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Increased cerebrospinal fluid ganglioside GM1 concentrations indicating neuronal involvement in all stages of HIV-1 infection

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Measurements of cerebrospinal fluid (CSF) concentrations of gangliosides can be used as markers of central nervous system (CNS) neuronal involvement. We have analysed the CSF concentrations of the four major brain gangliosides GM1, GD1a, GD1b, and GT1b at different stages of HIV-1 infection. CSF samples were collected from 44 HIV-1-infected patients and from 24 HIV-negative, healthy controls. A significantly higher mean CSF concentration of the ganglioside GM1 was found in HIV-1-infected patients than in HIV-negative controls (27 and 19 nmol/l, respectively, $P < 0.01$). The HIV-infected patients also had a higher mean GM1 proportion of the total ganglioside concentration (11% compared with 8.5%, $P < 0.01$). Nine out of 27 patients with asymptomatic HIV-1 infection, three of ten with AIDS without neurological complications, and three of seven with AIDS dementia complex had CSF GM1 concentrations above the mean+2SD in the HIV-negative control group. Conclusion: Biochemical signs of ongoing neuronal involvement could be found in about one third of HIV-1-infected patients. The same frequency was found regardless of stage, although the highest levels of CSF gangliosides were found in patients with AIDS.

Keywords: HIV-1; gangliosides; GM1; CSF; CNS; AIDS dementia complex

Introduction

Human immunodeficiency virus type 1 (HIV-1) belongs to the group of viruses named lentiviruses, which share the features of neurotropism and neurovirulens in its natural host. Like some other lentiviruses, such as visna virus in sheep, HIV-1 causes a slow, progressive disease of the central nervous system (CNS). In addition, opportunistic infections and tumours in the CNS are common in advanced stages of HIV-1 infection due to the immunodeficiency. Among the CNS complications directly attributable to HIV-1, the most common is AIDS dementia complex, a subcortical dementia characterized by abnormalities in cognition, motor performance, and behaviour (Price and Brew, 1988).

In AIDS dementia complex, the neuropathological abnormalities are predominantly found in the white matter and in subcortical structures, while the cortex is comparatively spared. Similar abnormalities are, however, also fairly often found in nondemented AIDS patients (Geleziunas *et al*, 1992; Navia *et al*, 1986). Neocortical damage with loss of synapses and large cortical neurons accompanied by gliosis, has been found in addition to white matter changes in brains from patients with AIDS dementia complex (Masliah *et al*, 1992; Navia *et al*, 1986; Wiley *et al*, 1991).

The pathogenic mechanisms behind the development of dementia in HIV-1 infections are still largely unknown. Since neuroimaging studies are too insensitive to detect abnormalities in early HIV-1 infection (Dooneief *et al*, 1992), and since autopsy studies are impossible to perform in this group, interest has been paid to find cerebrospinal fluid (CSF) abnormalities reflecting CNS involvement.

Gangliosides are sialic acid-containing glycosphingolipids located in the outer plasma membrane in all human cells. The concentration is highest in the brain and about three times higher in the cerebral cortex than in the cerebral white matter. The total brain concentration is up to 1000 times higher than in any other extra-neural organ (Svennerholm, 1980). Brain gangliosides are shedded into the extracellular space and thus found

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in the CSF. Alterations in the CSF concentrations of gangliosides might be used as markers of degenerative processes affecting neuronal membranes (Blennow *et al*, 1992; Davidsson *et al*, 1991).

We have measured the four major brain gangliosides GM1, GD1a, GD1b, and GT1b in the CSF in asymptomatic HIV-1-infected individuals, in patients with AIDS with and without dementia, and in neurologically healthy HIV-negative controls.

