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Data has been developed indicating that antibodies specific for the AIDS virus are being synthesized within the CNS in response to the infection. About half of the AIDS and ARC patients show this. Abnormal serum bands in the cerebral-spinal fluid of some asymptomatic infected patients has also been found. Investigation of the neurologic aspects of cytomegalovirus retinitis in AIDS patients is being done in collaboration with others.

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MULTIDISCIPLINARY LABORATORY

(To Study Early Markers of HIV Infection)

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Preamble to Annual Report

Our first publication in December 1985 (Resnick L, DiMarzo-Veronese F, Schupbach J, Tourtellotte WW, Ho DD, Muller R, Shapshak P, Vogt M, Groppman JE, Markham PD, Gallo RC: Intra-blood-brain-barrier synthesis of HTLV-III-specific IgG in patients with neurologic symptoms associated with AIDS or AIDS-Related Complex. N Engl J Med 313: 1498-1504, 1985) was the basis for this proposal to the Army DOD. We learned from this pioneering study that the CNS could be infected with or without neurologic symptoms and signs and independent of the disease progression, i.e., seropositive asymptomatic, ARC or AIDS. Accordingly, while we were recruiting to fulfill the requirements of the grant proposal awarded October 1, 1986, we decided to study another group of specimens (blood/CSF obtained from ours and others AIDS Centers around the world). Of the 52 patients studied about half had CNS signs and symptoms (all different than those reported in our 1985 publication). This study was recently published in the January issue (Resnick L, Berger JR, Shapshak P, Tourtellotte WW: Early penetration of the blood-brain-barrier by HIV. Neurology 1988, 38: 9-14.) I have based the Annual Report on this publication since the work was done during grant period year one. Unfortunately, all these patients are lost to follow-up.

Additionally, I was asked to present these data to the Infectious Disease and Neurologic Unit at WRAIR September 17, 1988.

Furthermore, the next day I presented to George E.T. Stebbing, Col. USA, MC, Chairman, Committee entitled "VA/DOD Committee on Continuity of Care and Research (AIDS)" at the Pentagon.

Because I was under the impression that the Annual Report replaced the 4th quarterly report (July 1 to September 30, 1987) we did not send forward a report. I have subsequently learned that it is required, and it is included in this mailing.

The fourth quarter was different from the third quarter. We finished the manuscript which was published in January 1988 Neurology, and we picked up momentum in entering patients into our longitudinal study since we recruited 2 physician scientists, one a board certified neurologist and the other a board certified infectious disease internist who completed a project on AIDS the previous year and hence knew the Los Angeles Gay Community Network.

(1) Statement of the problem under study.

To provide a better understanding of the natural history and pathogenesis of neurologic syndromes related to HIV infection, HIV isolation and antibody studies are being performed from blood and CSF of seropositive patients in various stages of disease development ranging from asymptomatic to ARC to AIDS.

(2) Background and review of appropriate literature.

AND

(3) Rationale.

A wide variety of neurologic diseases occur in association with human immunodeficiency virus (HIV) infection.[1-3] Evidence is accumulating from clinical and laboratory studies that neurologic complications may result from the underlying immune dysfunction caused by HIV or as a consequence of HIV infection of the nervous system.[4-6] Progressive dementia, of unexplained origin, in HIV seropositive patients is probably the most common neurologic sequela, occurring in as many as 60% of patients with AIDS.[7-8] Various unexplained neurologic processes such as vacuolar myelopathy, acute and chronic aseptic meningitis, peripheral neuropathy, and spinal myoclonus are being recognized with increasing frequency in patients who are seropositive for HIV. [9-12]

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These HIV-associated neurologic diseases may appear in the absence of immunologic abnormalities as the primary or only manifestation of the infection.[13]

Previous studies describing the recovery of HIV from the CSF and neurologic tissues have focused largely on patients with neurologic manifestations associated with AIDS and ARC.[14,15] Our research will provide a better understanding of the natural history and pathogenesis of neurologic syndromes related to HIV infection, since HIV isolation and antibody studies are being performed from blood and CSF of seropositive patients in various stages of disease development, ranging from asymptomatic to ARC to AIDS. So far we have studied 52 patients, 29 had various neurologic signs and symptoms, and 23 had no clinically overt neurologic abnormalities.

