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Evidence of Active Demyelination During Early Stage of HIV-1 Infection

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RESUMO

O sistema nervoso central é considerado um alvo precoce na infecção pelo vírus da imunodeficiência adquirida humana (HIV-1). Este trabalho teve por objetivo determinar por enzima imunoensaio (ELISA) a presença de reatividade imunológica para proteína básica da mielina (MBP) e para a seqüência de aminoácidos 68-84 da MBP exposta somente após degradação parcial ou total da MBP. Foram analisados soro e líquido cefalorraquiano (CSF) de 20 indivíduos HIV positivos, sendo 14 pacientes com o complexo demencial da AIDS (CDC estágio IV) e 6 indivíduos assintomáticos (CDC estágio II). Os grupos controle incluíram pacientes HIV negativos com várias doenças neurológicas como: demência multi-infarto, esclerose múltipla e indivíduos sem qualquer alteração neurológica ou cognitiva. A eletroforese em SDS-PAGE mostrou acentuada produção oligoclonal de imunoglobulinas no CSF dos pacientes com esclerose múltipla e dos indivíduos infectados pelo HIV. Intensa produção intratecal de IgG para o fragmento 68-84 da MBP foi observada de forma consistente, principalmente nos pacientes com ADC e HIV+ assintomáticos. Observou-se um paralelismo entre o grau de comprometimento clínico-neurológico/cognitivo, inclusive o aparecimento de placas de desmielinização com a intensidade da reação imunológica intratecal para os componentes do sistema nervoso central. Evidência de reatividade imunológica intratecal para o epítopo imunodominante 68-84 da mielina durante os estágios iniciais da infecção pelo vírus HIV-1, indica um comprometimento precoce do SNC associado a processo de desmielinização ativa silenciosa.

ABSTRACT

The central nervous system is considered an early target for the human immunodeficiency virus type 1 (HIV-1). Serum and cerebrospinal fluid (CSF) from 20 HIV positive patients, including 14 with AIDS-dementia complex (CDC stage IV) and 6 asymptomatic individuals (CDC stage II) were analysed by enzyme immunoassay for detection of antibodies to native myelin basic protein (MBP) and for the aminoacid sequence 68-84 exposed after partial degradation of native MBP. Control groups included HIV-1 negative patients with degenerative and/or vascular dementia, chronic multiple sclerosis (MS) and individuals without any sign of neurological or cognitive disturbances. Serum and CSF samples from MS and HIV-1 infected patients showed several oligoclonal bands running in the gamma region. AIDS-dementia complex (ADC) patients and HIV-1 infected asymptomatic individuals had increasingly high intrathecal IgG specific antibody titres for the aminoacid sequence 68-84 of MBP. Such alteration consistently paralleled development of neurological disturbances and appearance of CNS demyelinating plaques. Preferential immune recognition of this myelin epitope within the CSF during early stages of HIV-1 infection might point for an ongoing process of active demyelination and ultimately indicate subclinical CNS involvement.

KEY WORDS: AIDS, Demyelination, Intrathecal oligoclonal bands, Autoimmunity

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INTRODUCTION

The human immunodeficiency virus HIV-1, causes a severe transmissible form of immunodeficiency infection, frequently associated with neurological disturbances[1,2,3]. Myelopathy and sensory neuropathy are commonly observed in 20% of AIDS patients whereas dementia affect approximately 10% of patients with progressive disease and constitute a separate group named AIDS-Dementia Complex (ADC). Neurological disturbances could be caused directly by the virus, by an underlying opportunistic infection or as consequence of metabolic CNS dysfunction. Although the primary infection could be entirely asymptomatic in more than half of all HIV-infected individuals, acute mononucleosis-like symptoms and CNS opportunistic infections are usually observed in the majority of AIDS patients. In this sense, ADC should be a diagnosis of exclusion with CNS infections and tumors being excluded by examination of cerebrospinal fluid (CSF) and CT/MRI-scan of the brain[3].

