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ISOLATION AND CHARACTERIZATION OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE-I FROM PATIENTS WITH TROPICAL SPASTIC PARAPARESIS

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A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

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ABSTRACT

ISOLATION AND CHARACTERIZATION OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE-I FROM PATIENTS WITH TROPICAL SPASTIC PARAPARESIS. Guy M. McKhann II and Clarence J. Gibbs Jr., Laboratory of Central Nervous System Studies, National Institutes of Health, Bethesda, MD. (Sponsored by G. D. Edith Hsiung, Department of Laboratory Medicine, Yale University).

Endemic tropical spastic paraparesis/HTLV-I associated myelopathy (TSP/HAM) is a slowly progressive chronic neurological disorder that is manifested clinically by spastic paraparesis frequently in association with bowel and bladder dysfunction. It is one of the most common chronic neurologic disorders in many endemic foci around the world. Human T-lymphotropic virus type-I (HTLV-I) has been etiologically associated with TSP/HAM based primarily on the high prevalence of antibodies to HTLV-I in the serum and CSF of affected patients. We report here the isolation of HTLV-I from peripheral blood lymphocytes and cerebrospinal fluid derived mononuclear cells of TSP/HAM patients by stimulation with interleukin-2 and cocultivation with umbilical cord blood mononuclear cells. Established cell lines contained HTLV-I antigen as demonstrated by immunoflourescence and cell-associated virus particles as observed by electron microscopy; low level reverse transcriptase activity was detected in cultures expressing viral antigens. Virus infected cultures were transformed in vitro as determined by their loss of contact inhibition and their continuous growth in the absence of exogenous IL-2. Of particular significance was our isolation of HTLV-I from three family members with TSP/HAM of varying duration and clinical severity.

This study resulted in the first isolation of virus from a cluster of

TSP/HAM patients within the same family, the first isolation of virus from a childhood case of TSP/HAM, and the first in vitro transformed HTLV-I-infected cells from TSP/HAM patients. In addition, the methodology applied in this investigation subsequently has been used to consistently isolate virus from HTLV-I infected persons from widely separated geographic locations. This work supports a causative role for HTLV-I in the pathogenesis of TSP/HAM and helps to extend the spectrum of disease that may result from HTLV-I infection.

INTRODUCTION

Endemic tropical spastic paraparesis (TSP) is the most common chronic neurological disorder in many tropical and subtropical regions of the world [Rodgers-Johnson et al, 1989b]. This disorder is characterized by slowly progressive spastic paraparesis frequently in association with bowel and bladder dysfunction. It occurs in geographically widely separated endemic foci [Roman, 1987]. A number of processes have been postulated to be involved in the pathogenesis of TSP in individual endemic areas including nutritional deficiencies [Cosnett, 1965; Grieve et al, 1967], neurotoxic ingestions [Cruickshank, 1956], and a variety of microbial infections [Minchin, 1940; Rodgers, 1965; Robertson and Cruickshank, 1972; Roman et al, 1987]. None of these hypotheses has been able to account for the disease in all of the geographic foci in which it occurs [Roman et al, 1985b].

Over the past five years, it has been established that TSP patients have a high prevalence of antibodies to human T-lymphotropic virus type-I (HTLV-I) in both serum and cerebrospinal fluid (CSF) [Rodgers-Johnson et al, 1988b]. This important finding is the one piece of etiologic evidence that unifies the varied patient populations with endemic TSP [Rodgers-Johnson et al, 1985]. Additionally, patients from nontropical southern Japan with a chronic neurological disorder that is clinically and histopathologically indistinguishable from TSP also have antibodies to HTLV-I in their serum and CSF [Osame et al, 1986a]. This syndrome, HTLV-I associated myelopathy (HAM), is now felt to be identical to TSP [Roman and Osame, 1988]. Together, these chronic encephalomyeloneuropathies are designated TSP/HAM and have been found in more than 40 countries [Rodgers-Johnson et al, 1990].

HTLV-I, the first retrovirus isolated from humans [Poiesz et al, 1980a], was previously implicated as the causative agent of adult T-cell leukemia (ATL) [Yoshida et al, 1984; Gallo, 1985]. It had never been linked to neurologic

disease until its serologic association with TSP/HAM was demonstrated.

At the time this project was initiated, serologic evidence strongly associated HTLV-I with TSP/HAM. Virus isolation was an essential step in establishing an etiologic role for HTLV-I in the pathogenesis of TSP/HAM. However, isolation of virus from patients with this disease was previously reported to be difficult, and only individual case reports of successful isolation existed [Hirose et al, 1986; Defreitas et al, 1987; Rodgers-Johnson, 1988].

The purpose of this investigation was to isolate HTLV-I from peripheral blood lymphocytes and mononuclear cells of the CSF of TSP/HAM patients. Utilizing techniques similar to those that were previously successful in isolating HTLV-I from ATL patients, isolation was accomplished through cocultivation of patient cells with umbilical cord blood lymphocytes in the presence of interleukin-2. This study proved to be especially significant in that it led to the isolation of HTLV-I from three family members living in the endemic TSP focus of Tumaco, Colombia with spastic paraparesis of varying duration and severity. Successful isolation of the virus from the 13-year-old son represents the youngest TSP/HAM patient isolation to date. The techniques used in this investigation have subsequently been applied to allow consistent isolation of virus from HTLV-I infected persons.

Nomenclature

The nomenclature of chronic tropical neurological disorders of uncertain etiology is confusing and requires explanation. These disorders have been reported under various designations for the past 100 years but are now divided into two clinical groups. Tropical ataxic neuropathy (TAN) is an endemic form of disease manifested by predominately proprioceptive dysfunction with variable mild pyramidal signs and optic and auditory nerve involvement. Tropical spastic paraparesis (TSP) occurs in two forms, epidemic and endemic. Both epidemic and endemic TSP present clinically as a predominately spastic disorder with

frequent bowel and bladder dysfunction and variable posterior column and spinothalamic tract involvement [Rodgers-Johnson, 1988]. HTLV-I associated myelopathy (HAM) is a disorder that is endemic in non-tropical regions of Japan and is identical to the endemic form of TSP [Roman and Osame, 1988].

The terms chronic HTLV-I myelitis [Roman, 1989] and HTLV-I encephalomyeloneuropathy [Rodgers-Johnson et al, 1989] have recently been suggested to incorporate TSP and HAM under the same heading. However, the World Health Organization currently recognizes TSP/HAM as the official nomenclature for this disease process, and it is this designation that is used throughout the thesis.

Historical background

Chronic tropical neurological disorders of uncertain etiology were first reported over one hundred years ago by Strachan who described 510 Jamaicans with "a form of multiple neuritis prevalent in the West Indies" [Strachan, 1888; Strachan, 1897]. These patients had combined sensorimotor findings predominated by burning pain of the soles of the feet, ataxic gait, impaired lower extremity proprioception, and decreased or absent knee jerk reflexes. Strachan believed malaria to be responsible [Strachan, 1888], but the clinical picture was also compatible with arsenic intoxication from agricultural pesticides [Heyman et al, 1956]. These cases were reviewed twenty years later by Scott, and he concluded that a variety of clinical entities were involved including beri-beri, pellagra, and "Jamaican peripheral neuritis" [Scott, 1918].

In 1918, Scott described an acute outbreak of "central neuritis" in 21 Jamaican sugar cane cutters whose clinical picture was similar to Strachan's patients. Although he felt that his patients represented an acute form of Strachan's syndrome resulting from toxic ingestion, the acute clinical presentation suggested a postviral acute sensory neuronopathy syndrome [Sterman et al, 1980; Roman et al, 1985b]. The first detailed autopsies were

performed on two of Scott's patients who died with terminal diarrhea. The pathologic report detailed widespread changes consisting of diffuse spinal cord fatty degeneration and fibrosis that was most marked in the posterior columns and cerebellar tracts, extensive dorsal root ganglion fatty deposition and pigmentation, and optic greater than auditory nerve involvement [Scott, 1918].

Since these initial two reports, hundreds of cases of unusual chronic tropical neurologic disorders (later designated tropical myeloneuropathies [Roman, 1985b]) with similar clinical manifestations were described predominantly in underdeveloped areas of Africa [Wright, 1928; Stannus, 1930] and World War II prisoners [Cruickshank, 1946; Gibberd, 1980; Gill and Bell, 1982] These reports causally implicated vitamin deficiencies [Wright, 1928; Wright, 1936], malnutrition [Walters, 1947; Money, 1959], and/or tropical malabsorption (tropical sprue) [Keele et al, 1946; Elder, 1947]. Some investigators suggested that toxic ingestion or exposure was responsible [Cruickshank, 1946].

A clinical classification of this group of disorders was finally made in 1956 by Cruickshank who reported on 100 Jamaican patients seen at the University of West Indies over a three year period. He detailed two clinical pictures: 80% of his patients had predominantly lower limb spasticity and weakness while 20% were characterized by lower limb ataxia [Cruickshank, 1956]. Cruickshank's observations paved the way for the division of tropical myeloneuropathies into tropical ataxic neuropathy (TAN) and the two forms of tropical spastic paraparesis (TSP), epidemic and endemic.

Tropical Ataxic Neuropathy

TAN, a term coined by Osuntokun [Osuntokun, 1968], is an endemic form of myeloneuropathy most prevalent in parts of Africa. Patients typically complain of "burning feet" or other lower extremity paresthesias or dysesthesias and on examination are found to have severe impairment of position, vibration, touch,

and pressure sensation that is more pronounced in the distal lower extremities. Ankle and knee jerk reflexes are diminished or absent. Mild pyramidal signs and optic and auditory nerve involvement are sometimes present [Roman et al, 1985b]. The etiology of TAN in certain foci remains unclear, but in Nigeria and other parts of Africa, TAN has been clearly demonstrated to result from ingestion of improperly prepared or immature cassava root at times of food shortage. Cassava, a root vegetable that exists in two forms, Manioc utilissima and Manioc palmata, is the dietary staple in these regions. Improperly prepared or immature cassava contains high levels of cyanogenic glucosides, consumption of which results in TAN [Osuntokun, 1985; Cliff et al, 1985].

Epidemic Tropical Spastic Paraparesis

In contrast to the proprioceptive dysfunction with sensory ataxia that predominates in TAN, lower limb spastic paraparesis with weakness and hyperreflexia is characteristic of endemic and epidemic TSP. Epidemic TSP, which has only been reported from Africa, presents with the rapid onset of bilateral spastic paraparesis, sometimes in association with visual disturbance and speech disorders. Like TAN, epidemic TSP usually results from consumption of undercooked or poorly prepared cassava at times of drought [Casadei et al, 1984], although an infectious agent is still thought to be the cause in Zaire [Carton et al, 1986].

Endemic Tropical Spastic Paraparesis

Epidemiology

Following the initial description of Jamaican neuropathy by Cruickshank [Cruikshank, 1956], endemic foci of TSP were reported in various parts of the world under geographically specific designations including South African paraplegia [Cosnett, 1965], Seychelles paraplegia [Roman et al, 1987a; Roman et al, 1987b], South Indian paraplegia [Mani et al, 1969], and 'paraparesis

espastico del Pacifico' in Colombia [Zaninovic et al, 1981]. Additionally, Osame described clinically similar HAM patients from temperate regions of southern Japan [Osame et al, 1986a; Osame et al, 1987a]. It was initially thought that HAM and TSP were different disorders and that TSP was confined to tropical regions of the world, but it is now clear that TSP is found in some nontropical areas (Figure 1) [Rodgers-Johnson et al, 1989] and that TSP and HAM are the same disease [Roman and Osame, 1988].

Consistent with the population distribution of the endemic foci, most TSP patients are black and from lower socioeconomic groups in tropical regions [Rodgers-Johnson et al, 1990]. However, the ethnic, socioeconomic, and geographic distributions of the disease were expanded when it was realized that TSP and HAM are the same disease and that TSP is not restricted to tropical regions. In addition to Japanese and blacks in endemic regions, the disease has been reported in Indians, Chinese, mulattoes, and Caucasians [Roman, 1987], in Caribbean migrants to the United States [Bhagavati et al, 1988] and the United Kingdom [Cruickshank et al, 1989], and also in blacks in the United States who have never travelled to endemic areas [Bhagavati et al, 1988]. A non-tropical endemic foci of TSP/HAM has been recognized in Chile [Cartier-Rovirosa et al, 1989], and the disorder has been reported in upper and middle class Caucasians in the desert climate of Lima, Peru [Johnson et al, 1988]. Isolated cases have been reported in southern Italy [Annunziata et al, 1988] and France [Gessain et al, 1988].

