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Antibody response against HERV-W env surface peptides differentiates multiple sclerosis and neuromyelitis optica spectrum disorder

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Abstract

Background: A specific humoral immune response against HERV-W envelope surface (env-su) glycoprotein antigens has been reported in serum of patients with multiple sclerosis (MS). However, it has not been evaluated to date in patients with neuromyelitis optica spectrum disorder (NMOSD).

Objective: The objective of this paper is to investigate whether antibody (Ab) response against HERV-W env-su antigenic peptides differs between NMOSD and MS.

Methods: Serum samples were collected from 36 patients with NMOSD, 36 patients with MS and 36 healthy control individuals (HCs). An indirect ELISA was set up to detect specific Abs against HERV-W env-su peptides.

Results: Our data showed that two antigenic peptides, particularly HERV-W$env_{93–108}$ and HERV-W$env_{248–262,}$ were statistically significantly present only in serum of MS compared to NMOSD and HCs. Thus, the specific humoral immune response against HERV-W env-su glycoprotein antigens found in MS is widely missing in NMOSD.

Conclusion: Increased circulating serum levels of these HERV-W Abs may be suitable as additional biomarkers to better differentiate MS from NMOSD.

Keywords: Neuromyelitis optica spectrum disorder, multiple sclerosis, HERV-W env antigenic peptides, immune response

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Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the central nervous system (CNS) with an unclear immune-mediated pathogenesis. Several data support the hypothesis of a possible infectious stimulus as a trigger of the pathogenic cascade.[1–3] Given the lack of specific biomarkers, MS diagnosis is generally accomplished if precise clinical and neuroimaging criteria are satisfied and any other diagnostic possibilities have been excluded.[4,5] Nevertheless, around 5%–10% of MS diagnoses have to be reconsidered after two years.[6,7] Neuromyelitis optica (NMO) has been considered for many years a more severe variant of MS, mainly involving the spinal cord and optic nerves. However, the discovery of a specific antibody (Ab) directed against aquaporin-4 (AQP4-immunoglobulin (Ig)G), the main channel protein that facilitates the transport of water at the astroglial cell level in the CNS, has conferred to the pathology an identity different from MS.[8] At present, the term NMO spectrum disorders (NMOSD) also includes patients with AQP4-IgG positivity and signs/symptoms related to involvement of CNS regions other than the spinal cord and optic nerves, such as brainstem, diencephalon and subcortical white matter.[9,10] Moreover, a variant AQP4-negative is known, which can be diagnosed when definite clinical and neuroimaging criteria specific for NMOSD are satisfied.[9] These "seronegative" forms represent approximately 12% and are likely due to the known fluctuations in the serum autoantibody titer below the identification

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threshold. Interestingly, anti-myelin oligodendrocyte glycoprotein Abs (MOG-IgG) have been detected in 10%–15% of the seronegative NMOSD forms, suggesting that different autoantigens other than AQP4 may play a role in this disorder.[11]

Currently, NMOSD remains the main differential diagnosis for MS. Thus, the identification of biohumoral markers able to differentiate between NMOSD and MS is highly desirable for improving diagnosis and selecting the most appropriate and effective treatment.

Recent studies have shown that the expression of human endogenous retrovirus of the W family (HERV-W) could play a role in MS pathogenesis.[12–16] In particular, HERV-W env proteins' effects in MS may involve the activation of innate immune pathways and facilitate an abnormal pro-inflammatory and super-antigenic immune response.[17–19] Moreover, serum Abs directed against the glycoprotein encoded by this retrovirus may be useful, in clinical practice, as serum biomarkers indicative of MS.[20] Given these findings, we studied the role of Ab response against HERV-W envelope surface (env-su) peptides in NMOSD. In this paper, we document for the first time a different serum humoral immune response against immunogenic peptides derived from HERV-W env proteins between MS and NMOSD patients.