(4) Experimental methods.

Patients. Lumbar punctures were performed on 52 patients seropositive for HIV by enzyme-linked immunosorbent assay (ELISA) and Western blot. Permission for lumbar puncture was obtained in all patients. Physical examinations and laboratory studies were performed, and all patients were classified according to clinically defined groups.[16] Forty-eight men and 4 women (ages 21 to 59 years; mean, 38 years) were studied, 20 with AIDS, 20 with ARC, and 12 seropositive patients from high-risk groups with no clinical evidence of AIDS or ARC. Twenty-three patients had no clinical evidence of neurologic abnormalities. Twenty-nine patients exhibited clinical evidence of neurologic disease: 24 had unexplained neurologic disease and 5 had neurologic disease accompanied by infection with detected CNS opportunistic pathogens. Fourteen patients had dementia, 3 had myelopathy, 3 had aseptic meningitis, 3 had peripheral neuropathy, 1 had spinal myoclonus, 2 had CNS toxoplasmosis, and 3 had cryptococcal meningitis. All patients had known risk factors for HIV infection: 44 were homosexual and 8 were intravenous drug abusers.

Serum and cerebrospinal fluid samples. Serum and CSF samples were collected simultaneously under sterile conditions and stored at -70°C for the performance of the immunologic procedures for detection of HIV antibodies. For virus isolation studies, CSF specimens were placed at 4°C immediately after lumbar puncture and cultured within 24 hours of collection. Three patients were excluded because their CSF specimens had evidence of blood contamination.

Virus isolation. HIV isolation studies from CSF were routinely performed as follows: 3 to 5 ml of CSF was centrifuged at 600g for 15 minutes and the cell pellet was resuspended in 0.5 to 1.0 ml of CSF. The suspension was cocultivated with 5 x 10^6 Ficoll-Hypaque-separated normal peripheral blood mononuclear cells pretreated with 5 µg/ml phytohemagglutinin (Sigma) and 25 µg/ml DEAE-dextran (Sigma). The cultures were maintained in T25 flasks containing RPMI-1640 medium supplemented with 25% heat-inactivated fetal bovine serum, L-glutamine (2 mM), and 10% interleukin 2 (Cellular Products) at 37°C in a 5% CO_2 atmosphere. Every 3 to 5 days, the medium was changed, viable cells wre determined by the trypan blue exclusion method, and cell density was adjusted to 1 x 10^6/ml. Supernatant fluids were assayed for reverse transcriptase activity twice weekly as described.[17] Reverse transcriptase assays resulting in radioactivity counts above 10,000 cpm/ml of cultured medium with paired background counts under 1,500 cpm/ml were considered positive. The findings of elevated reverse transcriptase activity on two successive determinations in association with cell cytopathic effects and, in four instances, the presence of syncytia (multinucleated giant cells) provided evidence for the presence of HIV in culture.[18] Cultures negative for the presence of HIV were discarded after 10 weeks.

In six specimens, in addition to the routine isolation technique, 3 to 5 ml of CSF was ultracentrifuged at 100,000 g for 1 hour. The pellet was resuspended in 0.5 to 1.0 ml of CSF and the suspension was cultured as described in the routine methods.

Blood cultures to detect HIV were performed as previously described.[17]

Detection and quantitation of HIV-specific IgG in cerebrospinal fluid and serum. HIV ELISA and Western blot analysis were performed as previously described.[19] HIV-specific IgG antibodies in CSF and serum samples were quantitated by an ELISA in which a known quantity of affinity-purified human HIV-specific antibodies was used as a reference. The purification of these antibodies was performed as previously described.[19] The affinity-purified antibodies were serially diluted (in quadruplicate) in HIV antibody-negative CSF or serum and served as standards on the assay plates on which the samples were tested.

Oligoclonal IgG band determination. Oligoclonal IgG bands were detected by isoelectric focusing electrophoresis as described.[20] Oligoclonal igG bands are defined as IgG bands found exclusively or more intensely in CSF than in autologous serum.