The age of onset of TSP/HAM is usually from 35-49 years, although patients have been less than 20 years and over 70 years at the time of diagnosis. A few childhood cases have been reported from Japan, and a 13-year-old Colombian boy with early clinical signs and antibodies to HTLV-I in both serum and CSF was reported as part of this study [McKhann et al, 1989].

There is a female preponderance of approximately 3 to 1 in the majority of endemic areas, possibly because sexual transmission is predominately

unidirectional from males to females [Kajiyama et al, 1986]. Some earlier smaller studies of the same populations reported equal sex incidence [Montgomery and Cruickshank, 1964; Roman et al, 1987b, Rodgers-Johnson, 1990].

The prevalence of the disease varies somewhat between endemic foci from about 50 per 100,000 in the northern Atlantic region of Martinique to 128 per 100,000 in the Seychelles Islands. In Tumaco, Colombia the prevalence for the total population is 91 per 100,000 but increases to 281 per 100,000 if only those older than 25 are considered. These significant prevalence rates are comparable to the highest known prevalence of multiple sclerosis of 128 per 100,000 in the Orkney and Shetland Islands of Scotland [Roman, 1988].

Clinical Characteristics of TSP/HAM

Although there is variability, the predominant clinical features of TSP/HAM worldwide include the gradual onset of lower extremity weakness frequently in association with sensory symptoms ranging from burning to numbness to pins and needles [Rodgers-Johnson et al, 1989a]. Lumbosacral pain and urinary symptoms including frequency, urgency, nocturia, and incontinence are common; impotence, decreased libido, and constipation also occur frequently [Cruickshank, 1956; Montgomery and Cruickshank, 1964; Vernant et al, 1987]. Other variable manifestations include decreased hearing or vision and unsteadiness of gait.

On examination, patients have a spastic paraparesis with lower extremity hyperreflexia and a spastic gait. The spastic paraparesis usually develops insidiously and may be initially unilateral. Most patients have extensor plantar responses, bilateral clonus, and upper extremity hyperreflexia, but less than 50% of patients have arm weakness. Vibration sense is diminished slightly more frequently than position sense, and peripheral sensory changes including a sensory level or signs consistent with a root lesion may occur [Osame et al,

1987b; Rodgers-Johnson et al, 1988a; Said et al, 1988]. Other variable signs include diminished abdominal reflexes, nystagmus, intention tremor, optic atrophy, nerve deafness, ptosis, recurrent laryngeal nerve palsy, and facial weakness. Cognitive functions are preserved [Montgomery and Cruickshank, 1964; Rodgers, 1965; Zaninovic, 1988] (Table 1).

The characteristic clinical course of TSP is a slow progression over months to years, often with periods of plateau for several years. Severe disability usually results with 90% of patients requiring assistance to walk and 50% of patients wheelchair bound at 10 years [Cruickshank, 1989]. Patients have survived for up to 55 years after diagnosis [Osame et al, 1987b]. However, acute events similar to transverse myelopathy or vascular events have been reported. Prognosis is worse in those who become rapidly paraplegic because of predisposition to urinary tract infections or pulmonary emboli [Rodgers, 1965].

Pathology

There have been remarkably few postmortem studies of TSP/HAM patients. Nevertheless, pathologic findings were consistent in an early series of 15 Jamaican cases [Robertson and Cruickshank, 1972], a later report of 2 Jamaican cases [Piccardo et al, 1988], in single autopsies from the Seychelles [Roman et al, 1987a] and South India [Mani et al, 1941], and in Japanese reports [Akizuki et al, 1987; Nakazato et al, 1987]. The typical pathologic features consist of widespread meningoencephalomyelitis and spinal cord demyelination. The meningoencephalomyelitic changes, which are greatest at the spinal cord level, consist of perivascular inflammatory cuffing with plasma cells, lymphocytes, and histiocytes; microvascular proliferation; reactive astrocytic gliosis; and perivascular fibrosis with small vessel hyalinization. The spinal cord demyelination usually affects the lateral columns more than the posterior columns (Figure 2). In an early report on pathology, Robertson and Cruickshank commented on the similarity of the widespread central nervous

system disease in TSP/HAM to meningovascular syphilis. However, endarteritis obliterans, miliary gummata and other pathologic features characteristic of syphilis were never seen [Robertson and Cruickshank, 1972]. In patients with long term progressive TSP/HAM [Roman, 1988], the spinal cord pathology may resemble the vacuolar myelopathy seen in patients with the acquired immunodeficiency syndrome [Petito et al, 1985].

Laboratory Investigations and Diagnostic Tests

Most routine laboratory tests are normal in TSP/HAM including blood and urine chemistries, erythrocyte sedimentation rate, vitamin B12 level, and nutritional and metabolic parameters. Peripheral blood smears can be helpful in that 1-3% of lymphocytes may be atypical with convoluted rosette type nuclei [Rudge, 1989]. CSF parameters are frequently normal although a moderate predominately lymphocytic CSF leucocytosis with increased protein has been seen in 16-57% of patients [Rodgers, 1965; Vernant et al, 1987]. Atypical lymphocytes similar to those seen in peripheral smears are sometimes found in the CSF [Osame et al, 1986; Osame et al, 1987b]. CSF gamma globulin is frequently increased, and oligoclonal bands are seen [Roman, 1988].

Visual evoked potential studies are abnormal in up to 50% of patients [Cruickshank et al, 1989], although much lower frequencies have been reported [Bhagavati et al, 1988]. The marked symmetry of these latencies suggests that multiple small demyelinating lesions are present within both optic nerves, a finding that has been previously described in pathologic specimens. Minor brain stem auditory evoked potential abnormalities are commonly seen consisting of prolongation of various brainstem components with a normal component 1. Somatosensory evoked potentials are usually abnormal in testing of both the upper and lower extremities in a pattern that suggests bilateral involvement of the fasciculus gracilis [Cruickshank et al, 1989]. Although not a consistent finding in all studies, abnormal conduction velocities along sural and

peroneal nerves have been reported. These abnormal F wave latencies are most compatible with patchy demyelination/remyelination of peripheral nerves resulting from vasculitis along the vasa nervosum [Said et al, 1988].

Magnetic resonance imaging of patients, in comparison to appropriately age matched controls, has detected both cerebral and spinal cord lesions [Furukawa et al, 1989]. In the brain, minor periventricular and supratentorial white matter abnormalities are seen, occasionally accompanied by posterior fossa lesions. These brain lesions are usually less extensive than those seen in multiple sclerosis (MS) patients with a corresponding level of clinical disability [Cruickshank et al, 1989]. Results of magnetic resonance imaging (MRI) must be interpreted in relation to age because white matter abnormalities are found in over 30% of apparently normal individuals over 50 years [Brant-Zawadski et al, 1984]. Spinal cord change visualized by MRI, if present, is limited to dorsal column atrophy. Areas of increased signal in the spinal cord, as are sometimes found in MS, are not seen in TSP/HAM [Rudge, 1989].

Etiology

Early studies in Jamaica [Cruickshank et al, 1961; Rodgers, 1965] and South India [Mani et al, 1969] and more recent case control studies from Colombia [Roman et al, 1985] and the Seychelles [Roman et al, 1987a] failed to implicate diet, nutritional defiencies, neurotoxin exposure, housing, occupation, farming, pets, habits, or geographic location as possible contribtory factors in TSP pathogenesis. The etiology of TSP thus remained unclear.

A possible infectious etiology for the disease was first hypothesized three decades ago. Because of the pathologic similarity to meningovascular syphilis and because the geographic distribution of the disease on Jamaica is the same as that of previous yaws endemia, treponemal associations were postulated including atypical syphilis [Rodgers, 1965] and yaws [Montgomery, 1960]. (Yaws is a non-venereal spirochetal disease caused by Treponema pertenue

that is serologically indistinguishable from syphilis. It was previously prevalent in many areas where TSP/HAM is now found.) Early treponemal studies detected positive serology in greater than 60% of sera and 26.1% of CSF samples from Jamaican patients [Rodgers, 1965], in greater than 60% of sera and 19% of CSF samples from Colombian patients [Rodgers-Johnson et al, 1986], and in more than 42% of patients living in the Seychelles Islands [Roman et al, 1987a]. Early serologic testing in Martinique, South Africa, and India did not support a treponemal etiology [Vernant et al, 1987; Cosnett et al, 1965; Mani et al, 1969].

More recently, further testing detected Treponema pallidum antibodies in 11 of 24 sera but only 1 of 24 CSF samples from Jamaican patients [Rodgers-Johnson et al, 1985]. Furthermore, treponemal infection prevalence has declined in Jamaica over the past three decades while no corresponding decrease in the prevalence of TSP has been seen [Rodgers-Johnson, 1989]. An unexplained finding was the presence of antibodies to Borrelia burgdorferi, the etiologic agent in Lyme disease, in 44% and 24% of Seychelles and Jamaican sera, respectively [Roman et al, 1987a; Rodgers-Johnson et al, 1986].

A viral etiology for TSP was first postulated by Montgomery and Cruickshank in 1964 based on pathologic findings [Montgomery and Cruickshank, 1964]. Mani further noted in 1969 that Indian TSP was similar both clinically and histopathologically to visna-maedi lentivirus induced sheep encephalomyelopathy [Mani et al, 1969]. In addition, although most TSP patients' CSF samples are normal, studies from Jamaica and Martinique detected a predominately lymphocytic pleocytosis with increased protein in 16% to 57% of patients. Despite these suggestions of infection, early virological investigations were consistently negative [Montgomery and Cruickshank, 1964; Rodgers,1965; Montgomery, 1986].

The first serologic evidence of viral involvement in TSP was reported by Greaves et al in 1984 who noted high antibody titers to human T-cell

lymphotropic virus type I (HTLV-I) in one TSP patient as part of a large HTLV serological study in the United Kingdom [Greaves et al, 1984]. However, it was not until Gessain et al detected IgG antibodies to HTLV-I in 68% of patients with TSP in Martinique in 1985 that HTLV-I was considered as a possible causative agent [Gessain et al, 1985]. The significance of these results was greatly enhanced by the detection of high titers of HTLV-I antibodies in both serum and CSF of patients from two separate TSP foci, Jamaica and Colombia [Rodgers-Johnson et al, 1985]. In addition, Osame et al in 1986 reported high titers of HTLV-I antibodies in serum and CSF from 6 of 6 patients from non-tropical southern Japan with a chronic myelopathy clinically and histopathologically similar to TSP that they designated HAM [Osame et al, 1986].

Antibodies to HTLV-I were subsequently confirmed in the sera of a high percentage of TSP/HAM patients from all endemic foci tested, ranging from 80% in Martinique and the Seychelles to 92% in Jamaica to 98% in Colombia to 100% in Japan. In addition, 89% of Jamaican and 96% of Colombian TSP/HAM patients tested in our laboratory were found to have anti-HTLV-I reactivity not only in their serum but in their CSF as well. In contrast, the prevalence of antibodies to HTLV-I of the general population in endemic areas ranged from 3-5% in Martinique and Jamaica to 12% in the Seychelles to 16% in southern Japan [Montgomery, 1989] (Table 2).

Evidence for HTLV-I Infection in TSP/HAM

In addition to the strong serologic evidence etiologically associating HTLV-I with TSP/HAM, additional evidence included:

1) Intrathecal synthesis of IgG antibodies to HTLV-I in patients as evidenced by the presence of oligoclonal bands of HTLV-I specific IgG in CSF. The oligoclonal band pattern against HTLV-I in CSF differed from that found in serum. The CSF to serum albumin ratio was normal in these studies indicating

intact blood brain barrier function [Osame et al, 1987b; Ceroni et al, 1988].

2) The presence of abnormally activated CD4+ lymphocytes with lobulated nuclei in CSF and sera. These cells were morphologically indistinguishable from HTLV-I infected peripheral blood lymphocytes from ATL patients [Osame et al, 1986a; Johnson et al, 1988; Sarin et al, 1989] (Figure 3).

3) Increased numbers in peripheral blood of large CD3+ mononuclear cells expressing IL-2 receptor molecules consistent with HTLV-I mediated activation of the gene encoding the alpha subunit of the IL-2 receptor [Jacobson et al, 1988b].

4) Elevated spontaneous lymphoproliferative response in vitro of lymphocytes from peripheral blood of patients indicating abnormal activation of these cells [Jacobson et al, 1988b; Itoyama et al, 1988] .

5) Demonstration of HTLV-I antigens in peripheral blood lymphocytes by immunoflourescent staining using polyclonal antiserum from a chimpanzee infected with HTLV-I [Piccardo et al, 1988].