Materials and methods

Participants

The study protocol was approved by the local ethics committee (Prot. 2457/2017, Azienda Sanitaria Locale 1, Sassari, Italy). Among patients referred for serum AQP4/MOG-IgG analysis to the Laboratory of Neuropathology, University Hospital of Verona, Italy, we identified those eventually diagnosed with NMOSD. All patients consented to diagnostic procedures and biological sample storage at Verona Neuropathology Laboratory. Patients within one year of MS onset were enrolled at the Neurology Clinic of the University Hospital of Sassari. Age- and sex-matched healthy control individuals (HCs) were recruited at the Blood Transfusion Centre of Sassari. Peripheral venous blood of patients and HC donors was collected. All the participants provided written informed consent. Patients who underwent immunomodulatory/immunosuppressive therapy during the 12-month period prior to blood sampling were excluded. The diagnosis of MS and NMOSD was based on the established criteria.[4,9]

The cohort included 36 MS patients (27 females and nine males; mean age $\pm$ SD, 45.5 ± 8.0 years) diagnosed with relapsing–remitting MS (RRMS), who presented with at least two cerebrospinal fluid (CSF)-restricted oligoclonal bands, 36 NMOSD patients (31 females and five males; mean age $\pm$ SD, 51.1 ± 16.8 years) and 36 HCs. Sera of NMOSD patients were tested at the Laboratory of Neuropathology, University Hospital of Verona, Italy, for Abs to AQP4 using a commercially available cell-based assay (CBA) (Anti-Aquaporin-4 IIFT, Euroimmun, Lübeck, Germany), and for MOG-IgG using recombinant live cell-based immunofluorescence assay with HEK293A cells transfected with full-length MOG, as previously described.[21]

Peptides

In silico analysis performed by IETB Analysis Resource software allowed us to identify several antigenic peptides derived from HERV-W env protein (UniProtKB accession number: Q9UQF0): HERV-W $env_{93–108}$ (NPSCPGGLGVTVCWTY), HERV-W $env_{129–143}$ (VKEVISQLTRVHGTS), HERV-W $env_{161–180}$ (HTRLVSLFNTTLTGLHEVSA), and HERV-W $env_{248–262}$ (NSQCIRWVTPPTQIV) belonging to the surface region of the protein. All peptides were synthesized commercially (LifeTein, South Plainfield, NJ 07080, USA) with a purity $> 90\%$ and kept frozen in single-use aliquots (10 mM) at $-80°C$.

Enzyme-linked immunosorbent assay (ELISA) method

Indirect ELISA was carried out to detect specific antibodies (Abs) against HERV-W env peptides (assayed at 10 µg/ml). Ninety-six-well Nunc immunoplates were coated overnight at 4°C with 10 µg/ml of peptides diluted in 0.05 M carbonate-bicarbonate buffer, pH 9.5 (Sigma). Plates were then blocked for one hour at room temperature with 5% non-fat dried milk (Sigma) and washed twice with phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBS-T). Sera samples were subsequently added at 1:100 dilution in PBS-T for two hours at room temperature. After five washes in PBS-T, 100 µl of alkaline phosphatase-conjugated goat anti-human IgG polyclonal Ab (1:1000; Sigma) was added for one hour at room temperature. Plates were washed again five times in PBS-T and para nitrophenylphosphate (Sigma) added as substrate for alkaline phosphatase. Plates were incubated at 37°C in the dark for three to six minutes and the absorbance at 405 nm read on a VERSATunable Max microplate reader (Molecular Devices). Negative control wells