Intra-blood-brain-barrier total and HIV-specific IgG synthesis rates. IgG and albumin concentrations were quantified by electroimmunodiffusion,[21] and the intra-blood-brain-barrier total IgG synthesis rate (milligrams per day) was calculated[22] as:

$$\left[\left(IgG_{CSF} - \frac{IgG_{SERUM}}{369}\right) - \left(ALB_{SERUM} - \frac{ALB_{SERUM}}{230}\right) X \left(\frac{IgG_{SERUM}}{ALB_{SERUM}}\right)(0.43)\right] X\ 5,$$

where concentrations are in milligrams per deciliter, ALB denoted albumin; CSF, cerebral spinal fluid; 369 and 230, the average normal serum/CSF ratios for IgG and albumin, respectively; 0.43, the molecular weight ratio of albumin/IgG; and 5, the daily production of CSF in deciliters. Normal intra-blood-brain-barrier total IgG synthesis rate values are less than 3 mg/d. The HIV-specific intra-blood-brain-barrier IgG synthesis rate was calculated as the percentage of total intra-blood-brain-barrier IgG synthesis. Any detectable amount is abnormal.

(5) Results

CSF was obtained for HIV culture from a total of 55 patients. CSF cultured from 10 of 52 patients (19%) yielded findings consistent with HIV infection. Five of 23 (22%) CSF specimens were culture-positive for HIV in the neurologically asymptomatic group, in comparison with 5 of 29 (17%) CSF samples in the neurologically symptomatic group. Simultaneous blood collections for HIV isolation could be obtained from only 10 of the 23 neurologically asymptomatic and 13 of the 29 neurologically symptomatic patients. The incidence of positive HIV blood cultures was approximately the same in both groups (70%, 7 of 10 in the neurologically asymptomatic group and 9 of 13 in the neurologically symptomatic group). HIV was isolated from the CSF in 3 of 8 asymptomatic seropositive patients and in 2 of 4 seropositive patients with unexplained neurologic abnormalities as the only manifestations of HIV infection.

The frequency of HIV isolation from CSF was higher in seropositive patients with no clinical evidence of AIDS, 9 of 32 (28%), in comparison with 1 of 20 (5%) in patients with AIDS ($p < 0.05$). Recovery of HIV from blood cultures was successful in 10 of 15 patients (66%) without AIDS and in 6 of 8 patients (75%) with AIDs. Positive HIV CSF cultures demonstrated elevated reverse transcriptase activity usually *after* 10 days in culture (mean, 18 days), suggesting lower viral quantities than that found in peripheral blood, which exhibited detectable elevations in reverse transcriptase activity usually *within* 10

days of culture.[23] Recoverable HIV from CSF could be propagated in culture. Normal peripheral blood mononuclear cells that were incubated with the supernatant fluid of five HIV culture-positive CSF samples showed the presence of HIV, within 2 weeks of culture, in all cases.

Recovery of HIV from CSF was successful in 1 of 3 patients with myelopathy, 2 of 3 patients with peripheral neuropathy and 2 of 3 patients with aseptic meningitis. HIV could not be recovered from 14 patients with unexplained dementia nor from 5 patients with neurologic disease caused by detectable CNS opportunistic pathogens, cryptococcal meningitis, and cerebral toxoplasmosis. In six instances of AIDS-associated dementia, in addition to the routine isolation technique, attempts were made to increase the yield of isolation by concentrating virus in CSF. Ultracentrifugation of CSF followed by culture yielded no recoverable virus in these six cases. There was no association between the magnitude of CSF white blood cell counts and protein levels and the capacity to isolate virus. HIV was isolated from the CSF of two patients with normal CSF white blood cell counts and normal protein levels.

All 10 patients whose CSF was positive for HIV isolation exhibited evidence of elevated intra-blood-brain-barrier total IgG synthesis (range, 4 to 62 mg/d) and HIV-specific IgG synthesis (range, 1 to 7% of total IgG synthesis). Six of the 10 patients had detectable oligoclonal IgG bands in the CSF. There was no association between the magnitude of intra-blood-brain-barrier total IgG and HIV-specific IgG synthesis rates and the capacity to isolate virus in CSF. Thirty patients with elevations in intra-blood-brain-barrier total IgG (range, 3 to 81 mg/d) or HIV-specific IgG synthesis (range, 1 to 10% of total IgG synthesis) rates had no recoverable virus. In two instances, recovery of HIV from CSF was not associated with recovery from peripheral blood.