6) Three independent reports of virus isolation of HTLV-I or a HTLV-I like virus from, respectively, the CSF of a HAM patient [Hirose et al, 1986], peripheral blood of a patient with a chronic myelopathy of unknown etiology [Defreitas et al, 1987], and CSF of a Jamaican TSP patient [Rodgers-Johnson et al, 1988a].

Despite this evidence, isolation of HTLV-I from peripheral blood lymphocytes or CSF mononuclear cells of patients was reported to be difficult [Hirose et al, 1986; DeFreitas et al, 1987]. This was also the case in our laboratory as virus was initially successfully isolated from only 1 of 30 specimens from Jamaica or Colombia.

Human T-cell Lymphotropic Virus Type-I (HTLV-I)

Endemicity and Modes of Transmission

HTLV-I, the first retrovirus isolated from humans, is a type C retrovirus.

Antibody to this rather ubiquitous virus has been detected in 3-16% of the general population in southern Japan, the Caribbean, West Africa, and South America. Additionally, infection has been detected in 3-6% of asymptomatic Caribbean immigrants to the United Kingdom and in 4-5% of male homosexuals at risk for AIDS. Testing for antibody in blood donors in the United States has revealed only 0-0.1% positivity [Weber, 1989], while 1.9% of blood samples assayed at the Fukuoka Red Cross Blood Center in Japan were positive in 1983 [Okochi et al, 1984]. Following the initiation in 1986 of antibody screening of blood samples in Japan, the seroconversion rate resulting from transfusion decreased from 8.9% to 0.08% in Kyushu University Hospital [Okochi and Sato, 1989].

HTLV-I is unusual in that it remains endemic in geographic foci for long periods of time without any known vector of transmission with the exception of man. Several modes of transmission of HTLV-I have been implicated. HTLV-I seroconversion following blood transfusion was well demonstrated in Japan to be mediated by viable T-cells but not by plasma [Okochi et al, 1984]. Additionally, early epidemiologic studies in Japan showed an increased prevalence of HTLV-I in females in endemic areas [Tajima et al, 1982]. From these studies sexual transmission with a greater frequency of transmission from males to females than females to males was suspected. Evidence of sexual transmission included the observation that most wives of HTLV-I positive husbands seroconverted [Tajima et al, 1982], the demonstration of HTLV-I infected cells in semen [Nakano et al, 1984], and the finding of increased rates of seropositivity in sexually active homosexuals at risk for AIDS [Tedder et al, 1984; Robert-Guroff et al, 1984] and in promiscuous intravenous drug users in comparison to nonpromiscuous matched controls [Rezza et al, 1988]. The possibility of transmission from infected husband to wife over a ten year period was calculated to be 60.8% in contrast to the 0.4% chance of transmission from infected wife to husband over the same time period [Kajiyama et al, 1986].

In addition to blood borne and sexual horizontal routes of transmission, vertical transmission was suggested by reports of intra-familial clustering of HTLV-I carriers [Ichimaru et al, 1979] and of an association between carrier mothers and their children [Tajima et al, 1982]. Japanese investigators recently demonstrated by seroepidemiologic and virologic studies that transmission from mother to child by breast feeding is a major natural route [Hino and Doi, 1989].

Retroviral Characteristics

HTLV-I is characterized as a single stranded RNA virus that encodes a RNA dependent DNA polymerase (reverse transcriptase). Following infection of a susceptible cell, the reverse transcriptase "retrotranscribes" the viral RNA into proviral DNA for integration into the host's cellular genome. Following integration, the provirus has one of three fates: 1) to remain latent in infected cells either thru host or viral mediated inhibition of viral transcription; 2) to replicate resulting in viral progeny production and death of the host cell when the host is unable to inhibit viral transcription; and 3) to induce host cell transformation resulting in tumoral proliferation [de The, 1989].

There are three subfamilies of retroviruses. Spumavirinae have no known associated disease. In animals, Oncornavirinae are associated with hematological proliferations and connective tissue tumors, while Lentivirinae are associated with neurologic and/or pulmonary diseases [de The, 1989]. No retroviruses were known to infect humans until Gallo and his associates developed the methodology for successfully culturing T-cells in the presence of interleukin II (T-cell growth factor) [Morgan et al, 1976; Poiesz et al, 1980b]. Soon after this important advance, HTLV-I was isolated in 1980 by Poiesz, Gallo, et al from a patient with cutaneous T-cell lymphoma (CTCL) [Poiesz et al, 1980a]. A similar retrovirus, HTLV-II, was subsequently isolated from a patient with a T-cell variant of hairy cell leukemia [Kalyanaraman et al, 1982b]. Both HTLV-I and HTLV-II are oncornaviruses and as such are able to induce syncytia

and multinucleated giant cell formation in culture and transform T-lymphocytes in vitro. In vivo infection with either of these type-C retroviruses may result in abnormally proliferative or functionally impaired helper (CD4+) T-lymphocytes.

In contrast, HTLV-III (now named HIV-I) and HIV-II, the retroviruses associated with the acquired immunodeficiency syndrome, are lentiviruses and as such are genetically distinct from HTLV-I and HTLV-II and are cytopathic to CD4+ T-lymphocytes in vitro [Desgranges et al, 1989].

Evidence for Leukemogenesis

Following initial isolations of HTLV-I from patients with CTCL, it was demonstrated that HTLV-I could not be isolated from most CTCL patients and that many HTLV-I isolates were from patients who did not clinically have CTCL [Gallo, 1985]. The role of HTLV-I in human disease remained unclear until reports from Japan implicated the virus in the pathogenesis of adult T-cell leukemia (ATL). First described in 1977 by Takatzuki, ATL is an aggressive leukemia of malignant helper (CD4+) T-lymphocytes that is frequently associated with hypercalcemia and dermatologic manifestations [Uchiyama et al, 1977]. The incidence in Japan of ATL follows a north to south gradient with heaviest concentration on two southern islands, a pattern that is consistent with an infectious etiology [Yoshida and Seiki, 1990].

ATL is localized in three separate areas of the world: southwestern Japan [Uchiyama et al, 1977], the West Indies [Blattner et al, 1982], and central Africa [Hunsmann et al, 1983]. Because HTLV-I is endemic in these same areas, an association between HTLV-I and ATL was suspected. Sera from all ATL patients initially tested contained antibodies to HTLV-I, and monoclonal antibodies generated against HTLV-I proteins reacted with cultured ATL cells [Hinuma et al, 1981; Kalyanaraman et al, 1982]. A type-C retrovirus was isolated in 1984 from T-cell cultures of Japanese patients with adult T-cell leukemia (ATL) that was subsequently demonstrated to be identical to HTLV-I

isolates from the United States [Yoshida et al, 1984].

Additional evidence supporting a role for HTLV-I in leukemogenesis followed. The virus was isolated from a Japanese-American with a T-cell leukemia, and Caribbean born blacks who were recognized to have a concentration of T-cell leukemias were all found to be HTLV-I positive [Gallo,1985]. Studies of the biology and molecular virology of cell lines from ATL patients revealed that ATL is a disease of CD4+ helper T-cells. All leukemic cells from any individual ATL patient contained HTLV-I provirus integrated at the same genomic site, suggesting that infection occured prior to transformation of these cells into a lymphoproliferative clone [Yoshida et al, 1984]. Groups in Japan and the U.S. both showed that cultured ATL cells could transform a normal CD4+ cell line into an immortal clone [Wong-Staal and Gallo, 1985; Yamamoto and Hinuma, 1985]. Miyoshi was then able to induce malignancies in hamsters by inoculating cell lines transformed with HTLV-I from ATL patients [Miyoshi et al, 1984]. Furthermore, a virus closely related to HTLV-I, simian T-lymphotropic virus type I, was isolated from African green monkeys and found to be associated with Simian malignant lymphoma [Hunsmann et al, 1983, Miyoshi et al, 1982].

Biological Properties of HTLV-I Transformed Cells

Both ATL cell lines and cell lines immortalized by HTLV-I infection in vitro are predominately CD4+ helper T-cells that spontaneously express high levels of interleukin-2 (IL-2) receptor and grow in culture as multinucleated giant cells with lobulated nuclei. These cultures continue to grow in the absence of IL-2 or may require only low concentrations of exogenously added IL-2. Normal T-cell lines, in contrast, require phytohemagglutinin activation to induce IL-2 receptor expression, grow in culture as single cells, and require exogenously added IL-2 for continued growth [Popovic et al, 1983].

Genomic Characteristics

HTLV-I contains a magnesium-dependent reverse transcriptase, high molecular weight RNA, and both internal core and envelope glycoproteins. In contrast with other mammalian type-C retroviruses, HTLV-I has a small sized core protein (p24) and a glycosylated transmembrane protein (gp41) [Reitz et al, 1981; Kalyanaraman et al, 1981].

Like all type-C retroviruses, the HTLV-I genome contains 5' and 3' long terminal repeats (LTR's) and gag, pol, and env genes that encode viral structural proteins, reverse transcriptase, and transmembrane glycoproteins respectively. The gag gene encodes a 53,000 dalton polypeptide (p53) that is proteolytically cleaved into p19, p24, and p15 internal structural proteins. The product of the pol gene is a magnesium dependent reverse transcriptase (RT) that is synthesized in infected cells as a gag-pol product and detected as a 180,000-200,000 dalton polyprotein. The env gene product is a 62,000 dalton glycosylated protein that can be cleaved into outer envelope (gp46) and transmembrane (gp 21) proteins [Desgranges et al, 1989] (Figure 4).

HTLV-I has no known viral oncogene [Seiki et al, 1984b]. Furthermore, although all ATL cells from an individual patient contain HTLV-I provirus integrated at the same site, there is not a common region of proviral integration in leukemic cells from different ATL patients [Seiki et al, 1984a]. HTLV-I thus does not induce leukemogenesis by viral oncogenesis or insertional mutagenesis (in which proviral integration into a specific chromosomal locus activates an adjacent cellular oncogene- a cis-acting function). A trans-acting viral function mediated by viral proteins was hypothesized.

HTLV-I has a genomic segment that is unique to human retroviruses. Originally designated tat for transacting transcriptional regulation but now termed pX, this sequence is located between the env gene and the 3' LTR and has at least two important functions. It encodes regulatory proteins, $p40^{tax}$, $p27^{rex}$ and p21, that act in trans to exert both positive and negative control on

different stages of viral replication (Figure 4). One of these regulatory proteins, $p40^{tax}$, also transactivates host cell genes that encode interleukin-2 (IL-2) and the alpha subunit of the IL-2 receptor. The alpha subunits thus synthesized are able to combine with beta subunits normally present on the surface of T-cells to form functional IL-2 receptors [Yoshida, 1990]. HTLV-I mediated transactivation results in spontaneous production of IL-2 and expression of IL-2 receptors by infected cells. It explains the lack of dependence of HTLV-I transformed cell lines on exogenous IL-2 [Inoue et al, 1986]. Production of IL-2 and IL-2 receptors may be responsible for the early stages of ATL development [Yoshida and Seida, 1990].

Interestingly, creation of transgenic mice by transfection with the HTLV-I tat gene together with an associated promotor resulted in the development of multiple mesenchymal soft tissue tumors in the mice. No transfected mice in this murine transgenic model developed either leukemia/lymphoma or myeloneuropathy [Nerenberg et al, 1987].

PATIENTS

In the initial stage of this project, isolation of HTLV-I was attempted from peripheral blood of TSP/HAM patients and controls from the endemic foci of Jamaica and Tumaco, Colombia. Heparinized blood was shipped on ice from these areas by overnight mail for attempted viral isolation. The establishment of a successful system for isolation of HTLV-I from TSP/HAM patients coincided with the visit to the National Institutes of Health of a family of three from Tumaco with TSP/HAM of varying duration and clinical severity. The methodology used to isolate virus from TSP/HAM patients is detailed as it was applied to the members of this family.

Described here are the clinical findings and HTLV-I isolation from a married couple and one son who have lived their entire lives in the endemic TSP area of Tumaco on the southern Pacific coast of Colombia (Figure 5). They were seen by neurologists in Colombia and were brought to the Neuroimmunology branch of the Clinical Center, the National Institutes of Health, Bethesda, MD, in July 1987 for further evaluation.

Patient Histories

Patient 1

Patient 1, a 43-year-old man, had onset of disease twelve years earlier. His first symptoms were itching of the soles of the feet, hip pain on rising in the morning, nocturnal muscle cramps, occasional tremor, and numbness of the lower limbs. He developed gradually progressive weakness of the lower limbs over a period of seven years. He also developed urinary frequency and urgency, impotence, and constipation.

