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Intrathecal Synthesis of β_2 -Microglobulin and Lysozyme: Differential Markers of Nervous System Involvement in Patients Infected with Human Immunodeficiency Virus Type 1

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Summary: β_2 -Microglobulin and lysozyme were determined in paired serum and cerebrospinal fluid samples from 137 patients, using immunofluorometry and ELISA, respectively. Of these patients, 54 were infected by human immunodeficiency virus type 1 (HIV1) (including 20 AIDS dementia patients), 73 were HIV1-seronegative with neurological diseases (meningitis ($n = 10$), multiple sclerosis ($n = 29$), other neurological diseases ($n = 34$)) and 10 were controls. Intrathecal synthesis of β_2 -microglobulin occurred in each group. Conversely, lysozyme intrathecal synthesis was found only in meningitis (10/10) and in HIV1-infection (24/54). A pathological increase in β_2 -microglobulin intrathecal synthesis (≥ 2 mg/l) was observed in 45 patients (34 HIV1-infected patients and 11 HIV1-seronegative patients with neurological diseases). Serum concentration and intrathecal synthesis of β_2 -microglobulin were correlated only in the 20 AIDS dementia patients. The cerebrospinal fluid β_2 -microglobulin and lysozyme concentrations were correlated in the 54 HIV1-infected patients only. Blood CD4 + T-cell count was correlated negatively with β_2 -microglobulin intrathecal synthesis but not with lysozyme intrathecal synthesis. These data suggest that in the absence of any central nervous system opportunistic process the increase of β_2 -microglobulin intrathecal synthesis (≥ 2 mg/l) may be a reliable marker of central nervous system involvement in HIV1-infected patients. Intrathecal synthesis of lysozyme was related principally to HIV1-encephalitis and central nervous system opportunistic processes.

Introduction

Encephalitis due to human immunodeficiency virus type 1 (HIV1)-infection (i. e. HIV1-encephalitis or the AIDS-dementia complex) is mainly encountered in the late stages of infection (1). However, recent pathological studies have suggested that early involvement of the central nervous system may frequently occur (2–4). There are very few biological markers in clinical practice which effectively predict, diagnose, and follow neurological involvement in HIV1-infection.

β_2 -Microglobulin is a low molecular mass (M_r 11 800) protein non-covalently bound to class I molecules of the major histocompatibility complex. β_2 -Microglobulin restricts antigen presentation (5) and elevated serum β_2 -microglobulin concentrations appear to be highly predictive of the onset or worsening of HIV1-disease (6–9). β_2 -Microglobulin concentrations in cerebrospinal fluid (CSF) can be elevated in both asymptomatic HIV1-infected patients and AIDS patients (10, 11), suggesting that CSF β_2 -microglobulin concentrations could indicate clinically neurological

involvement. Recently, a strong correlation between CSF β_2 -microglobulin concentrations and AIDS-dementia complex severity was reported (12, 13).

It was shown that β_2 -microglobulin intrathecal synthesis is a physiological event in control subjects, and that its increase is correlated to neurological disorders in HIV1-infected patients. These results suggest that β_2 -microglobulin intrathecal synthesis may predict the spread of HIV1 to the central nervous system.

Patients

The 137 patients investigated were divided into three categories:

- 1) 54 HIV1-infected patients (48 men; 6 women; mean age 37 years) underwent diagnostic lumbar puncture at the Salpêtrière hospital. According to the 1986 Centers for Disease Control classification (14, 15), 5 patients had stage II, 5 had stage III, and 44 had stage IV HIV1-infection. Of the 44 stage IV patients, 15 met a single criterion (A: 4; B: 3; C1: 6; C2: 1 and D: 1) and 29 met between two and four criteria (tab. 1). The 20 patients with stage IV_B had AIDS dementia, of whom 15 (4 cases of cerebral toxoplasmosis and 11 of HIV1-encephalitis) had obvious central neurological involvement;
- 2) 73 HIV1-seronegative patients with neurological diseases, including:
 - i) 29 multiple sclerosis patients (according to Schumacher's criteria; 8 men, 21 women; mean age 44 years),
 - ii) 10 meningitis (8 men, 2 women; mean age 52 years): viral lymphocytic meningitis (n = 8), tuberculosis (n = 1); carcinomatous meningitis (n = 1),
 - iii) 34 other neurological disorders (23 men, 11 women; mean age 54 years): peripheral neuropathies (n = 12); dementias (n = 9); amyotrophic lateral sclerosis (n = 5); strokes (n = 5); myelopathies, (n = 3);
- 3) 10 control patients with no psychiatric or neurological disorders, and with serum and CSF quantities within normal limits (4 men, 6 women; mean age 49 years).

