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Soluble HLA Class I and Class II Molecule Levels in Serum and Cerebrospinal Fluid of Multiple Sclerosis Patients

Gilberto Filaci, Paola Contini, Sabrina Brenci, Paola Gazzola, Lorella Lanza, Marco Scudeletti, Francesco Indiveri, Giovanni Luigi Mancardi, and Francesco Puppo

ABSTRACT: Increased concentrations of soluble HLA class I and class II molecules (sHLA-I and sHLA-II) have been observed in infectious, inflammatory, and autoimmune diseases. Because autoimmune mechanisms are considered to play a role in the pathogenesis of multiple sclerosis (MS), we decided to dose sHLA-I and sHLA-II in serum and cerebrospinal fluid (CSF) of MS patients comparing their concentrations with those observed in serum and CSF of patients with other neurologic diseases (OND) without evidence of neuroradiologic involvement of central nervous system (CNS) and in serum of healthy donors. The serum concentrations of sHLA-I were higher in both MS and OND patients than in healthy donors ($P < 0.05$) whereas sHLA-II serum concentrations were lower in MS patients than in both OND patients and healthy donors ($P < 0.01$). Detectable amounts of sHLA-II were observed in the CSF of 45% of MS patients and in CSF of only 6% of OND patients ($P < 0.001$). In MS patients a significant correlation between sHLA-I serum and CSF concentrations was observed ($P < 0.01$), whereas sHLA-II serum and CSF levels did not correlate. In conclusion, alterations of sHLA-I and sHLA-II serum and CSF concentrations are present in MS patients and could be involved in the induction of enhanced susceptibility to develop MS or in MS pathogenesis. *Human Immunology 54, 54–62 (1997).* © American Society for Histocompatibility and Immunogenetics, 1997.

ABBREVIATIONS

sHLA-I	soluble HLA class I molecules
sHLA-II	soluble HLA class II molecules
MS	multiple sclerosis
OND	other neurological diseases
CNS	central nervous system
MBP	myelin basic protein
MOG	myelin oligodendrocyte glycoprotein
PLP	proteolipid protein
MAG	myelin associated glycoprotein
CSF	cerebrospinal fluid
Ig	immunoglobulin
MoAb	monoclonal antibody
DDIA	double determinant immunoassay
PBS	phosphate-buffered solution
BSA	bovin serum albumin
OPD	ortho-phenylenediamine
OD	optical density
BBB	blood brain barrier
HI-I	sHLA-I index
MRI	magnetic resonance imaging
EDSS	expanded disability status scale

INTRODUCTION

Soluble HLA class I and class II molecules (sHLA-I and sHLA-II, respectively) circulate in the serum of healthy subjects [1, 2]. The mechanisms leading to the presence of sHLA-I and sHLA-II in serum are not completely understood. It is possible that they are both shed from the membrane of damaged or lysed cells and secreted from activated immune cells. In fact, the serum levels of sHLA-I and sHLA-II increase during acute rejection episodes following organ transplant [3–6] and acute graft-versus-host disease following bone marrow transplantation [6, 7], as well as during phenomena involving immune activation, like infectious [6, 8] and autoim-

From the Department of Internal Medicine (G.F., P.C., S.B., L.L., M.S., F.I., F.P.) and the Department of Neurological Sciences and Neurorehabilitation (G.L.M., P.G.), University of Genoa, Genoa, Italy.

Address reprint requests to: Professor Francesco Puppo, Dipartimento di Medicina Interna (DIMI), Universita' di Genova, viale Benedetto XV n.6, 16132 - Genova Italy.

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mune diseases [9]. Furthermore, it has been reported that sHLA-I and sHLA-II may play an immunomodulatory role, mainly by downregulating activated immune cells [10].