Examination revealed no impairment of intellectual function. His cranial nerves were normal. Power was unimpaired in the upper limbs, but he had profound weakness of the lower limbs with spasticity, clonus, and bilateral

Babinski responses. Hoffmann's sign was positive in both upper limbs, and his abdominal reflexes were diminished. Superficial and deep sensation were normal. He was able to walk with a spastic gait using one cane.

His previous medical history and family history were noncontributory.

Patient 2

Patient 2, the 44-year-old wife of patient 1, first presented with symptoms of the disease eight years earlier when she was unable to expel the placenta after child delivery. Soon thereafter she developed numbness and slowly progressive weakness of the legs that was more pronounced on the right. More recently, her upper limbs became fatigued with continued usage although they were not subjectively weak. She also developed burning in the legs, polyuria, loss of libido, and constipation.

On neurologic examination, her intellectual functioning was normal. Cranial nerves were intact. She had a spastic paraparesis with generalized hyperreflexia, bilateral positive Hoffmann's signs, ankle clonus, and positive Babinski responses. In addition, she had marked impairment of vibration sense below the second thoracic dermatome. She was able to walk with the aid of one cane.

Her past medical history was significant for seven pregnancies with five living children. She was admitted to a hospital 8 years earlier for headache and abdominal pain. Two years ago she had herpes zoster affecting the mid-thoracic region.

Patient 3

Patient 3, the 13-year-old fourth grade son of patients 1 and 2 had a three year history of mild nocturnal cramps in his right more than his left leg and occasional numbness of his lower limbs. He had no bowel or bladder problems. In the past two years he had developed some photophobia and impairment of

visual acuity but had been unaware of any decreased ability to study or to play soccer.

On neurological examination, his intellectual function was normal, and his cranial nerves were intact. He had hyperreflexia of all four limbs which was more marked in the lower limbs. His power was good in the upper limbs, but he had minimal weakness of the right lower limb and right ankle clonus. His abdominal reflexes were brisk, and both plantar reflexes were extensor. Sensation was normal to all modalities. He walked and ran unaided but with a suggestion of a mild spastic gait.

There was no family history of neurologic disease. The extended family and household contacts of these patients were without clinical evidence of TSP/HAM.

MATERIALS AND METHODS

Antibody Determinations: ELISA and Western Immunoblot Assays

Both serum and CSF from the three patients were tested for IgG antibodies to HTLV-I using the Dupont/Biotech enzyme-linked immunosorbent assay (ELISA) kit (Biotech Research Labs, E.I. Dupont, Wilmington, DE) [Saxinger and Gallo, 1983]. Endpoint titrations were done on both serum and CSF. Positive ELISA reactions were confirmed by Western immunoblot using a commercially available kit for the detection of antibodies to the major proteins of HTLV-I (Biotech research Labs, E.I. Dupont, Wilmington, DE). Serum and CSF from each of the three patients were also assayed for IgM antibodies against HTLV-I using small columns of quarternary aminoethyl-Sephadex A-50 ion exchanger for IgM separation (Isolab Inc., Akron, OH) [Johnson and Libby, 1988]. Alkaline phosphatase and biotin-labeled goat antibodies to human IgM were used for ELISA and western blot procedures, respectively (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD).

Cell Separation and Establishment of Cultures

Peripheral blood lymphocytes from the father and the mother and several HTLV-I seronegative disease-free controls from the endemic TSP area of Tumaco, Colombia, were separated from heparinized blood by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation. Peripheral blood lymphocytes from the son were obtained by leukopheresis (supplied by Dr. Dale McFarlin). The cells were cultured by techniques similar to those used to isolate HTLV-I from peripheral blood lymphocytes of ATL patients [Poiesz et al, 1980b; Miyoshi et al, 1981]. Minor technical modifications included the addition of alpha interferon and both recombinant and cell-derived interleukin-2 to the culture medium.

Cells from all three patients were initially stimulated with 2 μg/ml

phytohemagglutinin (Wellcome Diagnostics, Dartford, Eng]) [Nowell, 1960] for 4 days at a concentration of $1X10^6$ cells/ml in culture medium containing an enriched mixture of: RPMI 1640, 20% heat-inactivated fetal bovine serum, 1% L-glutamine, 50 μg/ml gentamycin, 5 μg/ml hydrocortisone, 400 μg/ml sheep anti-human alpha interferon (Interferon Sciences, New Brunswick, NJ), 10% cell-derived IL-2 (Advanced Biotechnologies, Rockville, MD) and 20 units/ml recombinant IL-2 (Amgen, Thousand Oaks,CA) [Popovic et al, 1983].

Following stimulation, peripheral blood lymphocytes from the father, the mother and controls were either grown independently in culture medium or cocultivated with an equal number of phytohemagglutinin stimulated umbilical cord blood lymphocytes; peripheral blood lymphocytes from the son were cocultivated with cord blood lymphocytes, but were not grown independently due to the low number of cells obtained after the leukopheresis. Cultures were fed enough culture medium biweekly to maintain a cell density of $1X10^6$ cells/ml as monitored by Trypan Blue exclusion.

A small number of mononuclear cells isolated from 2 ml of cerebrospinal fluid from the son were cocultivated with $1X10^6$ cord blood lymphocytes. The cocultivation was phytohemagglutinin stimulated for 4 days and then grown in culture medium with biweekly feeding as needed. HUT-102 and HUT-78 cell lines (obtained from Dr. Prem Sarin, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health) were maintained as HTLV-I positive and negative controls, respectively.

Preparation of Cord Blood Lymphocytes

Cord blood lymphocytes were separated from umbilical cord blood (Advanced Biotechnologies, Rockville, MD) by Ficoll-Hypaque density gradient centrifugation. Separated lymphocytes were then either grown independently as a control culture or cocultivated with peripheral blood lymphocytes or CSF-derived-mononuclear cells from the patients.

Cellular Transformation: Requirement for Interleukin-2

Cultures testing positive for HTLV-I by immunofluorescence were assayed for transformation by monitoring growth in culture medium free of both cell-derived and recombinant exogenous IL-2 [Popovic et al, 1983]. Growth was determined by Trypan Blue exclusion. Transformed cultures were reassayed for viral antigen by immunoflourescence.

Detection of the Viral Antigens by Immunofluorescence

For detection of viral antigens by indirect immunoflourescence, cultured cells were initially centrifuged out of culture medium, washed with phosphate buffered saline, air dried onto 8 well Toxoplasmosis slides ($2x10^4$ cells/well), and fixed in methanol:acetone (1:1) for 15 min at room temperature. Fixed cells were then absorbed for 45 minutes at room temperature with 10% normal goat serum in phosphate buffered saline and washed three times with complete changes of buffered saline. After air drying, the cells were incubated at 37^{o} C for 30 min with either mouse monoclonal antibody to HTLV-I p19 or mouse monoclonal antibody to HIV p24. The slides were rinsed for 45 min in buffered saline/.25% Triton X-100 with 3 changes and air dried. Flourescein isothiocyanate labelled goat antibody to mouse immunoglobulin (IgG) (Cappel, Cochranvile, PA) was applied for 30 min at 37^{o} C, followed by overnight washing in buffered saline/.25% Triton X-100. Slides were then counterstained with 0.02% Evans blue, washed for 30 min in buffered saline, mounted, and examined with a Zeiss flourescent microscope [Sarin et al, 1985].

Reverse Transcriptase Assay Conditions

To obtain viral pellets for reverse transcriptase testing, 2 ml of culture supernatant was mixed with 1.0 ml of 30% polyethlene glycol 8000 and 0.2 ml of 4M NaCl. After overnight incubation at 4^{o} C, the mixture was spun at 2500 RPM for 30 minutes. The pellet was resuspended in 200 µl of cold buffer (50

mM Tris.HCl, pH 7.5, 1 mM dithiothreitol, 20% glycerol, 0.35M KCl, 0.25% Triton X-100) and vortexed. The sample was frozen at -70° C until ready for use.

To assay reverse transcriptase activity in the viral pellets, 20 µl of viral pellet solution was added to 30 µl of reaction mixture containing 50 mM Tris.HCl (pH 7.5), 5 mM dithiothreitol, 100 mM potassium chloride, 0.01% Triton X-100, 10 ug/ml (dT)15(A)n as template primer and [^{3}H] deoxythymidine triphosphate, and incubated for 1 hr at 37° C. The reaction was stopped by adding 10 µl of yeast transfer RNA (5mg/ml) and 2 ml of 10% trichloroacetic acid solution containing 10 mM sodium pyrophosphate. After standing in ice for at least 15 minutes, the samples were filtered on millipore filters, washed 5 times with 5% trichloroacetic acid, washed once with 70% ethanol and dried under a heat lamp. Dried filters were added to scintillation fluid, and radioactivity was counted in a ß-scintillation counter [Sarin et al, 1985].

Electron Microscopy

Cell lines were prepared for electron microscopy as follows. Cultures were centrifuged gently at 500xg at room temperature. The supernatant was discarded and replaced with 2.5% glutaraldehyde freshly prepared in cacodylate buffer, pH 7.4. The pellet was then processed as a solid tissue as follows: postfixation in 1% osmiun tetroxide for 2 hours, dehydration through graded ethanols, clearing in propylene oxide, and embedding in Embed [Electron Microscopic Sciences, Fort Washington, PA]. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with a Phillips 300 transmission electron microscope [Liberski et al, 1988]. All electron microscopy was performed by Dr. Pawel Liberski from our laboratory.

RESULTS

Antibodies to HTLV-I in Serum and CSF

Serum and CSF from the three patients contained IgG antibodies against HTLV-I as detected by ELISA. Serum and CSF from the father showed the highest reactivities, with endpoint titers of 1:2560 in serum and 1:128 in CSF. The endpoint titration of antibodies to HTLV-I in the mother was 1:640 in serum and 1:8 in CSF while in the son it was 1:320 in serum and 1:4 in CSF. Serum from the son but not his parents contained IgM antibody against HTLV-I using ELISA at a dilution of 1:10, indicative of a primary humoral response against the virus. All samples were confirmed positive against HTLV-I specific proteins by Western blot. Serum and CSF from the three family members demonstrated antibodies against p19, p24, p28, p32, p36, p42, gp46 and p53. In addition samples from the mother and father were reactive with p15 (Figure 6).

Among the additional household relatives, HTLV-I antibodies were found in the serum of the 46 year old clinically unaffected sister of patient 2 and in the serum of her 7 year-old daughter. No other family members were positive.

Expression of Viral Antigens and Cellular Transformation

Peripheral blood lymphocytes from the father and mother, peripheral blood lymphocyte/cord blood lymphocyte cocultivations from all three patients, and the CSF mononuclear cell/cord blood lymphocyte cocultivation from the son all showed positive immunofluorescence when labelled with the murine monoclonal antibody against HTLV-I p19 gag protein. In contrast to the red negative staining demonstrated by HTLV-I negative cultures of CBL or HUT-78, these cultures exhibited bright yellow-green flourescein isothiocyanate staining of p19 viral antigens in the cytoplasm of infected cells (Figure 7). HTLV-I isolates were obtained from peripheral blood lymphocytes of the mother and father without cocultivation with cord blood lymphocytes. In contrast, isolation of

HTLV-I from peripheral blood lymphocytes of adult T-cell leukemia/lymphoma patients has traditionally required cocultivation [Markham and Salahuddin, 1987]. This requirement for cocultivation differentiates HTLV-I isolated from ATL patients from HTLV-I isolated from TSP/HAM patients. We did not attempt virus isolation from the peripheral blood lymphocytes of the son without cocultivation due to the few cells obtained after the patient's leukopheresis.

All HTLV-I p19 positive cultures from the three family members became transformed, as defined by continued proliferation in the absence of exogenous IL-2. In addition, the immunoflourescent reactivity of these cultures against monoclonal antibody to HTLV p19 increased after selection for transformation in exogenous IL-2 free medium. The percentage of cells expressing viral antigens after transformation increased to as high as 85% (Figure7).

No cultures demonstrated any reactivity when assayed with a murine monoclonal antibody against HIV p24 gag protein either before or after transformation testing. Control cultures (cord blood lymphocytes, peripheral blood lymphocytes from HTLV-I seronegative controls from Tumaco, and peripheral blood lymphocyte/cord blood lymphocyte cocultivation controls) did not react with monoclonal antibody against HTLV-I p19 before transformation testing and died soon after IL-2 was removed from the culture medium.

Cell Growth and Morphology

Following phytohemagglutinin stimulation, all cultures from TSP/HAM patients and controls grew initially as predominantly single cell suspensions and small clumps of homogeneous lymphoid cells after a week in culture. The longevity of the single cell growth phase varied from 13 days (peripheral blood lymphocyte/cord blood lymphocyte cocultivation of the son) to 29 days (peripheral blood lymphocyte/cord blood lymphocyte cocultivation of the father). A change in the culture from single cell organization to growth in large clumps, often around a central giant cell, coincided with the expression of viral antigen

by the cells as detected by indirect immunoflourescence.