Materials and methods

Patients

The study included 44 HIV-1-infected patients, 27 of whom had an asymptomatic HIV-1 infection, 10 suffered from AIDS without neurological or psychiatric symptoms, and seven had symptoms in accordance with the criteria for AIDS dementia complex (MSK stage 1–3) (American Academy of Neurology AIDS Task Force, 1991; Price and Brew, 1988). Three of the patients with AIDS dementia complex had a history of substance abuse (alcohol in one, cannabis in one, and amphetamine in one), but in none of these could the neurological deterioration be attributed to the drug abuse. Ten patients were on antiretroviral therapy with zidovudine or didanosine with daily doses ranging from 400–600 mg and 300–500 mg, respectively. Thirty-two of the patients had earlier participated in a study where CSF sulfatide concentrations were measured (Gisslén *et al*, 1996).

Twenty-four HIV-negative patients admitted to a surgical unit for minor urological or ortophedic surgery under spinal anaesthesia participated as controls. Individuals with anamnestic or symptomatic signs of neurological or psychiatric disease were excluded, as were those with malignant, vascular, or systemic disorders. Clinical characteristics are presented in Table 1.

Methods

Lumbar punctures were performed in the morning, before breakfast, with the subjects in a lateral recumbent position. In patients with HIV-1 infection, approximately 20–25 ml CSF was collected in portions. Twelve ml CSF was collected from each control. After cell counting, the first 12 ml portion of CSF was centrifuged and stored in smaller fractions at -70°C until analysed. None of the samples analysed had a CSF red cell count above 30×10^6/l. Quantification of gangliosides was performed with a previously described method (Davidsson *et al*, 1991) with minor modifications. Lipids were extracted by adding methanol/chloroform to 0.5 ml CSF to obtain a final ratio of methanol/chloroform/CSF (4 : 8 : 3, by vol.). The extract was desalted by dialysis. Individual determinations of the gangliosides GM1, GD1a, GD1b, and GT1b were performed with an anticholera toxin-B subunit monoclonal antibody after chromatography and sialidase hydrolysis of GD1a, GD1b, and GT1b to GM1 and incubation with a cholera toxin-B subunit on high performance thin-layer plates. The gangliosides were quantified by densometric scanning of plates at 620 nm on a CAMAG TLC Scanner II. The proportions of the individual gangliosides were calculated as the concentration of each ganglioside divided by the total concentration of the four gangliosides. The peripheral $CD4^+$ cell count was measured by direct immunofluorescence on a flow cytometer.

The study was approved by the Research Ethics Committee at Göteborg University, Sweden.

Statistics

Statistical evaluations of comparisons between the different groups were performed by Mann–Whitney-U test.

Results

The total amount of the four major brain gangliosides did not differ significantly in CSF between HIV-1-infected patients and HIV-negative controls. When analysing the gangliosides GM1, GD1a, GD1b, and GT1b separately we found that HIV-1-infected patients had a higher mean concentration of GM1 than HIV-negative controls ($P<0.01$). Compared with controls, the mean proportion of GM1 (percentage of total ganglioside concentration) was higher ($P<0.01$) and the mean proportion of GD1b

Table 1 Age, CD4+ cell count, and number of patients with zidovudine treatment (zdv) and didanosine treatment (ddI) in the study group

		Age		*$CD4^+$ cell count*		*Treatment*	
	n	*mean*	*(range)*	*mean*	*(range)*	*zdv*	*ddI*
HIV-1 infection, all patients	44	38	(22–70)	294	(0–1040)	9	1
Asymptomatic HIV-1 infection	27	38	(22–70)	419	(40–1040)	1	0
AIDS without dementia	10	36	(24–57)	90	(10–400)	3	1
AIDS dementia complex	7	42	(33–59)	104	(0–420)	5	0
HIV-negative controls	24	41	(22–57)				

lower ($P<0.001$) in the HIV-1 group (Table 2). When subgroups of the patient population were compared, the mean concentration of CSF gangliosides was highest in patients with AIDS dementia complex, lower in neurologically healthy AIDS patients, and still lower in asymptomatic HIV-1-infected patients. These differences were not statistically significant, but the same pattern was valid for all four investigated gangliosides. The proportions of the different gangliosides did not differ in this respect (Table 3).

The number of patients in each group with CSF GM1 concentration and proportion above the mean+2SD in healthy controls (30.2 nmol/l and 12.1%) is shown in Figures 1 and 2.

The peripheral $CD4^+$ cell counts are presented in Table 1.

