Serological Studies of Patients with Multiple Sclerosis, controls, and Patients with Other Neurological Diseases: Antibodies to HTLV-I, HTLV-II, HIV, and STLV-III

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Introduction

Retroviruses have been associated with neurological disease in man. Recently, Kaprowski et al. [1] reported that outbreaks of mutliple sclerosis (MS) in Key West, Florida, and in Sweden seem to be associated with increased antibody for human retroviruses. In addition, homology studies under nonstringent conditions using CSF cells derived from MS patients reacted with human T-cell leukemia virus (HTLV)-I antigen. During the past 10 years more than ten different possible agents have been suggested as causes of MS [2]. These agents include a number of recognized viruses such as measles, canine distemper, scrapie agent, coronaviruses, and agents of unclear classification such as the MSAA (multiple sclerosis associated agent), bone marrow agent, and chimpanzee agent. We report here our studies of retrovirus antibody in a large number of sera and CSF collected from MS patients, matched controls, and patients with other neurological diseases (OND) prior to AIDS becoming a serious disease.

Review of the Literature

Retroviruses have been associated with a number of diseases in man and animals. In general these infections have been associated with leukemias, sarcomas, and immunosuppression. Neurological manifestations occur with some diseases. These agents have been listed as oncoviruses. In addition, retroviruses have been associated with slow virus diseases such as visna and progressive pneumonia (maedia). More recently immunosuppressive diseases caused by retroviruses have been recognized. These retroviruses are classified as nononcogenic or lentiviruses.

Retroviruses are enveloped viruses whose genome contain copies of high molecular weight, single-stranded RNA similar to messenger RNA. The virion contains various enzymes including reverse transcriptase (RNA to DNA). The retroviruses are classified into four groups: type A, B, C, and D, based upon dependency of

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Table 1. Retroviruses of humans and non-human primates

Human virus	Disease
HTLV-I	Adult T-cell leukemia, tropical spastic parapresis
HTLV-II	Hairy cell leukemia
HIV/HTLV-III/LAV	AIDS
HTLV-IV/LAV-II	Unknown
Non-human-virus	
STLV-I/HTLV-I	Lymphoma in captured macaques, antibody present in many wild monkeys in Asia and Africa
SIV/STLV-III	Immunosuppressive disease of monkeys
SRV-I	SAIDS in monkeys
SRV-II	Retroperitoneal fibromatosis
Mason-Pfizer monkey virus (MPMV)	Causes SAIDS-like disease in infant monkeys
SSV-I	Woolly monkey sarcoma virus or simian sarcoma virus
GALV	Gibbon ape leukemia virus or lymphosarcoma or myelogenous leukemia virus
MAC-I	Macaque endogenous virus, nonpathogenic
Squirrel monkey	Squirrel monkey endogenous virus, nonpathogenic
PO-I-Lu	Langur monkey endogenous virus, nonpathogenic

reverse transcriptase upon Mm^{2+} or Mg^{2+}, the characteristic of the nuclear capsules, and the maturation of the intact virus from the cells.

A list of retroviruses currently identified from human and nonhuman primates is presented in Table 1. The first human virus (HTLV-I) was isolated in 1980. Several other viruses have been isolated. All of these agents belong within the type C group. Most of the viruses cause disease in the host from which they are isolated. HTLV-I has been isolated from humans with adult T-cell leukemia and recently has been associated with tropical spastic parapresis (TSP). HTLV-II was isolated from an individual with hairy cell leukemia. It is serologically related to HLTV-I. HIV/HTLV-III/LAV is the most pathogenic, now being recognized as the etiological agent of AIDS. Primary subacute encephalopathy and neuropathy are also recognized as important signs of infection. HTLV-IV has recently been isolated from normal individuals in Africa. It is serologically closely related to HIV/HTLV-III.