The percentage of cells in a culture expressing viral antigen by immunoflourescence increased in proportion to the amount of cell clumping that could be detected micro- and macroscopically. Transformation could not be demonstrated until the large clumps formed. These characteristics are similar to those that have been described for HTLV-I induced transformation of umbilical cord blood lymphocytes by T cell lines from ATL patients [Popovic et al, 1983].

In contrast to the morphologic, antigenic, and growth characteristics exhibited by virally infected cultures following the single cell growth phase, cord blood lymphocyte cultures and cultures of peripheral blood lymphocytes from HTLV-I seronegative controls remained in the single cell growth phase for as long as they could be carried in culture.

Ultrastructural Examination

Electron microscopic examination of infected cell cultures derived from the family members revealed virus particles typical of type-C retroviruses associated extracellularly with bizarre giant cells. Mature virions measured 80-140 nm in diameter and were indistinguishable from standard HTLV-I viruses. Various stages of virus maturation were seen. Initially, virus formed crescent shaped buds at the plasma membrane. As the budding process continued, the membrane covered cores were closed, and mature virions were released containing electron dense nucleocapsid cores surrounded by a smooth envelope (Figure 8).

Reverse Transcriptase Activity

Reverse transcriptase activity was assayed in all cultures. In comparison to cultures of peripheral blood lymphocytes from HTLV-I seronegative disease free controls all of which had radioactivity counts less than 500, cultures from the family members had counts ranging from 1778 (CSF mononuclear cell

cocultivation from the son) to 6426 (peripheral blood lymphocyte cocultivation from the son). Counts from an HTLV-I infected cell line from an ATL patient (HUT 102) were consistently greater than 25,000. The background radioactivity count ranged from 390 to 423.

A radioactivity count of three times background was considered evidence of viral reverse transcriptase in the culture. Using this criterion, reverse transcriptase activity was detected in all cultures in which cells expressed viral antigens by immunoflourescence. These levels did not change significantly after cultures were grown in the absence of IL-2. Control cultures had no detectable enzyme activity.

It is unclear why the reverse transcriptase levels detected in cultures from TSP/HAM patients in this study were much lower than those detected in an ATL derived cell line (HUT 102). Subsequent studies performed in our laboratory detected significantly higher levels of activity in peripheral blood lymphocyte cultures from TSP/HAM patients. The activity in these cultures increased with time to a level comparable to that found in cell lines from ATL patients [Rodgers-Johnson, personal communication].

DISCUSSION

General Comments

A viral etiology has been proposed for many chronic neurological diseases including multiple sclerosis, Alzheimer's Disease, and Parkinson's Disease. Essential to implicating a viral pathogen in these diseases is the consistent isolation of virus from clinical cases. For diseases of postulated viral origin such as multiple sclerosis, the criterion of viral isolation has not been repeatedly fulfilled [Johnson, 1982]. In contrast, HTLV-I is etiologically associated with TSP/HAM, and, as shown by this study, virus can be consistently isolated from patients with this disorder.

In this investigation, methodology that was previously used to isolate HTLV-I from ATL patients was slightly modified to isolate virus from patients with TSP/HAM. HTLV-I was isolated from peripheral blood lymphocytes and CSF-derived mononuclear cells of a 13-year-old boy with early TSP/HAM and from peripheral blood lymphocytes of his 44-year-old mother and 43-year-old father, both of whom have disease of longer duration and worse severity. All three family members had IgG antibodies to HTLV-I in both serum and CSF, as detected by ELISA and confirmed by western immunoblot.

Successful isolation of a retrovirus was demonstrated by the presence of low levels of reverse transcriptase in cell culture supernatants combined with the presence of type-C retroviral particles budding off of the surface of infected cells as observed by electron microscopy. The retrovirus infecting these cell cultures was shown to be HTLV-I by positive staining when assayed by indirect immunoflourescence with a murine monoclonal antibody against the p19 gag protein of HTLV-I. In addition, subsequent studies in our laboratory by polymerase chain reaction DNA amplification showed that these isolates were definitely HTLV-I and not HTLV-II [Rodgers-Johnson et al, in preparation]. This was important because HTLV-II is an oncornavirus that is structurally and

antigenically similar to HTLV-I and may cross react with HTLV-I in some serologic assays [Wong-Staal et al, 1985]. HTLV-II is currently not etiologically associated with human disease.

The time in culture required to isolate virus varied inversely with the stage of disease of the respective family members. Over double the time in culture was required to isolate virus from the father, who first presented with TSP in 1975, than was needed to isolate virus from the son, who just recently developed upper motor neuron signs. The son represents an early stage of TSP, and the possibility that virus is more readily isolated from patients in the early stages of the disease should be further investigated. Transmission of HTLV-I to the son was most likely vertical via breast milk [Osame et al, 1987], but seroepidemiological studies need to be carried out to exclude other possible modes of transmission including insect vectors.

Significance of the Present Findings

This study was particularly significant for several reasons. Using methodology very similar to that previously established in the study of ATL, it resulted in consistent isolation of virus from TSP/HAM patients. Isolation had previously been reported to be difficult, and only individual case reports of successful isolation had been described [Hirose et al, 1986; DeFreitas et al, 1987]. This had also been the case in our laboratory as virus was successfully isolated from only 1 of 30 TSP/HAM patients [Rodgers-Johnson, 1988]. However, applying the methodology that was successful in this study, our laboratory has since isolated HTLV-I from TSP/HAM patients from Colombia, Jamaica, and Chile [Mora et al, in preparation]; from Jamaicans with polymyositis [Rodgers-Johnson et al, in preparation]; and from disease-free inhabitants of an HTLV-I endemic focus on New Guinea [Yanigahara et al, 1990]. Additionally, there are less than 10 cases recognized worldwide to date of patients who have had both ATL and TSP/HAM, and our laboratory has

isolated HTLV-I from one of these cases [Lee et al, 1989].

This study was the first isolation of HTLV-I from multiple TSP/HAM patients within the same family and the first isolation from an affected child. In addition, the 13-year-old son was the first childhood case of TSP/HAM reported outside of Japan. TSP/HAM had been reported in Japan in children as young as 6 years, but until this study, no childhood cases had been described outside of Japan, and virus had never been isolated from a child with TSP/HAM.

At the time of this investigation, TSP and HAM were thought to be separate disorders because of differences in age distribution, climate in which they occur, and response to corticosteroids. This study demonstrated that TSP and HAM have similar age distributions and thus provided evidence that they are not separate entities. The two syndromes were subsequently concluded to be identical based on similar clinical, laboratory, and histopathological findings [Roman and Osame, 1988].

This investigation also resulted in the first report of virally infected cells from TSP/HAM that were transformed as defined by continued proliferation in the absence of exogenous IL-2. HTLV-I transformed cells have previously been characterized by the production of both IL-2 and IL-2 receptors. These cells did not require exogenous IL-2 to proliferate. In contrast, non-transformed cells required exogenous IL-2 to proliferate, and these cells died when IL-2 was removed from the culture medium [Popovic et al, 1983]. Consistent with these criteria for transformation, our virus infected cultures did not require exogenous IL-2 to proliferate, and a high percentage of cells in these cultures expressed IL-2 receptors as determined by fluorescence activated cell sorting using a monoclonal antibody to the IL-2 receptor [Stone, personal communication]. Furthermore, removal of exogenous IL-2 from the culture medium significantly increased the percentage of cells expressing viral antigens as determined by indirect immunofluourescence. It is possible that the selective death of non-transformed cells in medium free of exogenous IL-2 enhanced the

percentage of cells in the cultures that were virally transformed.

Further Questions

Our results raise a number of questions including:

1) What further evidence exists that HTLV-I has an etiologic role in TSP/HAM?

2) Are the viral isolates from patients with TSP/HAM and ATL the same?

3) What is the significance of transformation relative to TSP/HAM?

4) What are the possible pathogenic mechanisms that result in ATL and TSP/HAM?

5) Is TSP/HAM a model for other chronic nervous system disease?

6) Can HTLV-I be implicated in other disease processes?

To answer these questions, I will discuss findings that have been described by members of our laboratory and other investigators subsequent to this study.

Evidence for an Etiologic Role of HTLV-I in TSP/HAM

With the isolation of HTLV-I from TSP/HAM patients, there is now compelling evidence implicating a role for HTLV-I in the pathogenesis of TSP/HAM. More than 40 geographic clusters of the disease have been recognized, all in areas of HTLV-I endemicity. In each of these foci there is a high prevalence of antibodies to HTLV-I in CSF and serum of affected patients [Rodgers-Johnson et al, 1990]. Early reports of abnormally activated lymphocytes with lobulated nuclei in the peripheral blood and CSF and of oligoclonal bands with HTLV-I specificity in the CSF of TSP/HAM patients have been confirmed repeatedly [Roman, 1990]. Additionally, the visualization by electron microscopy of HTLV-I-like particles in spinal cord cells of a Jamaican TSP/HAM patient was reported [Liberski et al, 1988].

In addition to the virus isolation in this investigation, isolates were obtained from peripheral blood and CSF of TSP/HAM patients by Jacobsen et al [Jacobson et al, 1988a]. Bhagavati and colleagues subsequently detected HTLV-I DNA in peripheral blood and CSF lymphocytes of patients with TSP/HAM by enzymatic DNA amplification using the technique of polymerase chain reaction [Bhagavati et al, 1988]. More recently, our laboratory detected HTLV-I DNA in cell lines from TSP/HAM patients using in situ hybridization [Beilke, personal communication].

This evidence strongly associates HTLV-I with TSP/HAM. However, the mechanism of virally induced pathogenesis is not known. No animal model of the disease exists as yet, but our laboratory has initiated experiments in this regard in non-human primates, laboratory rodents, and rabbits.

Comparison of Isolates from TSP/HAM and ATL

It is still unclear whether the viral isolates from TSP/HAM and ATL are the same. Established cell lines from the two diseases have the same in vitro growth characteristics consisting of abnormally activated cells with convoluted nuclei that express IL-2 receptors and grow in large clumps [McKhann et al, 1989]. We also found that cell lines from TSP/HAM patients will continue to proliferate in the absence of exogenously added IL-2, a property charecteristic of ATL cell lines. However, Jacobson et al reported that isolates obtained by activating T-lymphocytes using a monoclonal antibody to the T-cell receptor combined with stimulation with exogenous IL-2 were dependent on exogenous IL-2 for survival [Jacobson et al, 1988a].

Early DNA blotting studies reported that viral isolates from TSP/HAM and ATL were identical [Yoshida et al, 1987]. More recent work comparing the complete DNA sequence of provirus from the two diseases reported greater than 97% homology [Tsujimoto et al, 1988]. However, differences between TSP/HAM isolates and prototypical HTLV-I from ATL have been detected by

restriction mapping [Jacobson et al, 1988a; Sarin et al, 1989], and preliminary comparison between DNA that encodes reverse transcriptase in the two diseases suggested that up to 5 nucleotide substitutions exist [Bangham et al, 1988].

If in fact strain differences do occur, it remains to be determined whether these differences affect the pathogenesis of TSP/HAM versus ATL or whether they result from geographic separation of endemic foci. The theory that infection by a single strain of HTLV-I may result in two disease processes is supported by the recognition to date of several individuals from widely separated geographic areas who have developed both TSP/HAM and ATL [Groopman and Ferry, 1989; Rodgers-Johnson, personal communication].

Are Cells Lines from TSP/HAM Patients Transformed?

The significance of in vitro transformation relative to TSP/HAM must be examined. As discussed previously, our cell lines were transformed by the criteria of expression of IL-2 receptors by HTLV-I infected cells and continued proliferation in the absence of exogenous IL-2 [Popovic et al, 1983]. These properties have been shown in ATL-derived cell lines to result from transactivation of infected lymphocytes by one of the proteins encoded for by the HTLV-I pX region, $p40^{tax}$. This protein transactivates the genes for IL-2 and for the alpha subunit of the IL-2 receptor, resulting in spontaneous production of IL-2 and expression of IL-2 receptors [Inoue et al, 1986]. Because infected cells produce IL-2 and express IL-2 receptors, they spontaneously proliferate in vitro and continue to proliferate when exogenous IL-2 is removed from the cell culture medium. In support of transactivation occuring in TSP/HAM, recent application of the highly sensitive technique of combining splice excluded transcription with polymerase chain reaction genomic amplification has demonstrated greatly increased levels of messenger RNA encoding both IL-2 and the alpha subunit of the IL-2 receptor in peripheral blood lymphocytes from

TSP/HAM patients [Greenberg et al, 1990].

