Antibody to Human and Simian Retrovirus, HTLV-I, HTLV-II, HIV, STLV-III, and SRV-I Not Increased in Patients with Multiple Sclerosis

David L. Madden, DVM, PhD,* Francis K. Mundon, MS,† Nancy R. Tzan,* David A. Fuccillo, PhD,‡ Marinos C. Dalakas, MD,* Vincent Calabrese, MD,§ Tenesita S. Elizan, MD,‖ Gustavo C. Román, MD,# and John L. Sever, MD, PhD*

We have tested sera from patients with multiple sclerosis, matched controls, and those with other neurological diseases, as well as sera from patients with the acquired immunodeficiency syndrome and controls and patients with tropical spastic paraparesis (TSP) and controls for antibody to human T-lymphotropic virus type I (HTLV-I), HTLV-II, human immunodeficiency virus (HIV), simian T-lymphotropic virus type III, or simian retrovirus type I by immunofluorescent activity test, and for HTLV-I and HIV by the ELISA method. Sera from patients with multiple sclerosis and matched controls, and from patients with optic neuritis and Parkinson's or other neuromuscular diseases did not have antibody to any of the retroviruses tested. Specimens from TSP patients and some controls contained HTLV-I antibody. We conclude from our study that only TSP patients had serological evidence of infection with one of the retroviruses studied.

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Retroviruses have been associated with neurological disease in humans. Tropical spastic paraparesis (TSP) has been associated with human T-lymphotropic virus type I (HTLV-I), and a subacute encephalopathy and neuropathy has been associated with human immunodeficiency virus (HIV). Recently, Koprowski and colleagues [1] reported that outbreaks of multiple sclerosis (MS) in Key West, Florida, and in Sweden were associated with increased antibody for human retroviruses. In addition, homology studies under nonstringent conditions using cerebrospinal fluid (CSF) cells derived from MS patients reacted with HTLV-I antigen. We report here our studies of retrovirus antibody in a large number of sera and CSF samples collected from MS patients, matched controls, and patients with other neurological diseases before the acquired immunodeficiency syndrome (AIDS) became a serious disease.

Materials and Methods

We have several collections of sera from MS patients and matched controls and patients with other neurological diseases, obtained prior to 1980. Sera from 62 MS patients and 62 controls collected from Milwaukee, Wisconsin, prior to 1979 were available. Sera from 45 MS patients and 53 patients with optic neuritis along with CSF from 24 MS and 31 optic neuritis patients were collected by V. C. at Richmond, Virginia. Sera from 27 patients with postencephalitis Parkinson's disease, 26 age-matched controls, and 50 patients with idiopathic Parkinson's disease were collected by T. E. Sera from 117 patients with other neuromuscular diseases were collected by M. D. All samples except those from postpoliomyelitis patients were collected prior to 1980. Sera from 25 AIDS patients and controls, 25 homosexual individuals with HIV antibody but without clinical signs of disease, and 54 homosexual individuals without HIV antibody or clinical signs of disease were collected in collaboration with Dr Roger Detels in Los Angeles in 1982 through 1983. Sera

From the *National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD; †Electronucleonics Lab Inc., Columbia, MD; ‡Microbiological Associates, Bethesda, MD; §Medical College of Virginia, Richmond, VA; ‖Mount Sinai Medical Center, New York, NY; and #Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX.

Address correspondence to Dr Madden.

Retrovirus Immunofluorescent Activity in Serum from Patients with MS, AIDS, and TSP, and Controls

Study Group	No. of Patients Tested	HTLV-I	HTLV-II	HIV	STLV-III	SRV-I
MS patients	62	0/62	0/62	0/62	0/62	0/62
Controls	62	0/62	0/62	0/62	0/62	0/62
AIDS patients	25	0/25	NT	25/25	21[a]/25	0/25
Controls	25	0/25	NT	25/25	24/25	0/25
Controls	54	0/54	NT	0/54	0/54	0/54
TSP patients	20	17/20	NT	0/20	0/20	0/20
Other neurological diseases[b]	7	2/7	NT	0/7	0/7	0/7
Controls	9	0/9	NT	0/9	0/9	0/9

[a]All positive for HIV.
[b]Included are: peripheral neuropathy—1 negative; transverse myelopathy—1 positive; Guillain-Barré syndrome—1 negative; syphilitic dementia paralytica—3 negative; multiple sclerosis—1 positive.

MS = multiple sclerosis; TSP = tropical spastic paraparesis; HTLV-I (HTLV-II) = human T-lymphotropic virus type I (type II); HIV = human immunodeficiency virus; STLV-III = simian immunodeficiency virus, type III; SRV-I = simian retrovirus, type I; NT = not tested.

from 20 patients with 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, HTLV-II, HIV, simian T-lymphotropic virus type III (STLV-III), and simian retrovirus type I (SRV-I) antibody using an indirect immunofluorescence test. All sera from AIDS patients and controls and TSP patients and controls were tested for HTLV-I, HIV, STLV-III, and SRV-I antibody using the immunofluorescent activity (IFA) test. Sera from all individuals were tested for HTLV-I and HIV antibody using the ELISA test.