Many simian retroviruses have been identified; however, their disease spectrum is less well defined. STLV-I/HTLV-I are serologically and genetically similar. Recently this agent was isolated from three species of macaque with lymphoma. Many wild caught animals in Asia and Africa have HTLV-I antibody. An STLV-II/HTLV-II agent has not been identified. SIV/STLV-III has been isolated from monkeys at the New England Regional Primate Center. It has a one-way cross with HIV/HTLV-III, i.e., serum that reacts with HTLV-III antigen reacts with STLV-III antigen but monkey serum that reacts with STLV-III antigen may not react with HTLV-III antigen. This agent in monkeys produces an immunosuppressive disease similar to AIDS in man (infects the T4 cells) and at least one strain causes a severe

neurological disease. SRV-I is associated with the monkey SAIDS. This agent causes a generalized disease which in the acute form produces a leukopenia and anemia, with death occurring in 1–3 months. In the subacute disease the animals usually die of opportunistic infections in 3–12 months. Some antibody-positive animals may survive for years. No neurological disease has been associated with this infection. This virus is not serologically related to the other retroviruses. SRV-2 is associated with retroperitoneal fibromatosis. It also predisposes animals to opportunistic infections. It is not closely serologically related to the other retroviruses. Mason Pfizer monkey virus was isolated in 1970 from monkey breast cancer samples. When inoculated into infant animals it produced death. Its pathogenecity has not been well characterized. SSV-I was isolated from a woolly monkey with a sarcoma, and the GALV was isolated from a gibbon ape and causes lymphosarcoma or myelogenous leukemia. Three endogenous viruses have been isolated from monkeys. They are: MAC-1 virus, which was isolated from macaques, the PO-1-Lu virus, which was isolated from the Langer monkey, and the squirrel monkey virus, which was isolated from the squirrel monkey. These three are reported to be nonpathogenic for monkeys.

Several retroviruses have been associated with neurological disease in animals and man. The first recognized was that of visna and progressive pneumonia (maedia) of sheep. This disease syndrome has been recognized since the early 1930s and the virus identified in 1949. Recently, three diseases of man have been recognized. Tropical spastic parapresis (TSP) has been associated with HTLV-I virus and a subacute encephalopathy and neuropathy has been associated with HIV. An acute neurological disease in primates associated with SIV/STLV-III has been reported in rhesus monkeys. A summary of the information available about these three diseases is presented.

TSP has recently been associated with HTLV-I by antibody studies [3, 4]. The disease is prevalent in the Pacific lowlands of Columbia, Jamaica, Martinique, Seychelles, South Africa, India, other tropical countries, and Japan. It characteristically does not occur in those temperate zones in which MS has been reported. Clinically, TSP presents with a chronic spastic myelopathy of slow onset with minimal sensory deficits. TSP patients have a progressive involvement of the pyramidal tracts, bilaterally and symmetrically affecting only the lower extremities. This is manifested by difficulty in walking, spasticity, and hyperflexia of the legs. Spastic bladder, severe constipation, and impotence in males are common. TSP does not have the typical relapsing course or visual abnormalities common in MS. The CNS and spinal cord lesions of TSP on postmortem examination are significantly different from those observed in MS. Presence of oligoclonal bands in TSP have been reported. There are several contradictory reports on the effect of steroid treatment of TSP. Kogoshima in South Japan reports that the disease symptoms are diminished by steroids while Zaninovic reported that in Columbia steroids had no effect on the course of the disease.

Subacute encephalopathy and neuropathy have been described in HIV antibody positive patients [5, 6]. This virus has been isolated from brain tissue, CSF, spinal cord, and peripheral nerves. As many as 10% of the HIV antibody positive patients may present, with the first signs of disease being a neurological disorder. This initial neurological disorder usually occurs as a change in personality and abnormal

behavior; definable neurological signs may be present. In most of these patients other signs of AIDS developed later. In another 30%–40% of the AIDS patients, neurological disease – not due to opportunistic infections – occurs. The clinical syndrome includes dementia, gait disturbance, spastic paraparesis, and bilateral pyramidal tract signs. The pathological lesions observed consist primarily of a vacuolar myeloneuropathy associated with a subacute encephalitis. By in situ hybridization and immunohistochemical staining, virus-containing cells appear to be primarily large monocytic (macrophages) cells and multinucleated cells. These cells are not associated with the neuronal type of cells and are found primarily in frontal, temporal, and parietal regions. Oligoclonal bands have been found in a few cases in our laboratory. Dr. Ceroni of Pavia, Italy, while in our laboratory, has demonstrated the specificity of these bands for HIV.