Whether this process represents true transformation requires consideration. In contrast to ATL, a disorder in which monoclonal transformation of infected cells results in lymphoproliferation, TSP/HAM is not a clinically lymphoproliferative disorder. Transformation of HTLV-I infected cell lines implies monoclonal proviral integration. In support of transformation, studies have described monoclonal proviral integration in TSP/HAM [Gessain et al, 1989; Nakamura et al, 1989]. However, others have supported that the virus is polyclonally integrated in this disease [Jacobson et al, 1988a; Greenberg et al, 1989]. One recent study demonstrated that viral isolation from TSP/HAM patients produced nontransformed cell lines that died when exogenous IL-2 was removed from the culture medium [Jacobson et al, 1988a].

It is thus possible that cell lines infected with HTLV-I from TSP/HAM patients are not transformed in vivo. They may undergo a genetic event in vitro resulting in monoclonal proviral integration and transformation. Alternatively, these cell lines may be polyclonally infected resulting in persistently activated cells that spontaneously proliferate by producing IL-2 and expressing high levels of IL-2 receptor without actually being transformed. Consistent with this theory is the unusual increase in proliferation of unstimulated peripheral blood lymphocytes that has been reported in TSP/HAM patients from Peru [Johnson et al, 1988], Colombia [Jacobson et al, 1988b], and Japan [Itoyama et al, 1988]. The lymphocytes from these patients express high levels of IL-2 receptor and spontaneously proliferate significantly more than corresponding cells from HTLV-I seronegative controls, disease free HTLV-I seropositive carriers, or adult T-cell leukemia/lymphoma patients [Itoyama et al, 1988].

The mechanisms underlying transformation or persistent activation in TSP/HAM are not fully understood. Additionally, if HTLV-I infected cells are monoclonally transformed in vivo, why this transformation does not result in lymphoproliferation is not clear. Further molecular genetic studies of viral

isolates are needed to clarify these points.

Pathogenesis of ATL and TSP/HAM

As evidence accumulates implicating HTLV-I in the etiology of TSP/HAM, it is becoming clear that infection by this virus may result in a rapidly progressive lymphoproliferative malignancy, a slowly progressive spastic paraparesis, both, or neither. The factors controlling disease expression and mechanisms of disease pathogenesis are areas of intense scrutiny.

ATL most likely results from a direct pathogenic effect of HTLV-I in combination with other factors. The virus is monoclonally integrated and stably present. It can transform target T-lymphocytes both in vivo and in vitro. It is postulated that the virus acts as an inducer of ATL risk that when modified by the appropriate host genetic factors, virally induced immunosuppressive effects, and/or cytogenetic alterations result in progression to ATL. One theory is that HTLV-I mediated transactivation of genes encoding IL-2 and the IL-2 receptor is responsible for the early stage of ATL development, but that one of the above modifying factors is required for progression to the terminally differentiated leukemic state [Kramer and Blattner, 1989]. Evidence for this theory includes the finding that proviral DNA integrated into ATL cells is methylated and thus not being actively expressed [Kramer and Blattner, 1989] and the report that expression of HTLV-I pX genes is not required for terminal differentiation of ATL cells [Greenberg et al, 1990].

The pathogenesis of TSP/HAM is less clearly understood. Possible disease mechanisms include direct cytopathic effects of the virus on nervous system tissue and both humoral and cell-mediated immunopathogenic mechanisms. In support of direct virally induced neural tissue damage, affected patients were shown to have intrathecal evidence of HTLV-I infection [Ceroni et al, 1988] , and virus was isolated from their CSF mononuclear cells [Jacobson et al, 1988; McKhann et al, 1989]. In addition, electron microscopic visualization

of unidentified spinal cord cells detected HTLV-I-like particles [Liberski et al, 1988]. HTLV-I infection of cultured glial cells was also reported [Kim et al, 1988], but infection of cultured neurons has yet to be demonstrated. Direct neuropathic effects of HTLV-I may be similar to the destruction of neuronal tissue that occurs in subacute encephalomyelitis associated with human immunodeficiency virus infection [de la Monte et al, 1987].

A variety of immune mechanisms may be involved in TSP/HAM pathogenesis [Murphy and Blattner, 1989]. Cell mediated possibilities include: 1) activation of T-effector cells by HTLV-I infected T-helper cells resulting in neurologic damage and 2) alteration of T-suppressor cell function by virus infection resulting in uncontrolled T-effector cell damage. T-cell dysfunction may also induce disease in a permissive way. Popovic showed that HTLV-I infected cytotoxic T-lymphocytes are dysfunctional [Popovic et al, 1984]. Deficient cytotoxic T-cell function may allow persistent infection by other pathogens, antibodies against which may also damage myelin. This humoral molecular mimicry could explain the postulated cofactor role of treponemal and borrelial infections in endemic foci where a high prevalence of these infections exists [Rodgers-Johnson et al, 1990]. An alternate humoral mechanism of immunopathogenesis is the cross-reaction against myelin of antibodies to HTLV-I [Rudge, 1989].

Although direct evidence for an immunopathogenic mechanism of disease is lacking, several characteristics of HTLV-I infection in TSP/HAM support this process. HTLV-I specific antibodies are found in the CSF of TSP/HAM patients [Ceroni et al, 1988], and these antibodies are at much higher titers than in patients with ATL or HTLV-I seropositive controls [Rudge et al, 1989]. The pathology of TSP/HAM is very similar to that seen in ovine visna, an immune mediated demyelinating neuro-inflammatory disease [Mani et al, 1969]. In this disease, the lentivirus visna maedi infects sheep mononuclear cells. Once infected monocytes mature to macrophages, viral replication occurs, and

viral antigens are expressed on the cell surface. The resulting immune inflammatory response results in disease pathology the severity of which can be modified with steroids [Rudge et al, 1989]. Although HTLV-I is an oncornavirus, a similar mechanism may be involved. The reported response of patients in Japan and Colombia to high dose corticosteroid treatment and the less marked inflammatory changes in pathologic specimens following this treatment both support an immune mechanism [Rodgers-Johnson et al, 1989].

Additional disease manifestations of HTLV-I infection that are associated with TSP/HAM also support an immunologic pathogenesis. Systemic manifestations of HTLV-I infection that have been found in association with TSP/HAM include T-lymphocyte pulmonary alveolitis, uveitis, polymyositis, Sjogren's syndrome, arthropathy, necrotizing vasculitis, lymphocytic meningitis, and cryoglobulinemia [WHO report, 1989]. All of these processes may be immune mediated as a result of HTLV-I infection of T-lymphocytes.

Recent studies of HLA haplotype-linked immune responsiveness against HTLV-I in patients with ATL or TSP/HAM support immune modification of disease in both of these processes. Although as yet unconfirmed in other areas of the world, Usuku et al in Japan showed that certain human lymphocyte antigen (HLA) haplotypes associated with a high immune response to HTLV-I were found in 70% of TSP/HAM patients, while different HLA haplotypes associated with a low immune response to HTLV-I were found in patients with ATL. The authors suggested that high immune responsiveness was one of the most important factors in the development of TSP/HAM [Usuku et al, 1988].

An equally important question that remains to be answered is why so many HTLV-I infected people from areas of viral endemia do not develop either TSP/HAM or ATL. The lifetime risk for HTLV-I infected individuals of developing ATL has been estimated to be 1%. This risk increases to 3% to 5% for those infected before age 20 years [Murphy et al, 1989]. TSP/HAM has much higher prevalence in endemic areas than does ATL, but this likely represents the much

less rapid clinical course of TSP/HAM. Epidemiologic studies have suggested that childhood exposure may be more important for ATL development while adult and childhood exposure both confer risk of TSP/HAM development [Kramer and Blattner, 1989]. Other than these observations and the HLA immunogenetic data discussed above, little is currently known regarding factors affecting disease development.

Is TSP/HAM a model for multiple sclerosis?

A viral etiology for multiple sclerosis (MS) has been suggested for many years, and a number of possible agents have been proposed. Recently, the clinical similarity between TSP/HAM and the chronic progressive form of MS was recognized and raised the issues of whether these disorders are identical and whether HTLV-I plays a role in MS pathogenesis. The association between HTLV-I and MS was first reported by Koprowski and colleagues in 1985 who described MS patients from both Sweden and the southeastern U.S. who had antibodies against the p24 gag antigen of HTLV-I. T-cells from the CSF of these patients contained HTLV-I RNA sequences by in situ hybridization [Koprowski et al, 1985]. A second report appearing in 1986 described antibodies reactive to the p19 and p24 gag proteins of HTLV-I in 40% of Japanese MS patients [Ohta et al, 1986].

Taken together, the serologic and molecular biologic evidence of HTLV-I infection in MS patients combined with the clinical similarity between TSP and the chronic progressive form of MS necessitated that viral isolation be attempted from MS patients. Successful isolation would allow comparisons to be made between HTLV-I isolates from TSP and MS. As a first step in this process, virus isolation was attempted from 2 patients with clinical MS and serologic evidence of HTLV-I infection as part of this study. HTLV-I infected cell lines were not obtained from either of these patients using the same methodology that was utilized to isolate virus from TSP/HAM patients.

Failure to isolate virus from MS patients was not surprising considering the growing body of information that does not support a role for prototypical HTLV-I in MS. Poser reviewed the data concerning HTLV-I in MS pathogenesis and concluded that it is "highly unlikely that HTLV-I can be implicated as the etiologic agent of multiple sclerosis" [Poser, 1989]. More recently, preliminary results from a large blinded study looking at the role of HTLV-I in MS using polymerase chain reaction DNA amplification detected HTLV-I genomic segments in only 14 of 1368 peripheral blood lymphocyte samples from MS patients [Greenberg, personal communication].

HTLV-I and Other Diseases

HTLV-I has recently been associated with other disease processes including polymyositis and malignant fibrous histiocytoma. Sera of 11 of 13 biopsy proven cases of Jamaican polymyositis were reported to have high titers of antibodies to HTLV-I [Morgan et al, 1989]. HTLV-I infected patients with TSP/HAM and polymyositis had been previously described [Goudreau et al, 1988; Bhagavati et al, 1988], but this report associated HTLV-I with polymyositis alone. The authors suggested that HTLV-I be considered as a cause of polymyositis in areas of virus endemia. This etiologic association was supported by the recent isolation of virus from a Jamaican polymyositis patient [Rodgers-Johnson et al, in preparation]. Further studies are needed to determine whether this association is significant relative to disease pathogenesis.

A case was also reported of malignant fibrous histiocytoma in an individual with a history of both TSP/HAM and ATL [Lee et al, 1989]. Attempts to demonstrate HTLV-I antigen in sections of this patient's sarcoma were unsuccessful. However, the authors concluded that this tumor may have resulted from HTLV-I infection because it histopathologically was very similar to the multiple mesenchymal tumors that develop in transgenic mice transfected

with the tat gene of HTLV-I [Nerenberg et al, 1987]. Additionally, the chance of a malignant fibrous histiocytoma occurring randomly in a patient in association with TSP/HAM and ATL was calculated to be 1 in 10^{16}. If this tumor did result from HTLV-I infection, it further expands the spectrum of disease that may result from HTLV-I infection.

CONCLUSION

In a remarkably short period of time, a chronic neurologic disease of strikingly high incidence in certain areas of the world that was previously an epidemiologic mystery has been clarified. As HTLV-I is now recognized as the probable causative agent of TSP/HAM in addition to ATL, public health measures have begun to decrease transmission of the virus. Mandatory screening of all blood donors has begun in Japan and is being carried out in selected areas of the United States. In addition, recommendations against breast feeding by infected mothers have been made although they are impractical in many countries. The discovery that a virus known to cause leukemia also is implicated in the development of a chronic neurologic disorder by different pathogenic mechanisms raises the possibility that other infectious agents may be working in unsuspected ways to result in diseases of unknown etiology.

Table 1. Prevalence of Neurological Signs and Symptoms in 301 TSP patients.

Sign/Symptom	% Affected
Weakness/spasticity (lower limbs)	100
Urinary disturbance (e.g. frequency, urgency)	71
Constipation	65
Impairment of postural/vibratory sense	56
Positive jaw jerk	30
Impairment of light touch/pain sensation	28
Abnormal pupils	20
Muscle wasting	14
Visual impairment (sometimes optic atrophy)	14
Nerve deafness	9
Cerebellar signs (first noticed in upper limbs)	8

Adapted with permission from Rodgers-Johnson, 1989a.