Autoimmunity certainly plays a major role in the pathogenesis of Multiple Sclerosis (MS), a demyelinating disease of the central nervous system (CNS). Supports to this assessment come from genetic, histologic, and immunologic observations. In particular, there are evidences that the DR2 haplotype DRB1*1501-DQA1*0102-DQB1*0602, is commonly associated to MS in Caucasian subjects [11]. Moreover, both perivascular cuffing around CNS venules and infiltrating T lymphocytes, plasmacells, and mononuclear cells are found in acute MS lesions [12]. Finally, T lymphocytes and autoantibodies reactive to myelin basic protein (MBP) and other myelin proteins such as myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), or myelin associated glycoprotein (MAG) can be isolated from blood and cerebrospinal fluid (CSF) of MS patients [13–23], although autoreactive MBP-specific T cells and autoantibodies to MBP are also detected in the blood and CSF of normal donors [24]. Thus, autoantigen presentation by specific HLA molecules to autoreactive T lymphocytes appears as a key factor in the pathogenesis of the disease. Therefore, abnormalities in serum concentrations of sHLA-I and sHLA-II are expected in MS patients. Moreover, because the detectability of sHLA-I molecules in the CSF has been demonstrated [25], it could be of interest to investigate the presence and the biologic behavior of sHLA-I and sHLA-II molecules in the CSF of MS patients. However, to the best of our present knowledge, no information exists on the level of sHLA-I and sHLA-II in serum and CSF of MS patients. The aim of this study was to quantitate the concentrations of sHLA-I and sHLA-II molecules in the sera and CSF of MS patients and to compare their levels with those observed in normal control subjects and in patients affected by other neurologic diseases (OND).

MATERIALS AND METHODS

Sera and Cerebrospinal Fluids

Sera and CSFs were collected from 46 MS patients and frozen at −80°C until utilized. Patients' sex, age, and clinical features of disease are summarized in Table 1. All the MS subjects had a definite diagnosis of relapsing-remitting MS according to the criteria of Poser et al. [26]. In 35 of 46 MS patients sera and CSF were collected within 30 days from the onset of a clinical relapse, defined as the appearance of a new symptom or the worsening of an old one, accompanied by an appropriate neurologic abnormality lasting at least 24 h in the absence of fever, and preceded by a period of stability or improvement of at least 30 days. All MS patients did not assume steroids or immunosuppressive drugs.

CSF analysis showed an increased immunoglobulin (Ig) G index in 80% of cases and the presence of oligoclonal bands at isoelectrofocusing in 95% of cases. Sera and CSFs of 29 OND patients (Table 2) affected by diseases such as epilepsy, headache, psychiatric disorders, without clinical, laboratory (CSF examination including IgG index), and neuroradiologic (computerized tomography) evidences of organic involvement of CNS, were also collected and frozen as specified. The sera of 20 healthy subjects were used as controls.

Antibodies

The monoclonal antibody (MoAb) LGII-612.14, directed to a nonpolymorphic determinant of the β chain of HLA class II antigens [27], the MoAb Q5/13, recognizing a nonpolymorphic determinant of HLA class II antigens [28], and the MoAb NAMB-1 to β_2-microglobulin [29] were a kind gift of Dr. S. Ferrone (New York Medical College, Valhalla, NY). The monoclonal antibody W6/32 to a framework determinant of β_2-microglobulin associated HLA class I α-chain [30] was a kind gift from Dr. C. Russo (Cornell University, New York, NY). Peroxidase-conjugated sheep anti-mouse Ig antibodies were purchased from Amersham (Buckinghamshire, England).

Determination of sHLA-I and sHLA-II Molecules in Serum and Cerebrospinal Fluid

The levels of sHLA-I and sHLA-II in serum and CSF were determined by previously described double determinant immunoassays (DDIA) [31, 6], with minor modifications, using W6/32 and biotin-conjugated NAMB-1 MoAbs or LGII-612.14 and biotin-conjugated Q5/13 MoAbs, respectively.

Calculation of sHLA-I Index

sHLA-I and sHLA-II present in the CSF might be synthetized within the CNS or could come from blood through the blood brain barrier (BBB). To discriminate between these two possibilities we calculated an sHLA index as described [25]. By this calculation, the ratio between the concentration of sHLA molecules in CSF and serum is related to a variable (the CSF albumin/serum albumin ratio) that reflects the BBB status. The calculation was performed only for sHLA-I because CSF concentrations of sHLA-II in OND patients was below the detectability limit of our assay in all but 2 subjects. The sHLA-I index (HI-I) was calculated by the following formula:

TABLE 1 Clinical and laboratory data of MS patients

Patient no.	Initials	Sex[a]	Age (years)	Clinical course	EDSS[b]	serum sHLA-I (μg/ml)	serum sHLA-II (μg/ml)	CSF sHLA-I (μg/ml)	CSF sHLA-II (μg/ml)
1	T.R.	M	41	Relapse	3	0.68	0.04	0.02	0.01
2	D.A.M.	F	42	Relapse	3	7.46	0.06	0.05	0.06
3	G.C.	F	28	Relapse	3	6.2	0.05	0.09	0.01
4	G.G.	F	39	Relapse	2	1.48	0.04	0.07	0
5	T.R.	M	14	Relapse	4	1.1	0.01	0.02	0
6	P.S.	M	59	Stable	3.5	0.75	0.03	0.06	0.01
7	T.L.	M	29	Stable	3.5	5.46	0.4	0.09	0
8	F.M.F.	F	33	Relapse	1.5	1.12	0.03	0.01	0
9	C.E.	M	48	Stable	5.5	4.65	0.06	0.08	0
10	P.A.	M	69	Relapse	4	1.71	0.07	0.04	0
11	R.G.	M	34	Relapse	2	3.86	0.05	0.11	0.01
12	R.A.	F	45	Relapse	3	1.04	0.01	0.01	0.06
13	S.M.	F	52	Stable	3	1.77	0.01	0.01	0.01
14	P.M.	F	16	Relapse	3.5	3.64	1.04	0.03	0
15	S.A.	F	27	Relapse	3.5	6.64	0.01	0.06	0
16	M.G.	F	25	Relapse	3.5	0.44	0.11	0.01	0.07
17	N.S.	F	22	Relapse	4	0.6	0.98	0.03	0
18	D.C.	F	41	Stable	3.5	0.49	0.01	0.02	0.01
19	C.M.	F	37	Relapse	3.5	1.11	0.06	0.01	0
20	S.L.	F	30	Relapse	2	1.01	0	0.03	0
21	B.L.	F	46	Relapse	3.5	5.42	0.02	0.03	0.03
22	A.A.	F	54	Relapse	6	1.5	0.07	0.04	0.01
23	Z.M.	F	33	Relapse	3.5	0.5	3.84	0.01	0
24	M.C.	F	25	Relapse	3	2.58	0.01	0.01	0
25	R.T.	F	38	Relapse	2	0.96	0.04	0.01	0
26	D.P.	F	35	Relapse	3	0.61	0.13	0.02	0
27	T.P.	M	25	Relapse	3.5	2.6	0.01	0.01	0
28	M.A.	M	21	Relapse	2	1.56	0.02	0.02	0
29	B.M.	M	21	Stable	2	4.25	0.01	0.07	0.01
30	P.S.	M	28	Relapse	3	7.58	0.01	0.03	0
31	C.B.	M	39	Relapse	1	1.87	0.01	0.03	0.04
32	G.N.	F	24	Relapse	3	0.5	4.7	0.01	0.01
33	M.S.	F	26	Relapse	3	0.71	1.91	0.02	0.05
34	C.M.T.	F	46	Stable	1.5	3.6	0.01	0.23	0
35	M.R.	M	61	Relapse	3.5	2.24	2.73	0.05	0.02
36	Z.R.	F	52	Stable	5	3.18	0.14	0.05	0.01
37	B.E.	F	17	Relapse	2.5	1.42	0.01	0.01	0.01
38	M.M.	F	28	Relapse	1.5	0.97	0.15	0.01	0.02
39	S.M.	M	27	Relapse	2	1.14	0.01	0.01	0.01
40	B.G.	M	54	Stable	4	1.43	1.9	0.03	0
41	M.V.	M	35	Relapse	2	2.03	0.16	0.01	0
42	G.L.	F	42	Relapse	2	3.2	0.09	0.08	0
43	M.M.	F	48	Relapse	1.5	1.15	0.01	0.01	0.05
44	G.R.	F	16	Relapse	5	3.4	0.01	0.02	0
45	B.A.	M	21	Relapse	2	1.49	0.05	0.01	0
46	P.V.	F	55	Relapse	3.5	1.81	0.02	0.02	0

[a] Sex: M = male, F = female.

[b] EDSS: expanded disability status score.

$$\text{HI-I} = (\text{CSF sHLA-I/serum sHLA-I})/(\text{CSF albumin/serum albumin}).$$

The upper normal limit of the HI-I was considered as the mean of the sHLA indexes of OND patients plus 2 standard deviations and values exceeding this limit were considered as indicative of an intrathecal synthesis of sHLA-I molecules.