Information about the epizootiology, pathogenesis, and pathology of STLV-III infection in monkeys is limited. Virus has been isolated from the African green monkey STLV-III^{AGM} and the rhesus monkey STLV-III^{MAC}. The incubation period following inoculation until clinical signs develop is 3–9 months or longer. Passage through tissue culture systems attenuates the pathogencity of the virus. The neurological disease seems to be associated with certain isolates of STLV-III while other isolates produce disease without neurological involvement. Serial animal-to-animal passage of isolates which produce neurological disease appears to increase the frequene and severity of the neurological signs. The neurological disease is associated with an acute encephalitis. Death usually ocurs within a few weeks after neurological signs appear. Virus has been found in macrophages and multinucleated cells in the brain parenchyma.

Material and Methods

We have several collections of sera from MS patients and matched controls and patients with other neurological diseases collected prior to 1980. These sera and CSF are listed. Sera from 62 MS patients and 62 controls collected from Milwaukee, Wisconsin, prior to 1979 were available. Sera from 45 MS patients, 53 patients with optical neuritis, along with CSF from 24 MS and 31 optical neuritis patients were collected by V.C. at Richmond, VA. Sera from 27 patients with postencephalitis Parkinson's, 26 age-matched controls, and 50 idiopathic Parkinson's were collected by T.E. Sera from 117 patients with other neuromuscular diseases were collected by M.D. All except those from postpoliomyelitis were collected prior to 1980. Sera from 25 AIDS patients and 54 homosexual controls were collected in collaboration with R.D. in Los Angeles in 1982–1983. Sera from 20 cases of TSP and 16 control subjects were collected by G.C.R. in the Seychelles in 1985. All sera from Milwaukee were tested for HTLV-I and HTLV-II, HIV, and STLV-III antibody using an indirect immunofluorescence test. All sera from AIDS and TSP patients were also tested for HTLV-I and HIV using the IFA. All serum and CSF except for AIDS was tested for HTLV-I, HIV, and STLV-III antibody using an ELISA test. Sera from AIDS patients and controls were tested by ELISA for HTLV-III only.

All ELISA tests were performed using commercially available kits. For the HTLV-I ELISA a commercial kit was purchased from Dupont Co., Billary, MA

(manufactured by Biotech Research Lab Inc.). ELISA tests for HTLV-III were performed using a commercially available kit purchased from Organon Teknika Corp, Oklahoma City (Bionetics Lab Products, Charleston, SC). All positives were confirmed by immunofluorescence antibody (IFA). The IFA tests were performed utilizing HTLV-I in Hut 102 cells and HTLV-II and HTLV-III in H9 cells. These antigens were prepared by Electronucleonics Lab Inc., Columbia, MD. The STLV-III IFA antigen was prepared in our laboratory. Positive and negative serum was available to determine the specificity of the reactions.

Results

The results of testing patients with AIDS, TSP, MS, and controls is presented in Table 2. All 25 of the AIDS patients had HTLV-III antibody. In addition, 21 of the 25 patients reacted with the STLV-III antigen. Antibody to HTLV-I was not present in these individuals. None of the controls had antibody to any of the three antigens tested. Antibody to HTLV-I was found in 17 of the 20 individuals with clinical symptoms compatible with TSP. Two of the seven patients with other neurological disease had antibody. One of these patients was diagnosed as having transverse myelitis and one with clinically probable MS. None of the normal controls had HTLV-I antibody. None of the MS patients or control patients from Milwaukee, WI, had antibody to any of the four retrovirus antigens tested.

All 25 AIDS patients had HIV ELISA optical density (OD) readings above the cutoff of 0.39, ranging from 0.85 to 2.0, and were antibody positive. Two of the 54 control samples had an ELISA reading above 0.39 (5.5 and 8.5). Neither of these samples could be confirmed as being positive by IFA or Western blot. Thus, all 54 samples were negative. None of the TSP patients, patients with optic neuritis diseases, or controls had ELISA OD readings above 0.39; thus all were negative for HIV antibody.