Table 2. Prevalence of IgG Antibodies to HTLV-I in serum and/or CSF of patients with TSP/HAM and in the general adult population in areas of endemicity.

Endemic Region	% IgG Antibodies to HTLV-I		
	TSP/HAM patients		Population
	CSF	*Serum*	*Serum*
Jamaica	89	91	3-5
Colombia	96	98	10
Martinique	100	80	4
Trinidad	87	87	4
Seychelles	---	85	12
Japan (Kagoshima)	100	100	16

Modified with permission from Rodgers-Johnson et al, 1988b.
Antibody reactivity detected by enzyme-linked immunosorbent assay (ELISA) in all areas except Japan (gelatin particle agglutination test).

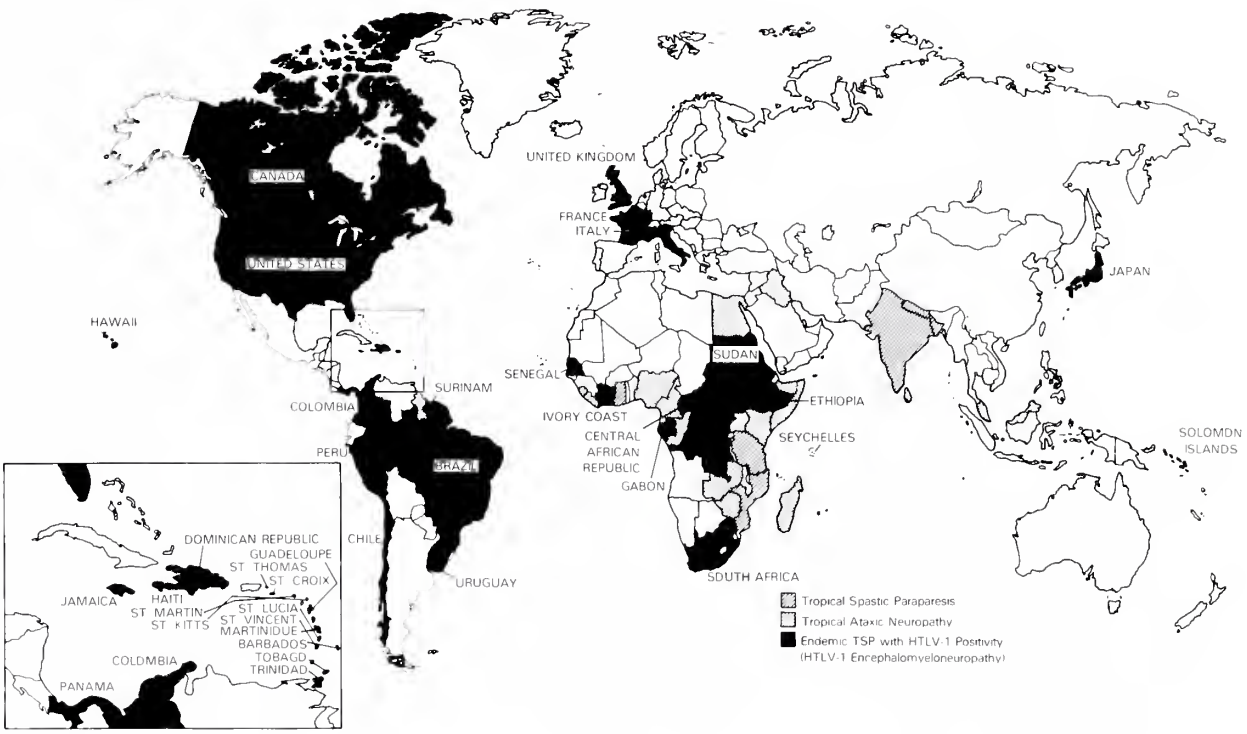

Figure 1. Worldwide distribution of tropical ataxic neuropathy (TAN) and tropical spastic paraparesis (TSP). Blackened areas indicate regions where a high prevalence of HTLV-I antibodies has been detected in TSP/HAM patients, while hatched regions are endemic TSP/HAM foci where HTLV-I seropositivity has not been fully tested. [Courtesy of Dr. Pamela Rodgers-Johnson, Laboratory of Central Nervous System Studies, National Institutes of Health].

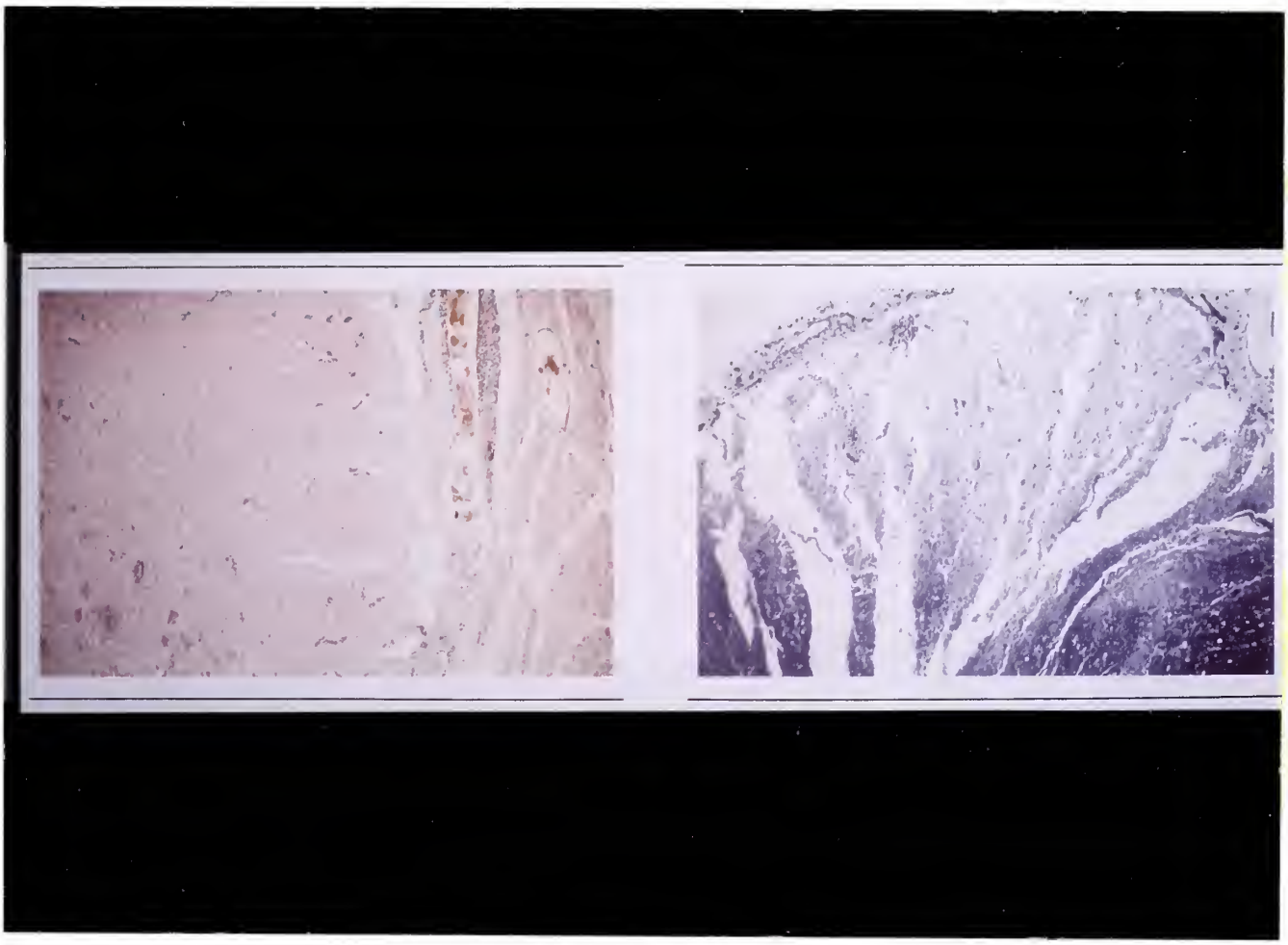

Figure 2. Histopathologic findings from spinal cord tissue of a 40-year-old Jamaican woman with TSP/HAM who died of a massive pulmonary embolism 5 years after the onset of disease. The left photomicrograph illustrates perivascular cuffing of a predominately lymphocytic infiltrate of mononuclear cells in the meninges and scattered in the gray and white matter of the parenchyma (hematoxylin and eosin, X16.5). The right panel shows prominent demyelination of the lateral column of the spinal cord (Luxol fast blue, X16.5). [Courtesy of Dr. Pedro Piccardo, Laboratory of Central Nervous System Studies, National Institites of Health].

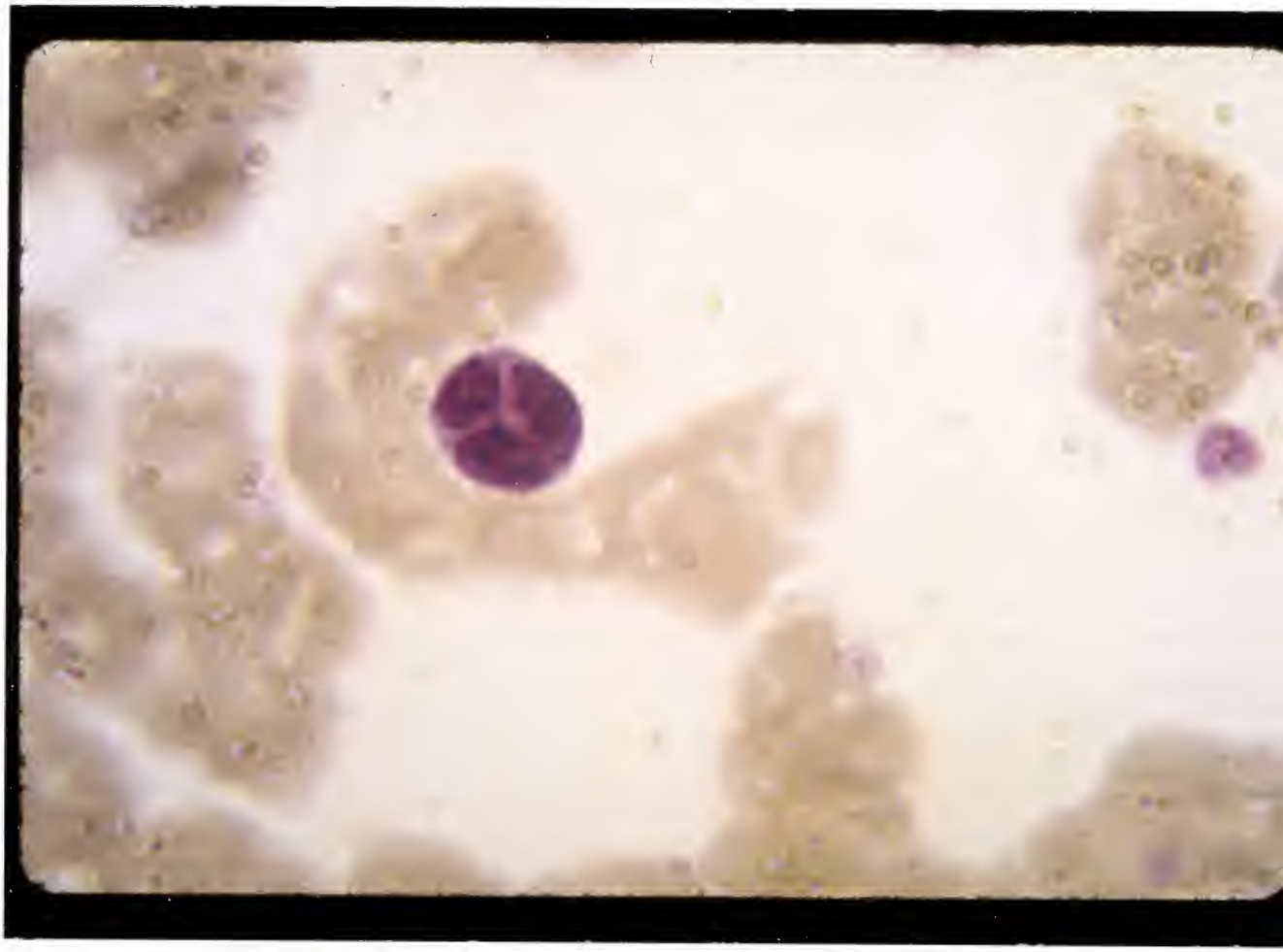

Figure 3. Light microscopic examination of peripheral blood from a 66-year-old Jamaican female with a 13 year history of TSP/HAM. The cell prominently displayed is an abnormal giant lymphocyte with convoluted rosette type nuclei. These cells, which are found in both CSF and peripheral blood of TSP/HAM patients, are morphologically similar to HTLV-I infected lymphocytes seen in peripheral blood of adult T-cell leukemia patients. [Courtesy of Dr. Prem Sarin, Laboratory of Tumor Cell Biology, National Institutes of Health].