Dissemination of HIV-1 into the brain tissue seems to occur by transmission of the virus itself or HIV-infected monocyte, dendritic, $CD4^+CD26^-$ cells across the blood-brain barrier[2,4,5]. The HIV gene product *tat* and high levels of cytokine (TNFα, TGFβ) modulate expression of adhesion molecules VCAM-1 and E-selectin thus favouring transendothelial migration of infected cells[6]. In addition, *tat* and TNFα also promotes viral replication and due to its neurotoxic effect upon neurons and glial cells[2,7,8] are implicated in producing ample variety of neurological disturbances. Distribution of viral-infected microglia in the basal ganglia and related nuclei resembles those observed in multisystem atrophy and may account for some of the clinical features of AIDS dementia complex. Demyelination associated with leukoencephalopathy in the CNS white matter[1,3], parallel the severity and duration of neurological symptoms. We have recently demonstrated[9] increased intrathecal immunoreactivity for myelin components in patients with multiple sclerosis, a chronic demyelinating CNS disease. The present study aimed to determine, in the serum and CSF samples of HIV-infected individuals, the presence of immune recognition for native MBP and immunodominant myelin-fragment corresponding to the aminoacid sequence 68-84. We observed that immunoreactivity for myelin components paralleled appearance of CNS demyelinating plaques and subsequent development of neurological disturbances in asymptomatic HIV-infected individuals.

MATERIALS AND METHODS

SUBJECTS

Twenty HIV-1 seropositive patients (14 males and 6 females) with mean age of 36,5 years (24 to 54 years) included 10 homosexual, 6 heterosexual, 3 bisexual and 1 heterosexual intravenous drug abuser. Clinical diagnosis was based on guidelines of the Centre for Disease Control (CDC).

Neurological examination consisted of routine physical examination, lumbar puncture, neuropsychological examination and CT/MRI scan of the brain. Cognitive abnormalities were estimated[10] by a global impairment score following application of a neuropsychological "mini mental state" (MMS) test and somatosensory evoked responses. Group 1 included 14 HIV^+ subjects with CNS involvement (neurological and cognitive alterations) and Group 2 had 6 asymptomatic HIV^+ individuals. ADC and HIV-infected patients were under treatment with zidovudine, acyclovir, sulfadiazine, pyrimethamine and/or rifamphicyn. All individuals, independent in self-care and treated in the out-patients, unit gave their informed consent.

HIV-1 negative control groups included six male patients with ages ranging from 30 to 63 with clinical symptons and signs of vascular or degenerative dementia (Group 3). Group 4 included 14 patients (3 males and 11 females) with ages ranging from 17 to 61 years (mean 37.4) with chronic multiple sclerosis, an inflammatory demyelinating disease of the CNS. Group 5 included HIV negative patients (n=12) with various neurological disorders (OND): Guillain-Barré syndrome, hydrocephalia, myelite, amyotrophic lateral sclerosis, neurosyphilis, cerebral trauma and asseptic meningitis. Control sera were collected from (22 to 26) HIV negative healthy blood bank donors without clinical and laboratory alteration. Control CSF samples were collected from 16 HIV negative individuals with herniated intervetebral disc.

VIROLOGICAL AND IMMUNOLOGICAL STUDIES

CSF and serum samples were analysed for the presence of HIV specific antibodies with commercial (Abbot Laboratory, USA) enzyme immunoassay (EIA) and western blot analysis (Pasteur, France). IgM and IgG antibodies to cytomegalovirus and herpes zoster were screened by EIA with commercial tests (Abbot Lab.,USA; Behring Diagnostica, São Paulo). IgM and IgG antibodies to *Toxoplasma gondii* were determined respectively with capture EIA and immunofluorescence assays. Samples were further assayed for VDRL, cryptococcal, fungal and mycobacterial antigenic reactivity. It was also included determination of CSF cellularity and nephelometry for albumin, IgG, IgM and IgA (Behring Diagnóstica, São Paulo).

