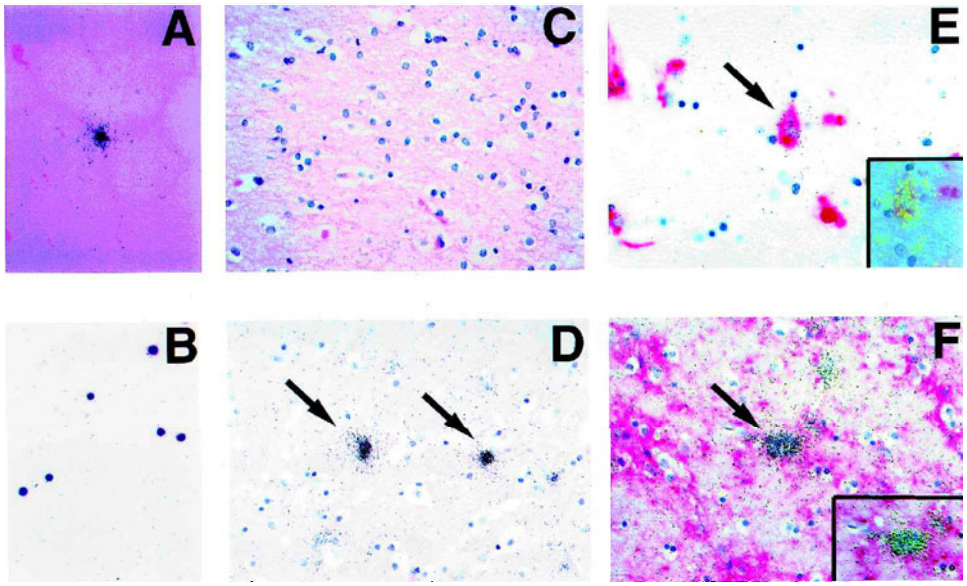

Figure 3 ISH for HIV-RNA with the antisense ^{35}-S HIV-1 probe (magnification 400 ×, hematoxylin and eosin counterstain except where noted). (**a**) positive control, H9 cells. Intense silver grain localization over the cell; (**b**) negative control, uninfected PBL. No silver grain localization over cells; (**c**) normal brain. No positive cell present; (**d**) dual infected brain. Multiple positive cells present, (**e**) dual infected brain stained with HAM-56, a macrophage/microglia marker (pink) and counterstained with hematoxylin only. HIV-RNA (silver grains, arrow) co-localized to a HAM-56 positive cell of macrophage/microglia lineage (pink) (brightfield microscopy). The insert shows the identical cell using polarized light and shows intense silver grain localization within the cell; (**f**) dual infected brain stained with GFAP (pink) and counterstained with hematoxylin only. HIV-RNA (silver grains, arrow) within a multinucleated giant cell. The insert shows the same cell using polarized light and shows intense silver grain localization. There was no co-localization of HIV-RNA within astrocytes (pink).

glia in the CNS of patients with HIV-associated dementia may result in neuronal or astrocyte dysfunction through indirect pathways from release of cytokines, toxins or viral proteins (Takahashi *et al*, 1996; Johnson, 1998).

Clinical manifestations of dual infected patients with neuropathological correlation are of interest because of the high rate of co-infection with HTLV-1 in certain HIV infected populations. There have been several reports showing that neurologic disease associated with co-infection can result in the clinical expression of either HAM/TSP, HIV-associated dementia or both diseases (Rosenblum *et al*, 1992; Harrison *et al*, 1997; Berger *et al*, 1991). Also, HIV infected patients can develop vacuolar myelopathy (VM), a spinal cord disease that is distinct pathologically from HAM/TSP, but may present with similar symptoms. Recently, patients infected with both HTLV-1 and HIV have a greater risk for developing myelopathy than those with HIV alone (Harrison *et al*, 1997). This is important in order for patients in appropriate risk groups to be screened for both HTLV-1 and HIV. There seems to be a reciprocal relationship between HTLV-1 and HIV. HTLV-1 may increase the risk of AIDS and increase HIV replication (Page *et al*, 1990; Cheng *et al*, 1998). However, this may not be the case *in vivo*, in which HIV viral load is similar between dual infected patients and patients with HIV alone (Harrison *et al*, 1996). Alternatively, HIV increased HTLV-1 levels in dual infected patients compared to patients with HTLV-1 alone (Beilke *et al*, 1998). We did not find evidence of over-expression of retrovirus in the CNS nor did we find cells of the same phenotype co-infected with both HTLV-1 and HIV. Considering the distinct neuropathology of HIV-associated dementia and HAM/TSP, the reciprocal relationship between HTLV-1 and HIV may increase the risk of neurologic disease via alterations in the immune response or cytokine expression in the CNS, rather than direct infection of both retroviruses on the CNS.

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