The IFA tests were performed using 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 and SRV-I antigens were prepared in our laboratory at the National Institute of Neurological and Communicative Disorders and Stroke, Bethesda. Positive and negative serum was available to determine the specificity of the reactions. All ELISA tests were performed using commercially available kits. For HTLV-I, a kit was purchased from Dupont Co, Billerica, MA (manufactured by Biotech Research Lab Inc); for HTLV-III, a kit from Organon Teknika Corp, Oklahoma City, OK, was used (manufactured by Bionetics Lab Products, Charleston, SC). All positive results were confirmed by IFA.

Results

The results of testing patients with MS, AIDS, TSP, and controls by IFA are presented in the table. None of the MS patients or control patients from Milwaukee had antibody to any of the five retrovirus antigens tested. All 25 of the AIDS patients had HTLV-III antibody. In addition, 21 of the 25 patients reacted with the STLV-III antigen. Twenty-four of the 25 individuals who were HIV-positive but without clinical signs reacted with the STLV-III antigen. Antibody to HTLV-I and SRV-I was not present in these individuals. None of the controls had antibody to any of the four antigens tested. Antibody to HTLV-I was found in 17 of the 20 individuals with clinical symptoms compatible with TSP. Two of the 7 patients with other neurological disease had antibody. In 1 of these patients, transverse myelopathy was diagnosed and 1 had clinically probable MS. None of the normal controls had HTLV-I antibody. None of the patients or controls had antibody to HIV, STLV-III or SRV-I antigens.

None of the patients with MS, optical neuritis, postencephalitis or idiopathic Parkinson's disease or other neuromuscular disease and matched controls had ELISA antibody to HTLV-I or HIV. All AIDS patients and HIV-positive but clinically normal homosexual patients had HIV ELISA optical density (OD) readings above the cutoff of 0.39, ranging from 0.85 to 2.0, and were thus antibody-positive. Two of the 54 control samples had an ELISA reading above 0.39 (0.55 and 0.85). Neither of these samples could be confirmed as being positive by IFA or Western blot. Thus, all 54 control samples were negative. None of the TSP patients, patients with other neurological diseases, or controls had ELISA OD readings above 0.39; thus, all were considered negative for HIV antibody.

None of the ELISA HIV OD readings on the samples from MS patients, patients with optic neuritis, controls, and patients with other neurological disease were above the cutoff of 0.39. An analysis of the OD readings obtained did not indicate that the readings were higher in the MS patients as compared to the controls.

Seventeen of the 20 TSP patients had HTLV-I ELISA OD readings above the cutoff of 0.36; 2 patients with other neurological disease also had readings above the cutoff. Three of the patients with clinical TSP, 5 with other neurological diseases, and the 9 controls had ELISA OD readings below the 0.36

cutoff and were considered negative. None of the HTLV-I ELISA readings on samples from AIDS patients or controls or from MS patients, patients with optic neuritis, controls, and patients with other neurological diseases 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 to the controls.

Discussion

Retroviruses have been associated with neurological disease in patients with AIDS and with TSP. The clinical signs and symptoms of TSP resemble, to some extent, MS. In our study of serum samples collected before HIV infection became prevalent in the United States, we could not demonstrate a serological relationship between the human or simian retroviruses and MS.

Several explanations for the differences of these findings and those reported by Koprowski and colleagues [1] are evident. First, it is possible that some patients in Key West have HTLV-I antibody because it is a tropical island on the edge of the TSP Caribbean belt. Second, modern society being so mobile, individuals who have lived or traveled to HTLV-I-endemic regions may develop antibody, return to more northern latitudes, and later develop MS independent of their HTLV-I status. Further, it should be expected that MS patients have lifestyles that are similar to the general population. Some will develop HIV antibody as a result of homosexual activity, heterosexual transfer, intravenous drug usage, and transfusion. Careful questioning may identify these individuals.

It has been suggested that the type of test used may influence the results. Koprowski [personal communication] believes that there is insufficient antigen on commercial plates to detect the possible cross-reactions with yet unidentified retrovirus. He believes that one must greatly overload the plates with antigen to identify the possible cross-reacting antibodies. Data from the use of commercial kits indicated that such kits are constructed to be overly sensitive; they identify 1 to 3% of false positives in order not to miss any true positives. Our laboratory has had experience in developing many ELISA tests for serological studies. We find it necessary to titrate the antigen and conjugate with positive and negative serum to get the right concentration of each to ensure maximum sensitivity and specificity. Altering one parameter, such as increasing antigen concentration, may increase nonspecificity and false-positive reaction. IFA is useful in confirming HTLV-I antibody and, by use of proper cell control, in detecting nonspecificity.

We conclude from our study that our MS patients did not have serological evidence of retrovirus infection. It is possible for some MS patients to have retrovirus antibody that is not related to MS. During the past 10 years, more than ten different possible agents have been suggested as causes of MS [2]. Among these are a number of recognized viruses, such as measles, canine distemper, scrapie agent, and coronaviruses, and multiple sclerosis–associated agents of unclear classification, the bone marrow agent, and the chimpanzee agent. The serological evidence for etiological association of a retrovirus with MS seems weaker than that available for measles, rubella, and coronavirus.

References

1. Koprowski H, DeFreitas EC, Harper ME, et al. Multiple sclerosis and human T-cell lymphotropic retroviruses. Nature 1985; 318:154–160
2. Sever JL, Madden DL. Viruses that do not cause multiple sclerosis. In: Boese A, ed. Search for the cause of multiple sclerosis and other chronic diseases of the central nervous system. Frankfurt/Main: First International Symposium of Hertie Foundation, 1979:414–424