Statistical Analysis

sHLA-I and sHLA-II concentrations were expressed as μg/ml (mean ± SD). Comparisons among groups were

TABLE 2 Clinical and laboratory data of OND patients

Patient no.	Initials	Sex[a]	Age (years)	Diagnosis	Serum sHLA-I (μg/ml)	Serum sHLA-II (μg/ml)	CSF sHLA-I (μg/ml)	CSF sHLA-II (μg/ml)
1	L.M.C.	F	38	Epilepsy	0.82	0.29	0.014	0
2	D.L.	F	59	Facial palsy	7.55	0.37	0.016	0
3	B.L.	F	63	TIA[b]	2.19	0.32	0.03	0
4	M.C.	F	29	Epilepsy	0.73	0.34	0.009	0
5	U.L.	M	52	Facial Palsy	3.52	0.26	0.035	0
6	B.P.	M	41	Epilepsy	0.85	0.32	0.011	0.07
7	T.S.	F	26	Muscle dystrophy	1.61	0.27	0.021	0
8	F.E.	F	75	Coma	3.86	0.33	0.03	0
9	V.G.	M	78	Epilepsy	0.72	0.31	0.013	0
10	G.S.	M	40	Parestesias	1.84	0.3	0.02	0
11	C.A.	F	82	Epilepsy	1.13	0.31	0.029	0
12	C.A.	F	64	Headache	0.71	0.29	0.024	0
13	C.G.	F	50	SAH[c]	2.96	0.28	0.019	0
14	O.M.A.	F	81	Confusion	2.45	0.27	0.035	0
15	D.A.	F	32	Polyalgias	2.9	0.21	0.041	0
16	E.M.C.	F	32	Catatonia	1.16	0.23	0.02	0
17	R.L.	F	80	Headache	1.5	0.19	0.02	0
18	F.O.	F	31	Headache	5.71	0.19	0.095	0
19	G.A.	F	83	Catatonia	1.11	1.52	0.047	0
20	S.S.	F	25	Headache	1.23	0.27	0.02	0
21	S.A.	M	68	Epilepsy	4.03	0.18	0.032	0
22	M.L.	F	71	Confusion	6.28	0.16	0.05	0
23	S.R.	M	49	Epilepsy	1.64	0.19	0.04	0
24	U.F.	F	71	Facial palsy	1.43	0.18	0.138	0
25	C.A.	F	67	Lipotimia	1.73	0.24	0.027	0
26	C.L.	F	65	Sciatica	1.97	0.11	0.02	0
27	M.S.	F	63	SAH	2.65	11.74	0.026	0.07
28	N.D.	F	30	Headache	2.4	0.07	0.028	0
29	I.R.	F	43	Epilepsy	3.08	0.05	0.038	0

[a] Sex: M = male, F = female.

[b] TIA: Transient ischemic attack.

[c] SAH: Subarachnoid hemorrhage.

performed by analysis of variance (ANOVA) procedure. Comparison between percentages of frequency was performed by Fisher exact test. Correlations among variables were calculated by Spearman correlation coefficient. A two tailed P value < 0.05 was considered as statistically significant.

RESULTS

Serum sHLA-I and sHLA-II Concentrations in Healthy Subjects, in Multiple Sclerosis and Other Neurologic Disease Patients

The serum concentrations of sHLA-I were significantly increased in both MS (2.3 ± 1.9 μg/ml) and OND (2.4 ± 1.7 μg/ml) patients with respect to healthy subjects (1.4 ± 1.1 μg/ml) ($P < 0.05$ and $P < 0.05$, respectively) (Fig. 1A). The serum sHLA-I levels were comparable in MS patients during a relapse (2.1 ± 1.8 μg/ml) and in MS patients with stable clinical course (2.8 ± 1.7 μg/ml) (Fig. 1B). Both values were significantly higher than that of healthy subjects ($P < 0.01$).

The serum concentration of sHLA-II in MS patients (0.4 ± 1.0 μg/ml) was significantly lower than that in both OND patients (0.6 ± 1.7 μg/ml) ($P < 0.01$) and healthy subjects (0.7 ± 0.7 μg/ml) ($P < 0.01$) (Fig. 1C). No statistically significant differences in sHLA-II serum concentrations were observed between MS patients during a relapse (0.4 ± 1.1 μg/ml) and those in a stable phase of the disease (0.2 ± 0.6 μg/ml) (Fig. 1D). The serum concentrations of sHLA-II in each MS patient subpopulation were significantly lower than those in OND patients and healthy subjects ($P < 0.001$).