Discussion

Although HIV-1 reaches the CNS at an early stage of the infectious course (Chiodi *et al*, 1988; Ho *et al*, 1985; Sönnerborg *et al*, 1989) whereby a progressive, chronic CNS infection is established (Elovaara *et al*, 1993; Gisslén *et al*, 1994), neurological symptoms rarely occur until several years later. Little is known about how the virus affects different cells in the brain during the asymptomatic period. Gray *et al*, found that neuropathological changes, such as cerebral vasculitis, lymphocytic meningitis, and myelin pallor with reactive astrocytosis, were frequent findings in brains from asymptomatic HIV-1-infected heroine addicts who died from overdoses but not often seen in HIV-negative drug-abusing controls (Gray *et al*, 1992). Neuropathological

Table 2 Cerebrospinal fluid gangliosides, mean ± SD, in HIV-1-positive patients at all stages of infection and in HIV-negative controls

		GM1	*GD1a*	*GD1b*	*GT1b*	*Total*
HIV-1 infected	(nmol/l)	27 ± 14.3[1]	60 ± 19.7	62 ± 19.5	92 ± 24.9	241 ± 68.7
(*n*=44)	(% of total)	11 ± 4.4[1]	25 ± 2.6	26 ± 2.5[2]	39 ± 5.5	100
Controls	(nmol/l)	19 ± 5.6	55 ± 11.9	62 ± 14.3	86 ± 13.6	223 ± 41.9
(*n*=24)	(% of total)	8.5 ± 1.8	25 ± 2.2	28 ± 1.7	39 ± 2.7	100

[1]Significant difference compared with controls, $P<0.01$
[2]Significant difference compared with controls, $P<0.001$

Table 3 Cerebrospinal fluid gangliosides, mean ± SD, in various groups of HIV-1-infected patients

	n	*GM1*		*GD1a*		*GD1b*		*GT1b*		*Total*
		(nmol/l)	*(% of total)*	*(nmol/l)*	*(% of total)*	*(nmol/l)*	*(% of total)*	*(nmol/l)*	*(% of total)*	*(nmol/l)*
Asymptomatic HIV-1 infection	27	25 ± 9.9	11 ± 4.1	56 ± 14.9	25 ± 2.2	58 ± 14.8	26 ± 2.4	86 ± 21.9	38 ± 4.6	225 ± 52.3
AIDS without dementia	10	28 ± 18.9	11 ± 4.6	60 ± 24.1	24 ± 3.1	63 ± 20.9	25 ± 3.2	96 ± 23.2	40 ± 6.5	248 ± 77.8
AIDS dementia complex	7	35 ± 20.0	12 ± 5.6	73 ± 25.5	25 ± 3.2	75 ± 29.0	26 ± 2.2	106 ± 34.1	37 ± 7.3	289 ± 94.5

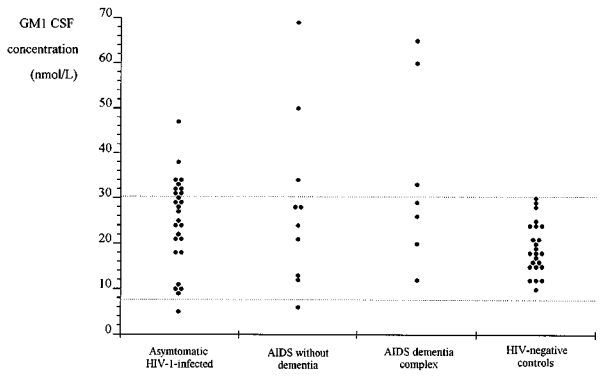

Figure 1 Cerebrospinal fluid concentrations of the ganglioside GM1 in various groups of HIV-1-infected patients and in HIV-negative controls. Mean ± 2SD in HIV-negative controls indicated.