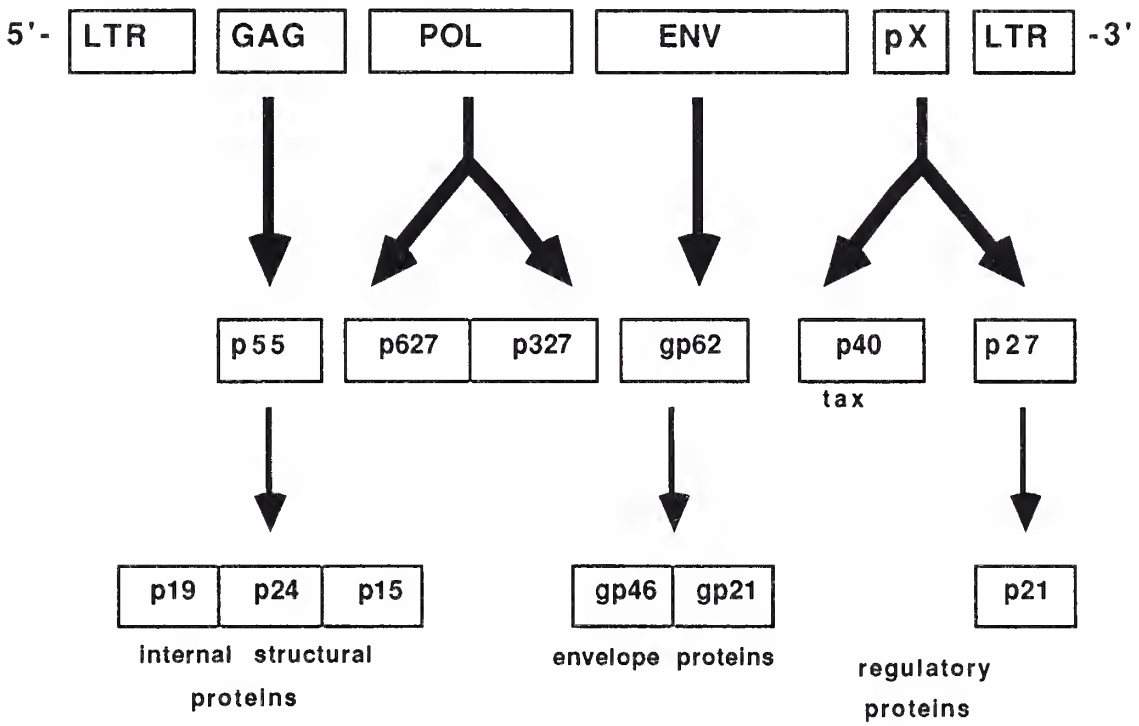

Figure 4: Schematic diagram of HTLV-I genomic structure.

Figure 5. Three members of a Colombian family from the endemic TSP/HAM focus of Tumaco. The father, mother, and son have TSP/HAM of varying clinical severity and duration, and all three have IgG antibodies to HTLV-I in their serum and CSF. [Courtesy of Dr. Carlos Mora, Laboratory of Central Nervous System Studies, National Institutes of Health].

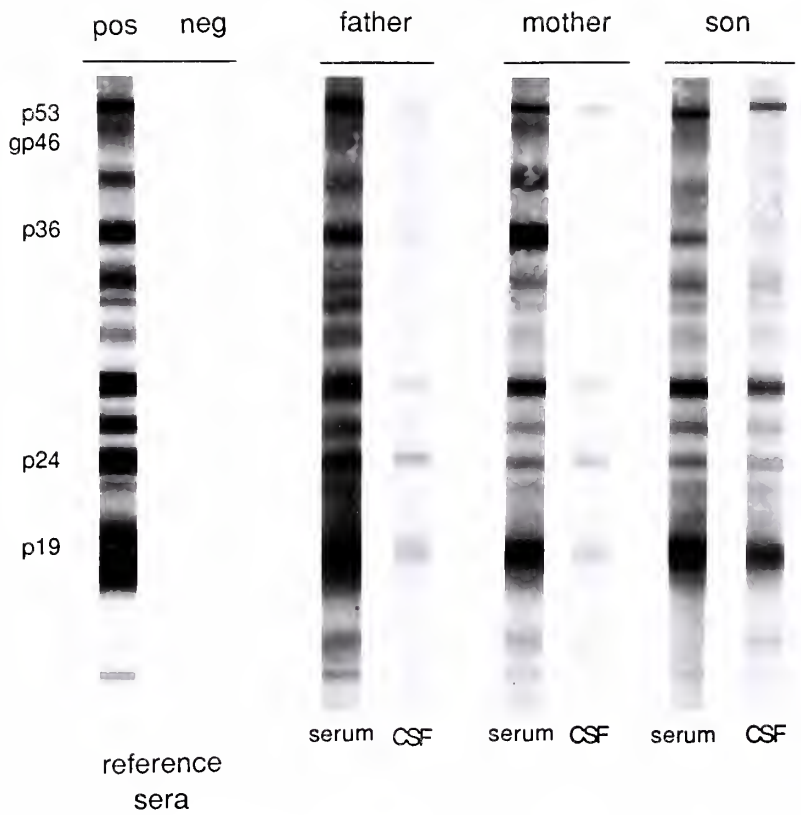

Figure 6. Western immunoblot for detection of antibodies against polypeptides of human T-lymphotropic virus type-I (HTLV-I). Human positive and negative refreence sera (left) are compared with serum and cerebrospinal fluid (CSF) of the father, mother, and son. Labelled bands include p19 and p24 gag internal structural proteins, gag precursor proteins p36 and p53, and the outer envelope glycoprotein gp46.

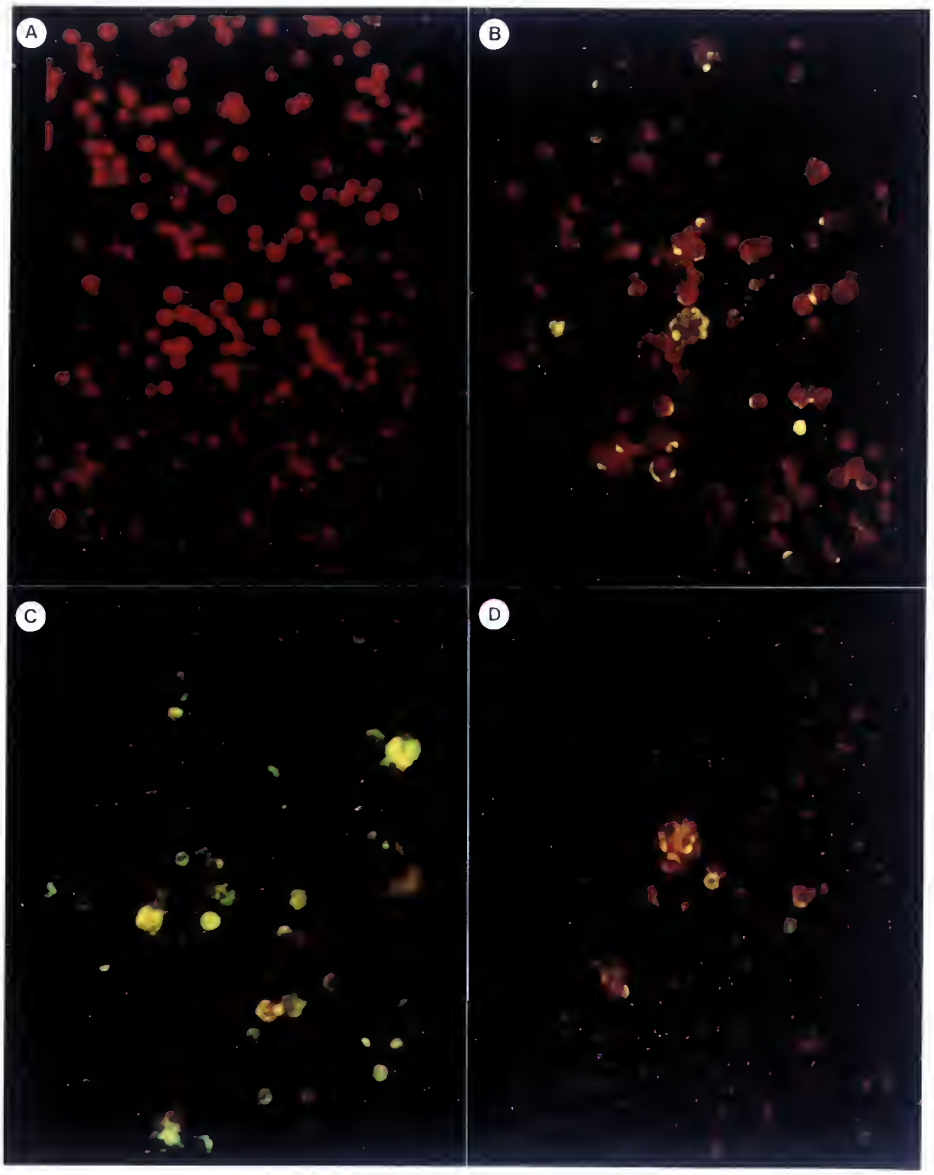

Figure 7. Immunoflourescence of cultured cells from TSP/HAM patients and controls following reaction with flourescein-labelled mouse monoclonal antibody to HTLV-I p19 antigen. A) Negative staining of cultured peripheral blood lymphocytes from an HTLV-I seronegative disease free control. B) Granular intracytoplasmic immunoflourescent staining of cocultivated peripheral blood lymphocytes from the son. C) Eight days following removal of exogenous interleukin-2 (IL-2) from growth medium of culture assayed in B), immunoflourescent reactivity and the percentage of cells expressing viral antigens has increased markedly. D) Similar staining pattern demonstrating HTLV-I antigen in cocultivated cerobrospinal fluid mononuclear cells from the son.

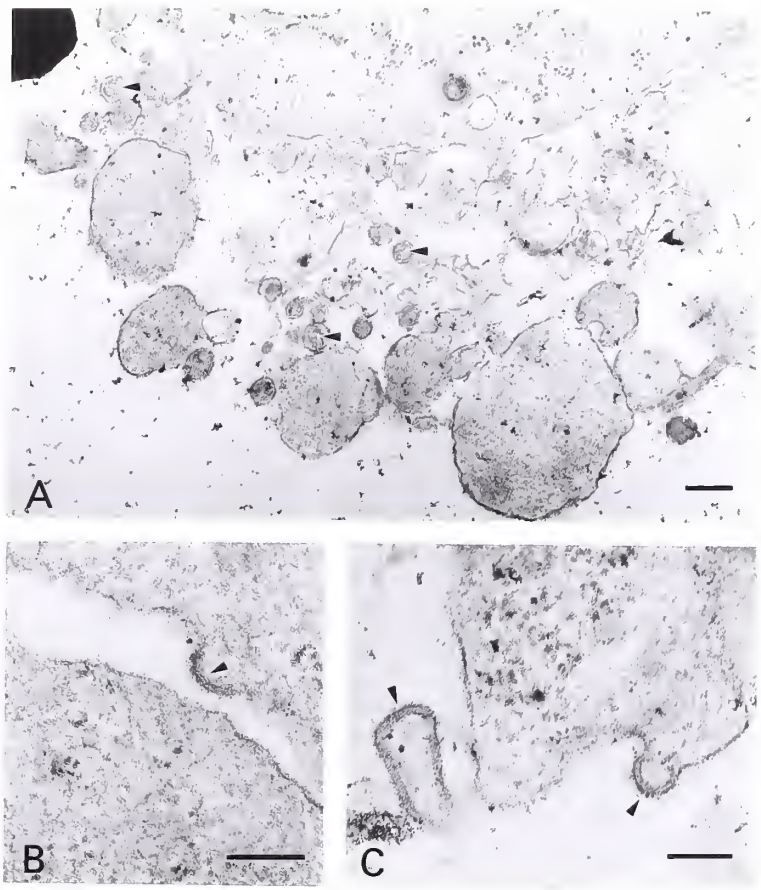

Figure 8. Electron microscopic examination of cultured peripheral blood lymphocytes from the mother showing: A) Extracellular mature virions that resemble HTLV-I like type-C particles with rounded nucleoid cores (arrowheads). B) Virion budding from the surface of infected cell (arrowhead). C) Two virions budding from the surface of infected cells (arrowheads). Bar= 0.2 μm.

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