CSF sHLA-I and sHLA-II Concentrations in Multiple Sclerosis and Other Neurologic Disease Patients

The CSF concentration of sHLA-I molecules was comparable in MS (0.03 ± 0.03 μg/ml) and OND patients (0.03 ± 0.02 μg/ml) (Fig. 2A). Nevertheless, the CSF concentration of sHLA-I was significantly increased in MS patients with stable disease (0.07 ± 0.06 μg/ml) with respect to both MS patients with relapse (0.029 ±

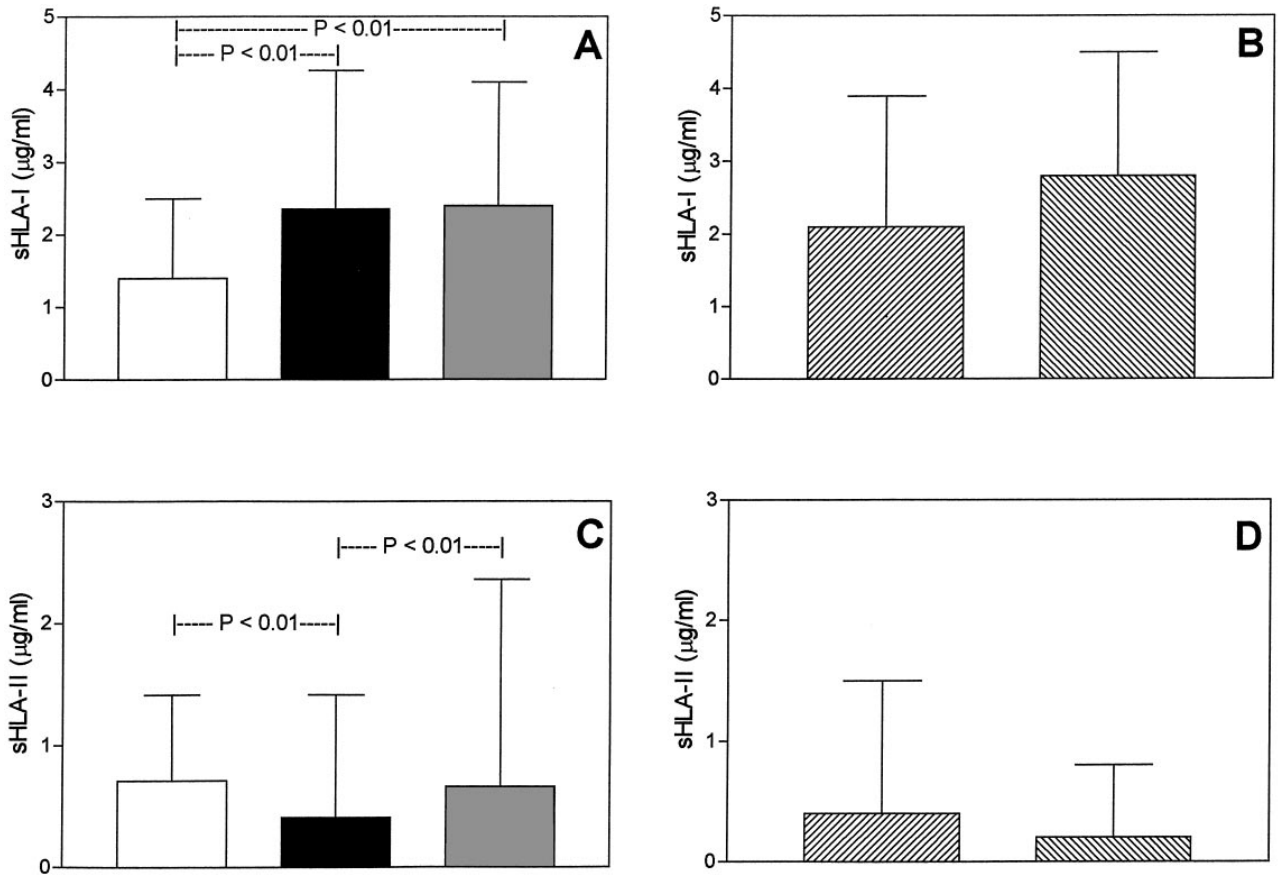


FIGURE 1 Concentrations of sHLA-I and sHLA-II in serum of healthy donors □, MS ■, and OND ▨ patients (**panels A and C**) and in serum of MS patients with a relapse ▨ or stable disease ▧ (**panels B and D**).