All patients exhibited antibodies directed against HIV in serum and CSF by ELISA and Western blot analysis. In all instances, paired serum and CSF samples had the presence of similar antibody reactivities directed against specific HIV antigens by Western blot analysis. All 10 CSF viral isolations were associated with antibodies directed against p24, the major core protein, and gp41, the transmembrane envelope protein of HIV, by Western blot analysis.[19] HIV could not be recovered from the CSF of 23 patients who demonstrated antibody reactiv ity directed against gp41 in the absence of reactivity to p24 by Western blot analysis.

The intra-blood-brain-barrier HIV-specific IgG synthesis rate was elevated in 39 of 52 patients (75%): in 18 of 23 (78%) with no neurologic manifestations and in 21 of 29 (72%) with neurologic abnormalities, 10 of whom had dementia. The frequency of an elevated intra-blood-brain-barrier HIV-specific IgG synthesis rate was significantly higher in the group of seropositive patients without AIDS, 28 of 32 (88%), in contrast with 11 of 20 (55%) in the group of patients with AIDS ($p=0.009$). In addition, a significantly higher frequency of an elevated HIV-specific IgG synthesis rate was seen in association with the presence of antibodies directed against p24 and gp41 in sera and CSF by Western blot analysis: 26 of 29 patients (90%), in comparison with 13 of 23 (57%) in patients with no detectable antibodies directed against p24 ($p=0.007$).

CSF oligoclonal IgG bands were detected in 31 of 52 patients (60%): in 12 of 23 (52%) with no neurologic manifestations and in 10 of 29 (66%) with neurologic abnormalities, 8 of whom had dementia. Oligoclonal IgG bands were detected more frequently in patients with antibodies directed against p24 and gp41 by Western blot analysis: 20 of 29 patients (68%), in comparison with 11 of 23 (48%) in patients with no detectable antibodies directed against p24. Two of 12 patients (16%) without evidence of an elevated intra-blood-brain-barrier HIV-specific IgG synthesis rate had detectable oligoclonal IgG bands in CSF, in contrast with 29 of 40 (72%) with an elevated HIV-specific IgG synthesis rate ($p<0.01$).

(6) Discussion and conclusion.

Our results indicate that infectious HIV can be isolated from the CSF of neurologically asymptomatic patients as well as neurologically symptomatic patients at various stages of infection ranging from asymptomatic to ARC to AIDS. Although other investigators have reported the isolation of HIV from the CSF of patients with ARC and AIDS, this is the first report describing the isolation of HIV from the CSF of asymptomatic seropositive patients. In contrast with our findings, Goudsmit et al[24] tested the CSF of 13 asymptomatic seropositive patients, but were unable to demonstrate HIV antigen by an ELISA.[24] One explanation for the difference in these findings is that early in the course of viral infection, patients have elevated titers of antibodies to HIV core proteins and form immune complexes that may block antigen detection by the ELISA antigen assay (Wade Parks, M.D., Ph.D., University of Miami School of Medicine, Miami, FL). This suggests that in the asymptomatic seropositive patients, virus isolation is a more sensitive method than the ELISA antigen assay for detecting HIV in the CSF.

In our patients, the yield of virus recovery from CSF was significantly greater in seropositive patients without clinical evidence of AIDS, 9 of 32 (28%), than in patients with AIDS, 1 of 20 (5%). Our rate of recovery of virus from the CSF of patients with AIDS is substantially lower than those reported previously. Levy et al[14] studied 14 homosexual patients with AIDS, and reported the isolation of HIV from the CSF of all12 (100%) patients with neurologic disease and in 1 of 2 patients without neurologic disease. Ho et al[15] studied 25 patients with AIDS and reported the isolation of HIV from the CSF in 12 of 18 patients (66%) with neurologic disease, but were unable to recover HIV from 7 patients without neurologic disease.

