Cerebrospinal fluid amounts of HLA-G in dimeric form are strongly associated to patients with MRI inactive multiple sclerosis

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ABSTRACT

Background-The relevance of HLA-G in dimeric form in MS is still unknown.

Objective-To investigate the contribution of CSF HLA-G dimers in MS pathogenesis.

Methods-CSF amounts of 78kDa HLA-G dimers were measured by western blot analysis in 80 MS relapsing-remitting MS (RRMS) patients and in 81 inflammatory and 70 non-inflammatory controls.

Results-CSF amounts of 78kDa HLA-G dimers were more frequent in RRMS than in inflammatory ($p<0.01$) and non-inflammatory controls ($p<0.001$) and in MRI inactive than in MRI active RRMS ($p<0.00001$).

Conclusion-Our findings suggest that HLA-G dimers may be implicated in termination of inflammatory response occurring in MS.

INTRODUCTION

Human Leukocyte Antigen-G (HLA-G) are immunonologically functional non-classical HLA-I proteins which have recently been indicated as potential immunomodulatory molecules in Multiple Sclerosis (MS). In fact, an overexpression of HLA-G and its inhibitory receptors (ILT-2 and ILT-4) was found in MS plaques and on cerebrospinal fluid (CSF) monocytes from MS patients.[1] In addition, high levels of intrathecally produced soluble HLA-G (sHLA-G) were observed in CSF of MS patients with magnetic resonance imaging (MRI) inactive disease,[2,3] in whom they were correlated positively with CSF concentrations of anti-inflammatory IL-10[2] and inversely with CSF concentrations of anti-apoptotic sFas molecules.[4] These findings argue for a tolerogenic role of sHLA-G in MS, favouring the remission of the disease. In this setting, the immunosuppressive function could be exerted by actively secreted HLA-G5 that was the preponderant sHLA-G isoform detectable in CSF of MS patients without evidence of MRI activity unlike sHLA-G1, the other most important functionally active sHLA-G isoform released after shedding of membrane-bound HLA-G1 from cell surface.[5] However, this hypothesis remains to be demonstrated since no data are currently available on the presence of HLA-G disulfide-linked dimers in CSF of MS patients. Indeed, HLA-G in dimeric form

exhibits higher affinity for ILT2 and ILT4 receptors and promotes a more efficient inhibitory receptor signaling compared to monomers[6,7] and, therefore, it is considered as the biologically active HLA-G form with the strongest immunosuppressive effects.[8] Based on these considerations, in this study we sought to investigate the distribution of CSF HLA-G dimers in MS and controls.

MATERIALS AND METHODS

CSF samples were prospectively collected in 80 consecutive newly diagnosed[9] definite relapsing-remitting (RRMS) patients (55 women and 25 men; mean age=38.7±10.9 years) and 151 age and sex matched neurological controls represented by 81 patients (55 women and 26 men; mean age=38.5±9.9 years) with other inflammatory neurological disorders (OIND) and 70 subjects (49 women and 21 men; mean age=39.9±10.3 years) with other non-inflammatory neurological disorders (NIND) (Supplementary Table), followed by the MS Centre of Ferrara (Italy) during the period from July 2010 to October 2014. All patients were imaged with a 1.5-Tesla MRI unit within 48 hours after sampling. MS patients were considered as clinically and MRI disease active if they had evidence of a relapse at admission and lesions showing Gd-enhancement on T1-weighted scans, respectively. Median Expanded Disability Status Scale was 2.0 (interquartile range=1.0-3.5; mean=2.1±1.5). Median duration of the disease was 7.0 years (interquartile range=1.0-39; mean=28±38.3). None of the patients had fever or other symptoms or signs of acute infections and had received any potential disease-modifying therapies during the 6 months before the study. None of the female MS patients was pregnant. All OIND and NIND patients were free of immunosuppressant drugs, including steroids. Informed consent was given by all patients before inclusion and the study design was approved by the Local Committee for Medical Ethics in Research. As previously reported,[2-5] CSF levels of sHLA-G were measured by enzyme-linked immunosorbent assay using the monoclonal antibody MEM-G/9 as capture antibody (Supplementary Material). After biotynilation and immunoprecipitation of CSF samples, CSF HLA-G monomers and dimers were identified by western blot analysis under reducing and non-reducing conditions in all sHLA-G positive RRMS, OIND and

NIND patients as described elsewhere[10] (Supplementary Material). After checking data for normality by using the Kolmogorov-Smirnov test, continuous variables were compared using Kruskal-Wallis and Mann-Whitney U test, categorical variables were compared by means of Chi-square test. Bonferroni correction was utilized for multiple comparisons. A value of $p<0.05$ was assumed as statistically significant.