0.02 μg/ml) ($P < 0.05$) (Fig. 2B) and OND patients ($P < 0.05$).

Detectable concentrations (0.025 ± 0.02 μg/ml) of sHLA-II molecules were found in the CSF of 21 of 46 (45%) MS patients and in CSF of only 2 of 29 (6%) OND patients (0.07 μg/ml). The difference between the percentages of frequency in the two populations was statistically significant ($P < 0.001$) (Fig. 3). Multiple sclerosis patients with a relapse showed higher concentration of CSF sHLA-II molecules (0.027 ± 0.02 μg/ml) than MS patients with stable disease (0.01 ± 0.0004 μg/ml), although the difference was not statistically significant ($P = 0.15$).

HI-I in Other Neurologic Disease and Multiple Sclerosis Patients

The HI-I of OND and MS patients were 3.35 ± 1.74 μg/ml and 4.05 ± 3.49 μg/ml, respectively. Because the latter value was lower than the upper normal limit of HI-I (6.85 μg/ml) we assumed that sHLA-I present in the CSF of MS patients were not intrathecally produced but came from peripheral blood.

Correlation Between the Concentrations of sHLA-I and sHLA-II in Serum and CSF of Multiple Sclerosis Patients

A statistically significant correlation was observed in MS patients between serum and CSF sHLA-I concentrations ($P < 0.01$) (Fig. 4A), whereas no correlation was detected between serum and CSF sHLA-II concentrations (Fig. 4B).

DISCUSSION

In the present study we have compared the concentrations of sHLA-I and sHLA-II in serum and CSF of MS patients with those in serum of healthy subjects and in serum and CSF of OND patients. The main results we have obtained are as follows: a) sHLA-I serum concentrations were significantly higher in MS patients than in healthy subjects but were comparable in sera and CSF of MS and OND patients; b) sHLA-II serum concentrations were significantly lower in MS patients than in both healthy subjects and OND patients, whereas detectable amounts of sHLA-II were found in

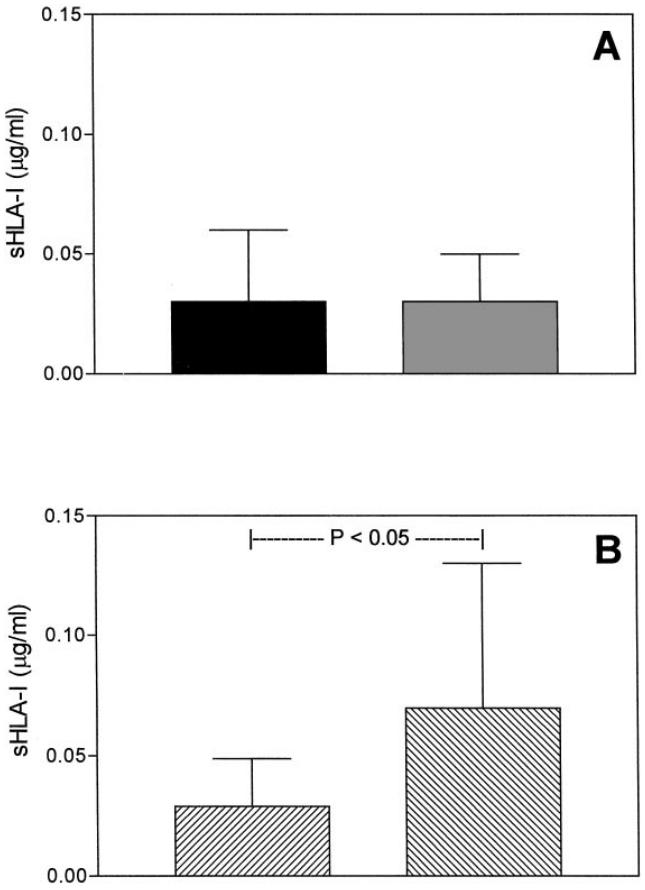


FIGURE 2 Concentrations of sHLA-I in CSF of MS ■ and OND ▨ patients (**panel A**) and in CSF of MS patients with a relapse ▨ or stable disease ▧ (**panel B**).