The results of the ELISA HIV tests on the samples from MS patients, patients with optic neuritis, controls, and patients with OND are presented in Fig. 1. None of

Table 2. Retrovirus immunofluorescent activity in serum from AIDS patients, TSP patients, and multiple sclerosis patients and controls

	No. of patients tested	HTLV-I	HTLV-II	HIV	STLV-III
AIDS patients	25	0/25	NT	25/25	21[a]/25
Controls	54	0/54	NT	0/54	0/54
TSP patients	20	7/20	NT	0/20	0/20
OND[b]	7	2/7	NT	0/7	0/7
Controls	9	0/9	NT	0/9	0/9
MS patients	62	0/62	0/62	0/62	0/62
Controls	62	0/62	0/62	0/62	0/62

[a] All positive of HIV

[b] PN 1 negative, TM 1 positive, GS 1 negative, PGP 3 negative, and MS 1 positive

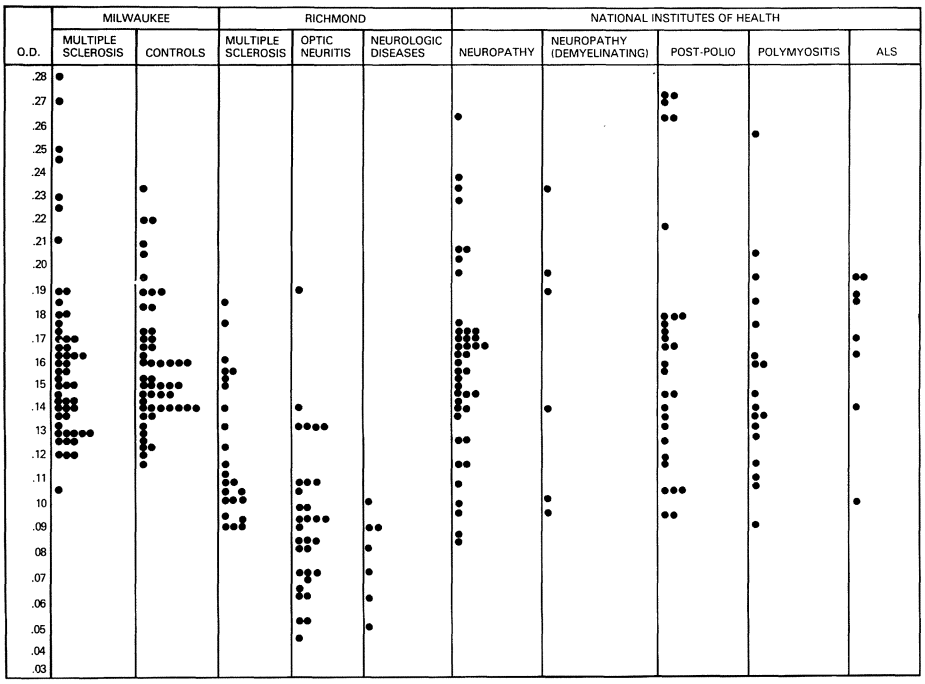

Fig. 1. HIV ELISA reactivity recorded as optical density (OD) on sera from MS patients, matched pal controls, and patients with other neurological diseases. The negative control cutoff was 0.39 and all of these samples were considerably below this level. The results obtained from the patients with Parkinson's disease and aged-matched controls were similar (data not shown). The OD readings on the CSF from multiple sclerosis, optic neuritis, and other neurological disease patients were lower but the distribution among the groups was similar (data not shown)

the OD readings were above the cutoff of 0.39. Analysis of the OD readings obtained did not indicate that the readings were higher in the MS patients as compared with the controls.

Seventeen of the 20 TSP patients had HTLV-I ELISA OD readings above the cut off of 0.36; 2 of the patients with OND had a reading above the cutoff. Three of the patients with clinical TSP, five patients with OND, and the nine controls had ELISA OD readings below the 0.36 cutoff and were considered negative.