CSF and serum samples were briefly incubated for 30 min at room temperature with liver extract (Sigma Chemical Co., USA) to avoid any interference in the analysis, due to antigenic cross-reactivity with unrelated antigens[11,12,13]. Protein content was further adjusted to corresponding normal levels with 0.14 M NaCl pH 7.2. Oligoclonal bands were assessed with dodecyl sodium sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a Mini-V 8.10 vertical system (GIBCO BRL, USA) at 200 V constant voltage for 60 min at 4°C using 10% polyacrylamide resolving gel, 4% polyacrylamide stacking gel and TGS (Tris-glycine SDS) running buffer pH 8.3. All reagents were purchased from GIBCO BRL, USA.

ANTIGENS AND REAGENTS

Purified native form of myelin basic protein (MBP) and the aminoacid (aa) sequence 68-84 of myelin corresponding to

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Tyr-Gly-Ser-Leu-Pro-Gln-Lys-Ser-Gln-Arg-Ser-Gln-Asp-Glu-Asn prepared from bovine brain were obtained from Sigma Chem. Co., USA. Goat anti-human IgG peroxidase conjugate and O-phenylenediamine (OPD) were both obtained from Abbott Lab., USA.

ENZYME IMMUNOASSAY (EIA)

Enzyme-linked immunosorbent assay was performed[9] in microplates (Linbro U shaped multiwell, ICN Pharmaceuticals Inc., USA) coated with 10 µg/ml of MBP or the aa sequence 68-84 of MBP dissolved in 0.14 M NaCl. After incubation for 3 hours, plates were washed (3X) and incubated for 60 min at 37 °C with blocking buffer containing 10 µg/ml of defatted milk pH 7.2. Microplates were then washed with buffer and incubated (60 min/ 37°C) with adjusted serum or CSF samples diluted in 0.14 M NaCl according to standard IgG CSF/serum levels. Thereafter microplates were washed with buffer and incubated (30 min/ 37°C) with peroxidase labelled goat anti-human IgG. After subsequent wash (3X) with buffer and incubation for 30 min at room temperature with O-phenylenediamine, absorbance (OD) was measured in a Quantum™ spectrophotometer with 492 nm filter. OD ≥ 0,315 referring to normal value plus 2 SD obtained in clinically healthy (HIV⁻) individuals were considered elevated for MBP and values ≥ 0,185 for the aa sequence 68-84.

STATISTICAL ANALYSIS

Data were evaluated for significance of differences by Student's T-test (two-tailed). Only p values less than 0.05 were considered significant.

RESULTS

CLINICAL FINDINGS

Clinical neurological abnormalities, neuropsychological impairment and/or brain atrophy indicative of CNS disease (Table 1) were evident from the beginning of the study in patients from group 1.

The mean lenght of time that elapsed between AIDS diagnosis and the neuropsychiatric/cognitive symptoms were 24 months (range 0 to 72 months). Patients belonging to this group showed cognitive abnormalities estimated by a global impairment score test battery of the MMS examination with values ranging from 23 to 30. Cerebral toxoplasmosis was evident in 3 patients from Group 1 and two patients from Group 2. Only one patient (Group 1) had cerebral cryptococcosis and none had lymphadenopathy syndrome. At the very beginning of the experiment, it was not observed any significant CNS abnormality in asymptomatic HIV-infected individuals (Group 2). However, during the course of the study, 5 out of 6 patients (83%) progressed into a more severe stage of the disease and developed neurological, cognitive and/or neuropsychological abnormalities.

DETECTION OF IgG ANTIBODY PRODUCTION FOR MYELIN COMPONENTS

Presence of several oligoclonal bands were detected (data not shown) by SDS-PAGE electrophoresis in paired CSF and serum samples of patients belonging to ADC and asymptomatic HIV-1 positive groups (1 and 2) and HIV-1 negative MS patients (Group 4). In contrast, HIV-1 negative patients with degenerative or vascular dementia (Group 3) and CSF control (Group 6) did not show any alteration in the electrophoretic pattern. Indication of local intrathecal IgG anti-myelin antibody production was consistently present not only in patients with multiple sclerosis, a chronic demyelinating CNS disease and also in asymptomatic HIV-1 infected individuals (Group 2). Interestingly, ADC patients belonging to group 1 showed (Table 2) only a slight increase of IgG anti-myelin antibody production in both serum and CSF samples, with CSF/serum ratio lower than Group 2 (CSF values = 0,676) and similar to that found (0,606) in control patients with OND (Group 5). IgG anti-MBP antibody production in normal serum and CSF from HIV-1 negative control individuals (Group 6) was respectively 0,061 (range 0,002-0,077) and 0,049 (range 0,031-0,080).