CSF of 45% MS patients and in CSF of only 6% OND patients; c) a statistically significant correlation was observed in MS patients between serum and CSF sHLA-I concentrations; and d) CSF sHLA-I concentrations were significantly lower whereas CSF sHLA-II concentrations were higher (although not significantly) in MS patients with relapse than in MS patients with stable disease.

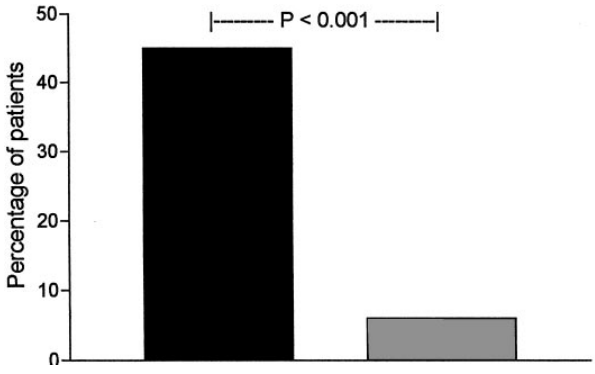
Autoimmune phenomena are considered to play a major role in the pathogenesis of MS. Both CD4+ and CD8+ lymphocytes reactive to antigens present in the CNS have been demonstrated in blood and CSF of MS patients [13–21]. Recently, cross-reactivity of MBP specific CD4+ T-cell clones with structural antigens of human Coronavirus 229E have been described [31], thus supporting the hypothesis that exogenous factors (i.e., viral antigens) in genetically predisposed individuals might induce autoimmunity by antigen-mimicking. In particular, autoantigen presentation and HLA restriction phenomena could be primarily involved in predisposing a genetic background to MS development. The elevated rate of concordance of MS manifestation in monozygotic twins (25%) [32] and the frequent association between MS and the DR2 haplotype DRB1*1501-DQA1*0102-DQB1*0602 [11] furtherly underline the pathogenetic role of the HLA haplotype in MS. Moreover, it is known that high or low serum levels of sHLA-I and sHLA-II may associate to particular HLA allospecificities [1, 33].

On these bases, the variations of serum sHLA-I and sHLA-II concentrations in MS patients, reported in this study, could acquire pathogenetic relevance. The results of recent studies suggest that sHLA-I and sHLA-II molecules may exert immunosuppressive activities on CD8+ and CD4+ lymphocyte functions, respectively [34, 35, 10]. The elevation of sHLA-I and the decrease of sHLA-II in MS sera prospect a potential scenario in which the coexistence of downmodulated CD8+ T-cell function (because of increased sHLA-I level) with poorly controlled CD4+ T-cell function (because of reduced sHLA-II concentrations) could predispose to the occurrence of chronic viral infections and long-term antigen release, thus favoring the selection of T-cell clones that are cross-reactive to self antigenic structures.

Alternatively, the observed alteration of sHLA molecule homeostasis could be just an epiphenomenon of the underlying autoimmune phenomena. In this view, the increased sHLA-I serum levels might be merely the consequence of the inflammatory status and might be due to either shedding from dead cells or secretion from activated cells. However, the decrease of serum sHLA-II levels is difficult to be simply interpreted as a secondary manifestation because a concordant increase of both sHLA-I and sHLA-II concentrations was detected in the serum of all infectious or inflammatory diseases studied until now [6].

The meaning of the elevation of serum sHLA-I levels in OND patients is difficult to explain because in these patients neither significant laboratory signs of peripheral or CNS inflammation were observed nor CNS lesions were detected at neuroradiologic examination. Our OND

FIGURE 3 Percentages of patients with MS ■ or OND ▨ with detectable sHLA-II in the CSF.