These differences are difficult to explain. It is unlikely that the low rate of HIV isolation from the CSF of our patients with AIDS is due to methods, since we were able to recover virus from the CSF of seropositive patients without AIDS, and the rate of HIV isolation from peripheral blood was comparable to those reported by other investigators.[25] We cannot exclude differences in the selection of patients and the timing of CSF sampling as possible explanations. Nevertheless, recovery and quantitation of HIV from CSF may prove beneficial as a marker for prognosis and in the monitoring of potential CNS antiviral therapy.

All CSF samples that had recoverable virus were from patients who exhibited serologic evidence of antibodies directed against HIV p24 and gp41 by Western blot analysis. These patients are generally earlier in their viral infection in comparison with patients with an absence of antibodies to p24.[26-28] As infection progresses, the host immune response may restrict the level of HIV replication in the CNS.

Specific antibodies synthesized intra-blood-brain-barrier are a by-product of plasma cells contained within the blood-brain-barrier and reflect the presence and intensity of a CNS immune response. The intra-blood-brain-barrier IgG synthesis rate equation corrects for diffusion of IgG from the serum across the blood-brain-barrier and also for possible damage to the barrier. Therefore, an elevated intra-blood-brain-barrier IgG synthesis rate provides evidence for de novo IgG synthesis within the CNS, and this evidence has been supported by the presence of unique CSF oligoclonal IgG bands.[22]]The presence of oligoclonal IgG bands in association with elevated intra-blood-brain-barrier HIV-specific IgG synthesis rates in the majority of seropositive patients confirms the presence of a local CNS immune response to infection by HIV in all stages of disease development and irrespective of neurologic disease.[19] The presence of an elevated intra-blood-brain-barrier HIV-specific IgG synthesis rate was detected more frequently in seropositive patients without AIDS, and could potentially be utilized to determined the timing of CNS infection by HIV.

Our findings correlate with experimental visna virus infection, in which it is easier to recover virus from the CSF of sheep during early infection whether or not the animal has clinically evident neurologic disease.[29] As time of infection progresses, visna virus

can only occasionally be recovered from the neurologic compartment by cocultivation with CSF cells or neurologic tissue. Examination of the CSF of infected sheep characteristically reveals a mononuclear pleocytosis, mildly elevated protein, and anti-visna antibody can be demonstrated within the CNS.

The data reported here are consistent with the hypothesis that HIV infects the CNS early in the course of viral infection and before the development of AIDS-associated neurologic abnormalities. The virus could penetrate the blood-brain barrier by migration of infected mononuclear cells into the parenchyma or, alternatively, via direct viral CNS invasion.[30,31] The manifestation of neurologic disease may be dependent on multiple factors, such as the duration of HIV infection of the CNS, concomitant infection with other agents, and the immune response within the CNS. Clearly, additional studies must be performed to establish the true incidence of subclinical CNS infection associated with HIV. Prospective studies need to be performed to determine the spectrum of neurologic disease and the immunologic and viral-related factors that may be predictive of impending neurologic disease in HIV-infected patients.

Conclusion: CNS dysfunction occurs frequently in patients with HIV infection. To better define the role of HIV in the pathogenesis of neurologic dysfunction, HIV isolation and antibody studies were investigated from the CSF in 52 seropositive patients, 29 with and 23 without neurologic signs and symptoms, in various stages of disease development ranging from asymptomatic to ARC to AIDS. HIV was recovered from the CSF of 5 of 29 (17%) patients with neurologic signs and symptoms and 5 of 23 (22%) neurologically asymptomatic patients. All patients with positive CSF HIV cultures had antibodies directed against HIV p24 and gp41 in serum and CSF by Western blot analysis and elevated intra-blood-brain-barrier total IgG and HIV-specific IgG synthesis rates. The frequency of CSF HIV isolation from the group of seropositive patients without AIDS, 9 of 32 (28%), exceeded that of patients with AIDS, 1 of 20 (5%) ($p<0.05$). These findings indicate that HIV infects the CNS early in the course of viral infection and prior to the development of HIV-associated neurologic abnormalities.

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