The results of the ELISA HTLV-I tests on the samples from MS patients, patients with optical neuritis, controls, and patients with OND are presented in Fig. 2.

None of the OD readings were above the cutoff of 0.36. Analysis of the OD readings obtained did not indicate that the readings were higher in the MS patients as compared with the controls.

Discussion

Retroviruses have been associated with several neurological diseases. HTLV-I has been associated with TSP, which occurs in the lower latitudes and Japan. This

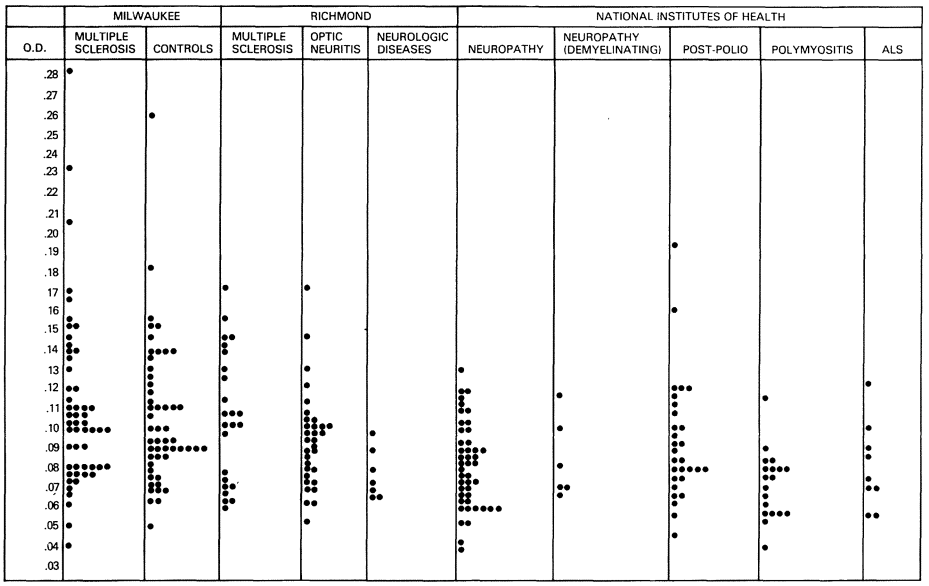

Fig. 2. HTLV-I ELISA reactivity recorded as optical density (OD) on sera from MS patients, matched pal controls, and patients with other neurological diseases. The negative control cutoff was 0.36 and all of these samples were considerably below this level. The results obtained from the patients with Parkinson's disease and aged-matched controls were similar (data not shown). The OD readings on the CSF from multiple sclerosis, optic neuritis, and other neurological disease patients were lower but the distribution among the groups was similar (data not shown)

association needs to be further confirmed by additional serological studies and virus isolation studies. The clinical signs and symptoms of disease resembles progressive multiple sclerosis but the pathological lesions are significantly different. HIV causes a neurological syndrome. This clinical syndrome as well as the pathology differs from MS. In our study on serum samples collected before HIV infection became prevalent we could not demonstrate a serological relationship of the human retrovirus as well as the simian retrovirus STLV-III with MS.

Several explanations for these differences and those reported by Kaprowski et al.[1] are evident. It is possible that some patients might have HTLV-I antibody since Key West is a tropical island on the edge of the TSP belt and have TSP, or an early stage of adult T-cell leukemia. Further, with modern transportation so easy, individuals who have lived or traveled to these HTLV-I regions may develop antibody and may return to the northern latitudes and develop MS independent of their HTLV-I status. Further, it should be expected that MS patients have life-styles that are similar to the general population. Some will develop HIV antibody due to homosexual activity, heterosexual transfer, drug usage, and transfusion. Careful questioning may identify these individuals. Thus, our findings in samples collected prior to 1980 are in agreement with the results reported by Hauser et al. [7] and Karpas et al. [8] and currently demonstrate that the recognized human related retroviruses are not etiologically related to MS.

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