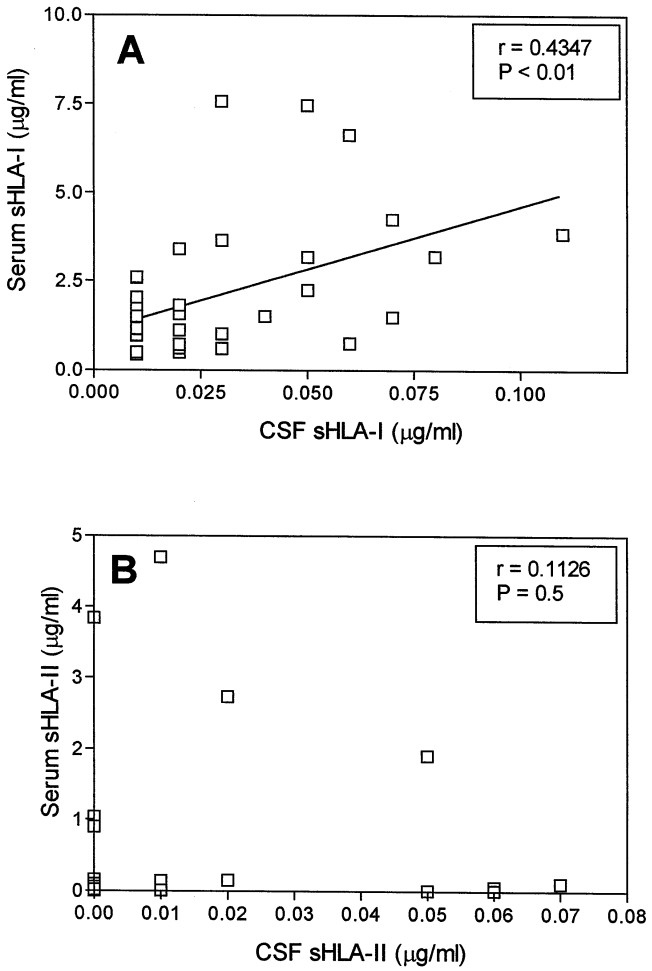


FIGURE 4 Correlations between serum and CSF concentrations of sHLA-I (**panel A**) and sHLA-II (**panel B**) in MS patients.

cases were showing clinical symptoms and signs of involvement of peripheral or central nervous system (i.e., facial palsy, epilepsy, and sciatica) and it may be hypothesized that in these cases an unspecific activation of the immune system could occur.

Comparable concentrations of sHLA-I molecules were found in the CSF of MS and OND patients. The existence in MS patients of a significant correlation between serum and CSF sHLA-I concentrations and the fact that the HI-I was below the HI-I upper normal limit support the hypothesis that in these patients sHLA-I concentrations found in the CSF come from blood through the BBB. Interestingly, significantly lower sHLA-I concentrations were detected in the CSF of MS patients with a relapse than in those with stable disease, thus indicating that intra-CNS variations of sHLA-I concentrations can be associated with different clinical phases of the disease.

sHLA-II molecules were undetectable in the CSF of the majority (94%) of OND patients, whereas they were detectable in almost the half (45%) of the MS patients, mainly in MS patients with flared disease. Thus, the presence of sHLA-II in the CSF of MS patients appears as a disease-related phenomenon. The fact that the highest sHLA-II concentrations were found in the CSF of MS patients with relapse perhaps relates the release of these molecules with phenomena (and phases) of local immune activation. Unfortunately, the limited volume of CSF available from each patient does not allow the purification of sufficient amounts of sHLA-II to test their effect on activated T lymphocytes. Because in MS patients no correlation was found between CSF and serum sHLA-II levels, it is likely that CSF sHLA-II molecules are produced within the CNS. They could be shed from HLA class II positive cells (microglia cells) dying in the CNS or they could be secreted by activated immune cells. It must be considered that simple clinical observation does not give us reliable information on the activity of the disease and the magnetic resonance imaging (MRI) with contrast enhancement with gadolinium often shows an active disorder with disruption of the BBB also during the apparently stable clinical phase of the disease. In fact, serial MRI with gadolinium is almost tenfold more sensitive than clinical examination to detect an ongoing disease activity [36] and therefore future efforts should be directed to correlate the amount of serum and CSF sHLA-I and sHLA-II, together with that of other soluble mediators of inflammation, to the MRI signs of disease activity. At the moment, therefore, it is not possible to affirm whether the absence of significant amounts of sHLA-II in CSF of about half of MS patients studied is related to different phases of the disease or is the expression of different forms of MS.

In conclusion, the results of our study show the existence of an imbalance between sHLA-I and sHLA-II concentrations in the serum and CSF of MS patients. Whether these alterations may play a pathogenetic role and whether their detection could have a diagnostic relevance in MS deserve further investigation.

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