CSF and serum samples were further analysed for detection of IgG antibodies against the fragment 68-84 of MBP. ADC patients (Group 1) with full blown disease and neurological disturbances showed significantly ($p < 0.001$) high CSF/serum ratio. Foremost, intrathecal IgG specific antibody production was higher than that observed in MS patients (Group 4). Likewise, asymptomatic HIV-1 infected individuals (Group 2) also showed an intense ($p < 0.01$) intrathecal antibody production (Table 3) for this myelin fragment. Indeed, CSF/serum antibody ratio in Group 2 was significantly higher ($p < 0.01$) than in MS patients. The mean of specific serum antibody levels in HIV-1 negative control subjects (Group 6) was 0,054 (0,006-0,093) and for CSF IgG antibody production was 0,063 (0,071-0,090).

It is unlike that CNS infections had caused such a marked intrathecal antibody production for both antigens because, apart from one ADC patient, all the others with cerebral toxoplasmosis or cerebral cryptococcosis showed in the serum and CSF, low antibody levels for MBP and the immunodominant epitope 68-84 of MBP.

DISCUSSION

Demyelination in the brain tissue of AIDS patients with neurological disturbances (Group 1) were diffuse, with CT/MRI scan showing inumerous small areas mainly in the subcortical region. In contrast, asymptomatic HIV-1 infected individuals with CT/MRI scan considered normal, did not have any clinic neurological alteration at the moment of the study. However, soon afterwards, 5 out of 6 patients (Group 2) showing increased intrathecal immunoreactivity for the 68-84 myelin fragment, developed characteristic neurological alterations.

There are reports[14,15] implicating cerebral toxoplasmosis and/or zidovudine treatment in the development of CNS demyelination and dementia in HIV-infected patients. The results

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reported herein suggest that this might not be the case, because all individuals were under treatment with this drug and only one patient with cerebral toxoplasmosis had increased intrathecal antibody production for myelin components. It is well established[11,12,13] that retroviral infection frequently cause T and B cell hyperactivity in response to HIV and unrelated antigens. In this sense, the electrophoretic pattern observed in the serum and CSF samples from all ADC and HIV-1 infected asymptomatic individuals showed a pattern of intrathecal polyclonal B-cell activation. Nonetheless, enzyme immunoassays carried out with these samples did in fact demonstrate that part of such clonal activation was due to specific IgG antibody production for MBP and also for the epitope 68-84. Indication of an ongoing process of active demyelination within the central nervous system in these individuals is supported by the fact that exposition of this MPB epitope occurs after partial or total damage of native molecule due to active CNS myelin breakdown[16,17]. In this regard it should be mentioned that the immunodominant aminoacid sequence 68-84 of MBP and other myelin epitopes[18,19,20] are considered encephalitogenic in various species.

Antibody producing cells isolated from the cerebrospinal fluid of MS patients with a chronic demyelinating disease[16] show preferential recognition for the aminoacid sequence 70-89 of human MBP. Likewise, immune recognition of the 82-102 MBP epitope is probably implicated in the development of clinic-neurological and cognitive alterations[17]. Although some authors[21] failure to demonstrate a relationship between intrathecal IgG production and acute demyelination, increased concentration of MBP in the cerebrospinal fluid of AIDS patients[22], paralleled clinical severity of the disease. Interestingly, we observed that the majority of asymptomatic HIV-1 infected individuals developed neurological/cognitive disturbances soon after detection of high intrathecal IgG antibody production for myelin components.

Dissemination of HIV-1 into the CNS seems to occur shortly after infection, either during the acute seroconversion or at the time of subclinical infection[2]. Therefore, it is possible the existence of a silent CNS involvement preceding the onset of clinical/neurological symptoms in HIV-infected asymptomatic individuals.