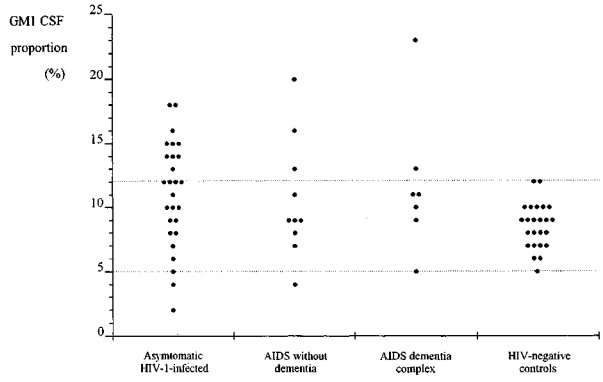

Figure 2 Proportion of the ganglioside GM1 of total cerebrospinal fluid gangliosides in various groups of HIV-1-infected patients and in HIV-negative controls. Mean ± 2SD in HIV-negative controls indicated.

findings at late stages of the disease predominantly consist of subcortical changes, but there is also evidence of diffuse cerebral atrophy with loss of neurons and synapses (Navia *et al*, 1986; Masliah *et al*, 1992; Wiley *et al*, 1991). The correlation between neuropathological changes and neurological impairment is, however, poor (Navia *et al*, 1986; Glass *et al*, 1993).

In our study we found a significantly higher mean CSF concentration and proportion of the ganglioside GM1, in HIV-1-infected patients than in HIV-negative controls. Although not statistically significant, the mean CSF concentrations of all four major brain gangliosides increased with increasing severity of infectious stage. One might expect considerable variations in CNS involvement between individuals since only a minor part of patients with AIDS develop dementia. Thus, it could be assumed that only some patients show biochemical signs of ongoing neuronal involvement. Looking at patients individually could be of greater interest than comparing mean-values between different groups of patients. Approximately one third of HIV-1-infected patients, both asymptomatic and patients with AIDS, have increased CSF concentrations of GM1. High proportions of GM1 and GD1a, and low proportions of GD1b and GT1b, but not appreciable high total concentration of gangliosides in CSF have been found in patients with Alzheimer's disease type I. Vascular dementia, which mainly involves the myelin did not show any increase of gangliosides (Blennow *et al*, 1991, 1992). Increased CSF concentrations of all the major brain gangliosides have recently been found in autistic children (Lekman *et al*, 1995). Patients with polyunsaturated fatty acid lipidosis with an almost complete absence of neurons in the brain have only minor amounts of these gangliosides in CSF (unpublished results). In normal ageing, the total concentrations of gangliosides, in CSF decreases, while the proportion of GM1 increases, with advancing age (Blennow *et al*, 1992). In our study, HIV-1-infected patients and controls did not differ significantly in chronological age.

CSF gangliosides are mainly derived from the CNS (Blennow *et al*, 1991; Davidsson *et al*, 1989, 1991). That CSF ganglioside levels reflect the CNS compartment is supported by the fact that the ganglioside pattern is different in CSF than in serum, where GM3 and GD3 dominates (Håkansson *et al*, 1985; Ledeen and Yu, 1972). Furthermore, no correlation has been found between the major brain gangliosides and blood brain barrier function, measured as albumin ratio (Blennow *et al*, 1991).

Gangliosides, like other glycosphingolipids, are continuously released from the outer plasma membrane into the intercellular space (Doljanski and Kapeller, 1976), which is in direct contact with the CSF. This process, called cell surface shedding, is increased in degenerative processes. The major brain gangliosides are enriched in the grey matter why it is likely to assume that increased CSF levels of gangliosides reflect neuronal involvement. Since the CSF is normally renewed about five times a day, CSF analyses are restricted to recognition of ongoing processes. This might explain why patients with AIDS dementia complex did not have higher CSF ganglioside concentrations than non-demented AIDS patients. The cerebral damages have probably occurred long before the clinical presentation. Thus, the measurement of CSF gangliosides seems not to be a useful marker for established AIDS dementia complex.

The results of this study indicate that the neurones in some patients are affected by a degenerative process already at the asymptomatic stage of HIV-1 infection. Continuous, low-grade, long-term neuronal damage might, besides the more prominent white matter damage, be a part of the pathogenic mechanisms behind neurological impairment in HIV-1 infection.

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