Methods

β_2 -Microglobulin assay

Paired serum and CSF samples were stored at -20°C . Renal function, as assessed by serum creatinine concentrations, was normal in all patients. β_2 -Microglobulin was determined using

the Delfia System β_2 -microTM (Pharmacia, Uppsala, Sweden), a solid-phase time-resolved fluoroimmunoassay based on competition between europium-labelled β_2 -microglobulin and sample β_2 -microglobulin for binding to a monoclonal antibody (16, 17). Linearity was observed between 0.2 and 32 mg/l of β_2 -microglobulin. The detection limit of the assay was 0.1 mg/l, the intra-assay precision less than 3.3%, and the inter-assay precision less than 7.5%.

Lysozyme assay

Lysozyme was determined in serum and CSF using a sandwich-enzyme immuno assay (Eurodiag, Magny en Vexin, France), with an anti-lysozyme monoclonal antibody adsorbed to polystyrene beads, and revealed by an anti-lysozyme polyclonal antiserum labelled with horseradish peroxidase (18). The assay was linear between 10 and 3000 $\mu\text{g/l}$ of lysozyme. The detection limit was 2 $\mu\text{g/l}$. The intra-assay and inter-assay precisions were less than 5% and 9%, respectively.

Albumin, immunoglobulins (A, G, M) and C4 complement component assays

These proteins were determined by electroimmunodiffusion in serum and CSF (19) using agarose alone or combined with hydroxyethylcellulose for IgA and IgM (20), or carboxymethylcellulose for IgG (21).

Assessment of intrathecal synthesis

Intrathecal synthesis of immunoglobulins and C4 complement component was taken as:

$$\left(\begin{array}{c} \text{CSF} \\ \text{concentration} \\ (\text{mg/l}) \end{array} \right) - \left\{ \left(\begin{array}{c} \text{physiological} \\ \text{filtration} \\ (\text{mg/l}) \end{array} \right) + \left(\begin{array}{c} \text{transudation} \\ (\text{mg/l}) \end{array} \right) \right\}$$

Maximal physiological filtration is the upper limit of normal (mean + two standard deviations) for each protein. Transudation was calculated from the normal mean CSF albumin concentration (210 mg/l in our laboratory using an electroimmunodiffusion assay) and the transudation equivalence of each protein in comparison with albumin (22, 23).

Lysozyme intrathecal synthesis can be calculated (in $\mu\text{g/l}$) similarly:

$$\left\{ \begin{array}{c} \text{CSF} \\ \text{lysozyme} \end{array} \right\} - \left\{ 60 + \frac{[(\text{CSF albumin} - 210) (\text{serum lysozyme})]}{28 \times 1000} \right\}$$

Tab. 1. Details of the 29 HIV1-infected patients (stage IV) with 2 to 4 criteria (CDC classification).

Associated stages	Number	Associated stages	Number	Associated stages	Number
A + B	(2)	B + C1	(2)		
A + B + C1	(2)	B + C1 + C2	(1)	C1 + C2	(1)
A + B + C2	(3)	B + C1 + D	(2)	C1 + D	(2)
A + B + C1 + C2	(3)	B + D	(1)		
A + B + C2 + D	(1)			Total	(3)
A + C1	(3)	Total	(6)		
A + C2	(3)				
A + C1 + C2	(3)				
Total	(20)				

where 60 $\mu\text{g/l}$ is the upper limit of the lysozyme concentration in control CSF (mean: $38 \pm 11 \mu\text{g/l}$) and 28 the transudation equivalence of lysozyme, based on its molecular mass.

This calculation method may not be suitable for β_2 -microglobulin intrathecal synthesis. Control serum/CSF ratios were very low, as previously reported (24), suggesting a physiological synthesis. Despite very similar molecular masses (M_r 11 800 for β_2 -microglobulin and 14 000 for lysozyme), the respective serum/CSF ratios in the controls were different (1.6 for β_2 -microglobulin and 50 for lysozyme) (tab. 2). Extrapolation between the normal serum/CSF ratio for albumin (M_r 63 000), which is 200, and the corresponding ratio for lysozyme, which is 50, gives an expected ratio of 40 for β_2 -microglobulin, which differs from that observed. Thus, if the CSF albumin is $\leq 210 \text{ mg/l}$, the β_2 -microglobulin intrathecal synthesis (mg/l) can be taken as:

$$\left(\text{CSF } \beta_2\text{-microglobulin} \right) - \left(\frac{\text{serum } \beta_2\text{-microglobulin}}{40} \right)$$

If plasma filtration is increased (CSF albumin $> 210 \text{ mg/l}$), the previous ratio must be multiplied by the ratio: patient's CSF albumin/210, taking into account the β_2 -microglobulin increase by plasma transudation. Since the molecular masses of β_2 -microglobulin (M_r 11 800) and lysozyme (M_r 14 000) are low compared with albumin (M_r 63 000), the results of both formulae were compared with the protein/CSF albumin ratio for each protein, and with the index calculated for IgG (25).