During process of active CNS demyelination, activated macrophages produce cytokines (e.g TNF α) and endogenous proteases capable of digest myelin components and cause the release of various fragments. Exposition of different immunodominant MBP epitopes to the local immune system is capable of inducing T and B cell clonal activation. Thus intrathecal production of immunoglobulins and cytokines may account for the maintenance of immunoreactivity for CNS components and probably demyelination[8,9,12,16]. The virus itself might also trigger an autoimmune demyelinating-like illness during the course of disease[23], although an interplay between viral and host factors would predict the development and progression of neurological disturbances[2]. There is increasing evidence[24,25] that, in conjunction with the cytopathic effect of HIV-1, a disturbance of self-nonself recognition is strongly associated with the emergence of autoreactive antibodies and lymphocytes. This would contribute to the wide spectrum of features of HIV disease, ranging from of a long-term asymptomatic infection to rapid progression. In conclusion, this paper shows that intrathecal immunoreactivity for the immunodominant epitope 68-84 of MBP should be considered as an early predictive indication of active CNS demyelination in HIV-1 infected individuals.

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TABLE 1 - Clinic-neurological characteristics of HIV-infected patients.

Neurological Disturbance	Group 1 (n = 14)	Group 2 (n = 6)
Duration of disease*	24.3 ± 19	2.2 ± 1.8
Cerebral toxoplasmosis	3	2
Cerebral cryptococcosis	1	0
Irritability	4	0
Hallucination	10	6
Forgetfulness	4	0
Convulsion	4	0
Chronic headache	9	0
Abdormal MMS°	10	0

° Mini Mental State test.

* Duration of disease in months.

Group 1 included 14 AIDS patients with neurological disturbances (ADC) and group 2 had 6 HIV-1 infected asymptomatic individuals.

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TABLE 2 - Detection of IgG anti-myelin antibody production

IgG anti-MBP	Group 1 n = 10*	Group 2 n = 6	Group 3 n = 6	Group 4 n = 14	Group 5 n=7	Group 6 n = 26**
Serum	0.377 (0.014 - 1.481)	0.260 (0.089 - 0.415)	0.182 (0.040 - 0.356)	0.236 (0.051 - 0.775)	0.366 (0.051 - 0.968)	**0.061** (0.002 - 0.086)
CSF	0.255 (0.018 - 0.483)	0.390 (0.233 - 0.666)	0.068 (0.010 - 0.307)	0.492 (0.011 - 1.777)	0.222 (0.124 - 0.450)	**0.049** (0.031 - 0.080)
CSF/Serum Ratio	0.676	**1.500**	0.374	2.085	0.606	ND

Results are expressed as mean value of absorbance for specific IgG anti-MBP antibody and range in the brackets. *CSF samples from 14 HIV+ patients. ** CSF from 16 HIV negative individuals with herniated intervetebral disc and sera from 26 blood bank donors. ND not done CSF/serum ratio from control group (Group 6).

TABLE 3 - Detection of IgG anti-fragment 68-84 of MBP

IgG anti-Frag. 68-64	Group 1 n = 10*	Group 2 n = 6	Group 3 n = 6	Group 4 n = 14	Group 5 n=7	Group 6 n = 26**
Serum	0.208 (0.028 - 0.645)	0.450 (0.253 - 0.916)	0.116 (0.012 - 0.275)	0.372 (0.068 - 0.801)	0.224 (0.076 - 0.339)	**0.054** (0.006 - 0.093)
CSF	0.843 (0.157 - 1.919)	1.440 (0.716 - 2.000)	0.097 (0.019 - 0.331)	0.959 (0.167 - 2.000)	0.407 (0.191 - 0.574)	**0.063** (0.071 - 0.090)
CSF/Serum Ratio	**3.867**	**3.200**	0.836	2.578	1.816	ND

Results are expressed as mean value of absorbance for specific IgG anti - fragment 68-84 of MBP and range in the brackets. *CSF samples from 14 HIV+ patients. ** CSF from 16 HIV negative individuals with herniated intervetebral disc and sera from 26 blood bank donors. ND not done CSF/serum ratio from control group (Group 6)

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