RESULTS

Detectable CSF levels of sHLA-G were more frequent ($p<0.00001$;Chi-square) in RRMS (58/80;72.5%) than in OIND (23/81;28.4%) and in NIND (12/70; 17.1%) patients. As shown in the Table, CSF concentrations of sHLA-G were different among RRMS and controls ($p<0.00001$;Kruskal-Wallis) since they were higher in RRMS than in OIND and NIND ($p<0.00001$) and equivalent between OIND and NIND. We standardized the Western Blot analysis on cell culture supernatants and cell lysates from 721.221 HLA-G1 transfected cells (Supplementary Figure). In sHLA-G positive patients, while a 39kDa monomeric band was present in 100% of RRMS and controls, a 78kDa dimeric HLA-G band was more represented in RRMS than in OIND ($p<0.001$) and NIND ($p<0.01$). When RRMS patients were stratified according to clinical and MRI activity, CSF concentrations of sHLA-G and CSF monomers and dimers distribution did not differ between clinically active and clinically stable RRMS. Conversely, CSF concentrations of sHLA-G were more elevated ($p<0.001$) in RRMS patients without than in those with Gd enhancing lesions, whereas CSF HLA-G dimers were more frequent in MRI inactive than in MRI active sHLA-G positive RRMS ($p<0.00001$). An additional HLA-G like band with a molecular weight of 53 kDa was detected, under reducing condition, in 6 clinically and MRI active RMMS, in 4 OIND and 3 NIND sHLA-G positive patients (Figure).

DISCUSSION

In this study, we confirmed previous data[2-5] showing that CSF levels of sHLA-G were higher in MS than in controls and predominated in MS patients without MRI evidence of active disease. These results further strengthen the possibility that sHLA-G can be implicated in immunomodulation of CNS inflammatory response operating in MS. However, whether sHLA-G molecules detected at intrathecal level in MS are functionally active is still to be proven. Therefore, the main finding of our investigation was the demonstration, for the first time, that HLA-G in dimeric form was present in CSF of MS patients and its distribution reflected the fluctuations of sHLA-G antigens because HLA-G dimers were more frequent in MS than in controls and in MRI inactive than in MRI active MS. In fact, the unique characteristic of both membrane-bound and soluble isoforms of HLA-G is the ability to form disulfide-linked dimers which are created through the generation of disulfide bonds between two cysteine residues at position 42 of the HLA-G alpha-1 domain. As these structures interacts with high affinity with HLA-G specific receptors and, thus, are believed to represent the biologically active form of HLA-G generating immunosuppressive effects.[6-8] These data strongly support the involvement of HLA-G in MS autoimmunity as anti-inflammatory molecules mediating the termination of inflammation. Intriguingly, elevated amounts of a 53 kDa HLA-G like protein were found in CSF of a small proportion of clinically and MRI active RRMS patients and, with a lesser extent, OIND and NIND patients. However, the actual biological significance of this molecule is currently elusive.[10] Future studies are warranted to elucidate the actual significance of CSF HLA-G dimers in MS, with particular attention to the choice of control subjects which, in our study, were slightly more heterogeneous than recently recommended.[11] In fact, although the search of a potential biomarker for MS was beyond the scope of the current investigation, this limitation could affect the consistency of our data. On the other hand, further efforts are required to obtain quantitative measurements of CSF HLA-G dimers in MS. In this way, a recently proposed densitometry analysis of chemoluminescent signals[8] could represent a reliable option.

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CONFLICT OF INTEREST STATEMENT

The Authors declare that there is no conflict of interest.

APPENDIX

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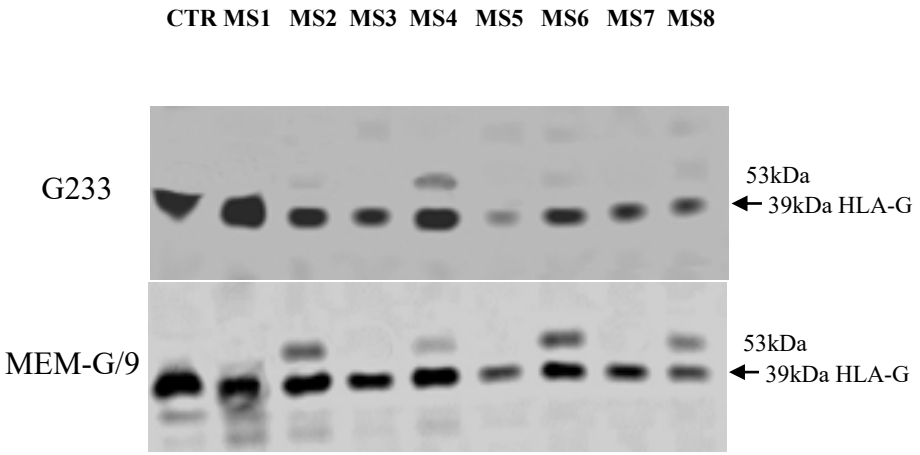