Peripheral blood CD4 + T-cell count

The absolute number of CD4 + T-cells in peripheral blood was determined by flow cytometry, in the HIV1-infected patients only.

Statistical analysis

Group comparisons were made using the U-test of Mann & Whitney, the χ^2 test, and the Spearman's rank correlation.

Results

β_2 -Microglobulin and lysozyme concentrations in serum and CSF

The mean serum and CSF β_2 -microglobulin and lysozyme concentrations in the patient groups are summarized in table 2. The mean serum β_2 -microglobulin concentrations in the HIV1-infected patients was significantly higher than in HIV1-seronegative patients with neurological diseases or in the controls. Similar differences were observed in CSF, and there was also a significant difference between the AIDS dementia and other HIV1-infected patients. There was no correlation between serum and CSF β_2 -microglobulin concentrations in the controls, multiple sclerosis, meningitis, or the 34 HIV1-infected patients without neurological disorders. In contrast, there was a highly significant correlation ($p < 0.001$) in the 20 AIDS dementia patients.

The mean serum lysozyme concentration in the HIV1-infected patients did not differ significantly from that of the HIV1-seronegative patients with neurological diseases, or from that of the controls. CSF lysozyme concentrations were significantly higher in both subgroups of HIV1-infected patients than in the control group.

Using the CSF β_2 -microglobulin/CSF lysozyme ratio proposed previously (26), we found a significant difference ($p < 0.01$) between both the meningitis patients (mean 17.8 ± 1.7) and the HIV1-infected patients (mean 58.5 ± 8.4) and the controls (mean 28.8 ± 3).

Tab. 2. Serum and cerebrospinal fluid β_2 -microglobulin and lysozyme concentrations (mean $\pm$ standard error of the mean) in the 3 patient groups.

Patients	n	CSF albumin serum albumin $\times 1000^*$	β_2 -Microglobulin (mg/l)		Lysozyme ($\mu\text{g/l}$)	
			Serum	CSF	Serum	CSF
HIV1-infected patients	54	8.10 ± 0.58	3.18 ± 0.31	3.0 ± 0.24	1658 ± 141	140 ± 30
without neurological disorders	34	7.58 ± 0.51	2.89 ± 0.24	2.56 ± 0.27	1541 ± 112	124 ± 35
with AIDS dementia	20	8.98 ± 1.31	3.69 ± 0.74	3.75 ± 0.41	1860 ± 333	169 ± 52
HIV1-seronegative patients with neurological diseases	73	8.15 ± 1.00	1.71 ± 0.08	1.60 ± 0.12	1560 ± 70	80 ± 10
Controls	10	4.40 ± 0.29	1.65 ± 0.14	1.04 ± 0.10	1915 ± 190	38 ± 4

Statistics (means were compared with Mann & Whitney's U-test):

54 HIV1 vs 10 Controls	$p < 0.001$	$p < 0.001$	$p < 0.001$	NS	$p < 0.001$
54 HIV1 vs 73 HIV1-seronegative	NS**	$p < 0.001$	$p < 0.001$	NS	NS
20 AIDS dementia vs 34 other HIV1	NS	NS	$p < 0.02$	NS	NS

* Blood-brain barrier index; normal ≤ 6.0

** Not significant

Intrathecal synthesis of β_2 -microglobulin and lysozyme

β_2 -Microglobulin intrathecal synthesis was observed in both the control group and all 127 other patients (tab. 3). Considering the standard deviation of β_2 -microglobulin intrathecal synthesis values in the controls (0.3 mg/l) and the pattern of distribution, it seems reasonable to consider that values ≥ 2 mg/l (more than 3 standard deviations above the normal mean) indicate a pathological increase. Such an increase was observed in 45 patients (34 HIV1-infected and 11 HIV1-seronegative patients with neurological diseases). The highest values were observed in HIV1-infected patients with AIDS dementia (tab. 3). Among these 45 patients, the increase in β_2 -microglobulin intrathecal synthesis was highly significant ($\chi^2 = 23.7$; $p < 0.001$) in the 34 HIV1-infected patients compared with the 11 HIV1-seronegative patients. Using this assessment, the sensitivity of this marker in AIDS dementia was 63% and the specificity 85%, with a positive predictive value of 79% and a negative predictive value of 76%. Finally, the ratio K (positive predictive value/1-negative predictive value) was 3.3, clearly illustrating the relative risk of neurological disorders for a HIV1-infected patient with a pathological increase of β_2 -microglobulin intrathecal synthesis.

Lysozyme intrathecal synthesis was much rarer, occurring in HIV1-infected patients and showing no difference between the 20 AIDS dementia and the 34 HIV1-infected patients without neurological disorders; it also occurred in 19 HIV1-seronegative patients with neurological diseases, of which 10 were infectious meningitis. In contrast to β_2 -microglobulin intrathecal synthesis, lysozyme intrathecal synthesis was not detected in control CSF.

Correlations of intrathecal synthesis of β_2 -microglobulin and lysozyme in the 54 HIV1-infected patients

In the 34 HIV1-infected patients with a pathological increase in β_2 -microglobulin intrathecal synthesis (≥ 2 mg/l), there was a correlation between β_2 -microglobulin and lysozyme intrathecal synthesis but no correlation with intrathecal synthesis of immunoglobulins (G, A, M) and complement C4. Conversely, in the 24 HIV1-infected patients with a lysozyme intrathecal synthesis, a significant correlation with IgG and IgM intrathecal synthesis ($p < 0.01$ and $p < 0.001$, respectively) was observed, but not with IgA or complement C4 intrathecal synthesis.

The mean blood CD4 + T-cell count was $183 \pm 23.7 \times 10^6/l$ in the 54 HIV1-infected patients without a significant difference between the means for the 20 AIDS dementia and the other 34 HIV1-infected patients. A negative correlation ($p < 0.02$) was found between the CD4 + T-cell count and serum β_2 -microglobulin concentration, but this correlation was better ($p < 0.001$) with CSF β_2 -microglobulin or β_2 -microglobulin intrathecal synthesis (fig. 1). Such correlations were not observed with serum lysozyme, CSF lysozyme or lysozyme intrathecal synthesis.

AIDS dementia and β_2 -microglobulin intrathecal synthesis in HIV1-infected patients

As previously demonstrated, CSF β_2 -microglobulin and β_2 -microglobulin intrathecal synthesis were significantly higher in the 20 AIDS dementia patients than in the other 34 HIV1-infected patients. The highest mean β_2 -microglobulin intrathecal synthesis (4.5 ± 0.5 mg/l) was observed in the 11 patients with HIV1-encephalitis.

Tab. 3. β_2 -Microglobulin and lysozyme intrathecal synthesis (mean $\pm$ standard error of the mean) in the 3 patient groups.

Patients	(n)	Intrathecal synthesis	
		β_2 -Microglobulin (mg/l)	Lysozyme (μ g/l)
HIV1-infected patients	54	2.91 ± 0.22	206.3 ± 61.4 (24)*
without neurological disorders	34	2.49 ± 0.27	209.2 ± 47.6 (13)
with AIDS dementia	20	3.63 ± 0.41	202.8 ± 60.2 (11)
HIV1-seronegative patients with neurological diseases	73	1.53 ± 0.12	100.4 ± 21.8 (11)
Controls	10	1.00 ± 0.10	0 (0)

* Means calculated in patients with intrathecal synthesis of lysozyme only: the number of patients is indicated in brackets. Intrathecal syntheses of β_2 -microglobulin were compared between the different groups with the U-test of Mann & Whitney.

54 HIV1 vs 10 Controls

$p < 0.001$

54 HIV1 vs 73 HIV1-seronegative

$p < 0.001$

20 AIDS dementia vs 34 other HIV1

$p < 0.01$

Fig. 1. Correlation between CD4 + T-cell count and
 a) serum β_2 -microglobulin ($Rho = -0.34$, $p < 0.02$)
 or
 b) β_2 -microglobulin intrathecal synthesis
 $Rho = -0.50$, $p < 0.001$)
 in 54 HIV1-infected patients

Discussion

Although β_2 -microglobulin and lysozyme have similar molecular masses and serum concentrations, their CSF concentrations are completely different in the controls and in the HIV1-infected patients. This raises two questions:

- i) the origin of β_2 -microglobulin in the CSF of normal subjects;
- ii) the significance and prognostic value of intrathecal β_2 -microglobulin and lysozyme synthesis in HIV1-infected patients, compared with the HIV1-seronegative patients with neurological diseases.