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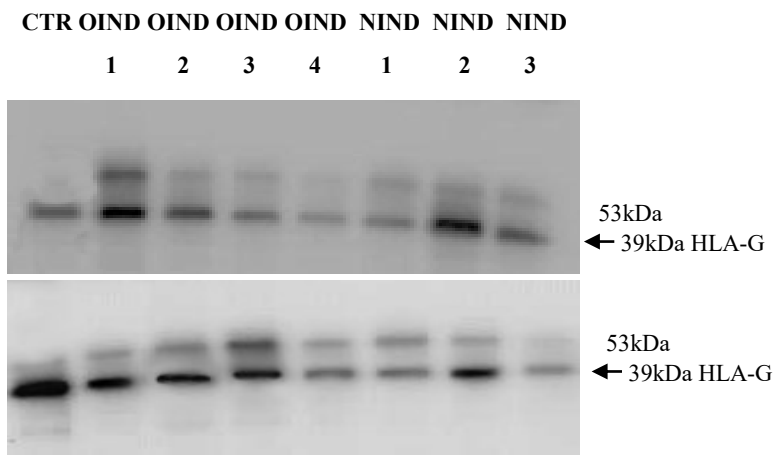

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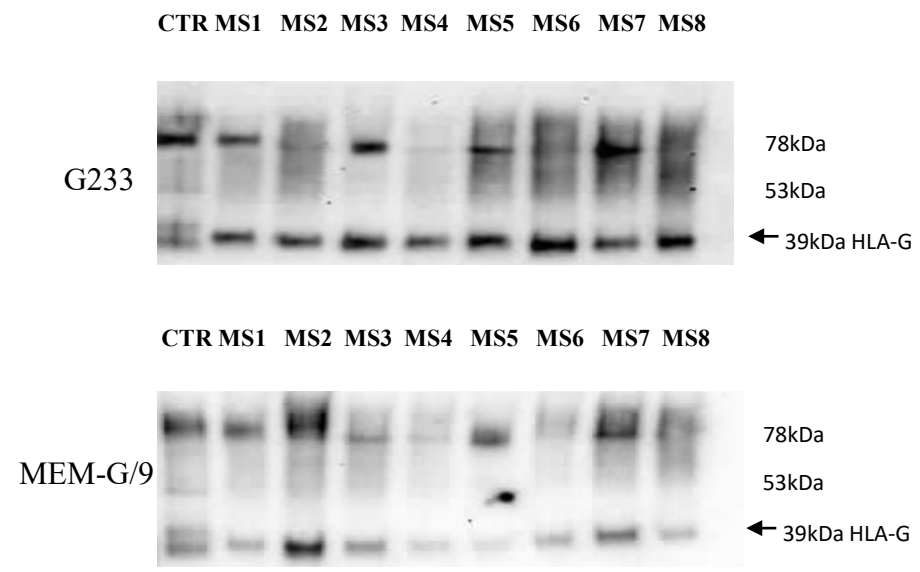

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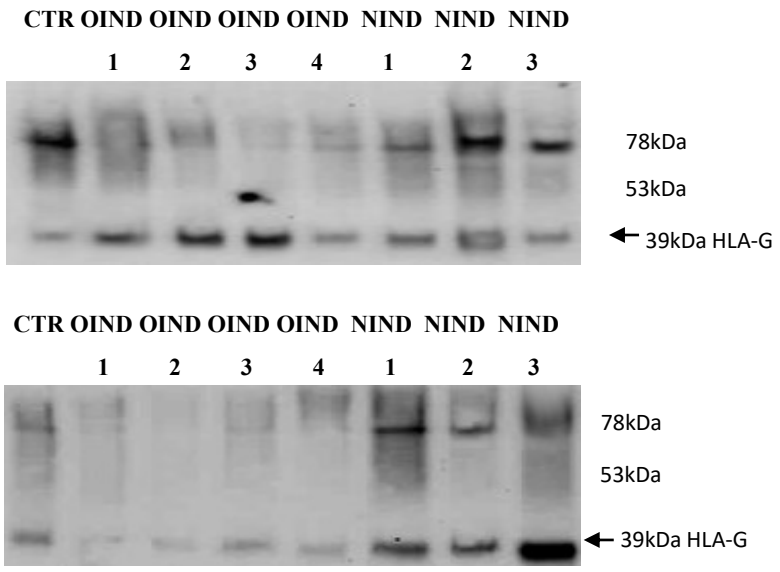

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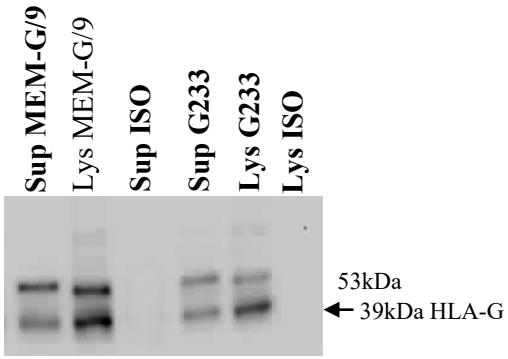

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