As we previously demonstrated, β_2 -microglobulin intrathecal synthesis is a physiological phenomenon, present in both controls and all the patients with neurological diseases. In contrast, lysozyme intrathecal synthesis appeared to be a pathological phenomenon related to an opportunistic process of the central nervous system, since it was not observed in the controls and rarely in the patients with neurological dis-

eases. β_2 -Microglobulin is found on the surface of lymphocytes in two molecular forms; the most abundant is associated with HLA class I antigen heavy chains, and the second is unbound to other membrane molecules (27). It was recently demonstrated that this free β_2 -microglobulin, in excess, promotes functional peptide association with purified soluble HLA class I molecules (28). Of the estimated 100 mg β_2 -microglobulin shed per day into the plasma, about half originates from lymphocytes (29). From our data the daily intrathecal production of β_2 -microglobulin is about 0.5 mg, given a daily CSF production of 500 ml.

During degradation of HLA heavy chains, the associated β_2 -microglobulin may be shed into the cerebral extracellular fluid. β_2 -Microglobulin is also expressed on the surface of glial cells (30), macrophages and endothelial cells (31). Whether or not the normal central nervous system expresses HLA class I antigens remains controversial, but the existence of physiological β_2 -microglobulin intrathecal synthesis is, in our opinion, in favour of such expression. The normal brain is equipped to respond to immune stimulation, although low levels of lymphocyte traffic (estimated, from CSF cytology in normal subjects, as 500 000 cells/day) probably occur. The precise origin of β_2 -microglobulin intrathecal synthesis requires further investigation.

In pathological events, stimulation of immune cells (in particular lymphocytes and macrophages) and the activity of specific cytokines (such as γ -interferon) may contribute to increasing β_2 -microglobulin concentrations via an enhancement of HLA class I expression on target cells. We suggest, like *Mc Arthur et al.*, that HIV1 replication within the brain and the accompanying inflammatory response increase the expression of class I molecules and account for the release of β_2 -microglobulin. In demented patients, there may be greater class I expression than in non-demented subjects, leading to the presence of elevated CSF β_2 -microglobulin (32). Inhibition of mitogenic responsiveness of peripheral blood mononuclear cells to phytohaemagglutinin, concanavalin A and pokeweed mitogen was recently demonstrated using monoclonal antibodies to human β_2 -microglobulin (33). Shedding of β_2 -microglobulin from cells is enhanced during proliferation and differentiation. Moreover, it was recently shown that β_2 -microglobulin possesses chemotactic activity for lymphoid cells and that it is identical to thymotaxin (34), which may be involved in migration of bone marrow cells into the central nervous system. Increased β_2 -microglobulin intrathecal synthesis might reflect a gradually increased lymphocyte mass and/or lymphocyte activity within the central nervous system. An alternative explanation is

that β_2 -microglobulin is released from necrotic tissue or proliferating glial cells. Such an increase was observed 10 years ago in patients with herpes encephalitis, mumps, and mononucleosis (26). Despite the fact that β_2 -microglobulin readily passes fenestrated blood vessels (eg. glomerular barrier or choroid plexus), such a mechanism cannot explain why the CSF β_2 -microglobulin was higher than the serum β_2 -microglobulin in 22 HIV1-infected patients. Such intrathecal synthesis was never observed with CSF lysozyme, and the major source of lysozyme is the lysosomes of polymorphonuclear leukocytes and macrophages. Assessment of lysozyme intrathecal synthesis (like complement C4 intrathecal synthesis) may offer a means of evaluating functional macrophage activity (35). IgG and IgM intrathecal synthesis are indeed linked to lysozyme intrathecal synthesis, but not to β_2 -microglobulin intrathecal synthesis. In other words, lysozyme intrathecal synthesis seems to be a marker of inflammation related to the HIV1 infection itself and the associated neurological opportunistic infections like toxoplasmosis, while any pathological (≥ 2 mg/l) increase of β_2 -microglobulin intrathecal synthesis pos-

sibly reflects cell death and hyperactivation of lymphoid cells, glial cells and astrocytes. This is supported by the significant difference between patients with AIDS dementia and other HIV1-infected patients without neurological disorders, and the correlation with the decrease of the CD4 + T-cell count. All these data suggest that in the absence of central nervous system opportunistic infections or neoplasms, a high replication within the brain and the accompanying inflammatory response of β_2 -microglobulin intrathecal synthesis greater than 2 mg/l have a good positive predictive value for the diagnosis of HIV1 dementia. Also, intrathecal synthesis of lysozyme may be a reliable predictor of HIV1 encephalitis and central nervous system opportunistic processes.

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