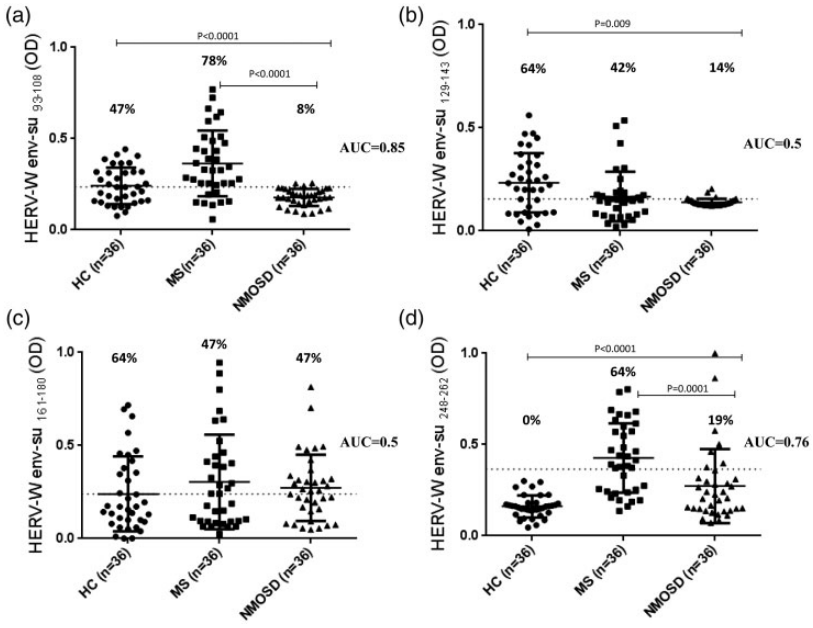

Figure 1. Prevalence of antibodies against peptides derived from human endogenous retrovirus of the W family (HERV-W) envelope protein in serum. Thirty-six neuromyelitis optica spectrum disorder (NMOSD) patients, 36 with multiple sclerosis (MS) and 36 healthy controls (HCs) were screened for immunoglobulin G antibodies by indirect enzyme-linked immunosorbent assay against HERV-W$env_{93–108}$ (a), HERV-W$env_{129–143}$ (b), HERV-W$env_{161–180}$ (c) and HERV-W$env_{248–262}$ (d) peptides. Scatter plots present median values with interquartile range. Area under receiver operating characteristic curve (AUC) as well as p values are displayed.

were obtained by incubation of immobilized peptides with secondary Ab alone, and their mean values subtracted from all samples. Positive control sera were also included in all experiments. Results are expressed as means of triplicate 405 nm optical density (OD) values.

Statistical analysis

The analysis was carried out using GraphPad Prism 6.0 software (San Diego, CA, USA). Continuous variables were presented as mean ±standard deviation (SD), and categorical variables as numbers and percentages. Kruskal-Wallis multiple comparison test was performed as post hoc for multiple comparisons, the Mann-Whitney U test was used to compare two groups, and a value of $p < 0.05$ was considered significant. The diagnostic value of the indirect ELISA assays was evaluated by the receiver operating characteristic (ROC) curve. The optimal cut-off values were chosen according to ROC analysis, setting specificity and sensitivity at 90% for all serum samples measured.

Results

Abs against HERV-W env peptides were evaluated in serum of patients from the three groups (Figure 1). Abs against HERV-W env-$su_{93–108}$ were found in the sera of 28 out of 36 (78%) MS patients and 17 out of 36 (47%) HCs whereas only three out of 36 (8%) NMOSD were positive (AUC = 0.85, HC vs MS vs NMOSD, $p < 0.0001$, MS vs NMOSD, $p < 0.0001$) (Figure 1(a)). Regarding the HERV-W env-$su_{129–143}$, 15 out of 36 (42%) MS patients, 23 out of 36 (64%) HCs, and five out of 36 (14%) among NMOSD patients were positive in serum (AUC = 0.5, HCs vs MS vs NMOSD, $p = 0.009$) (Figure 1(b)). Concerning HERV-W env-$su_{161–180}$, no statistical differences were found regarding positivity against this peptide that was recognized by 23 out of 36 (64%) HCs, by 17 out of 36 (47%) MS patients, and by 17 out of 36 (47%) NMOSD patients (AUC = 0.5, p = NS) (Figure 1(c)). Serum reactivity against HERV-W env-$su_{248–262}$ peptide was found in 23 out of 36 (64%) MS patients; however, only seven out of 36 (19%) NMOSD were positive, whereas none of the HCs was positive (AUC = 0.76, HCs vs MS vs NMOSD, $p < 0.0001$, MS vs NMOSD, $p = 0.0001$) (Figure 1(d)).

Among NMOSD patients, 25 were seropositive for AQP4-IgG and 11 were seronegative. All of them were seronegative for MOG-IgG. Only one of the AQP4-IgG–negative NMOSD patients showed Abs against HERV-W env-$su_{93–108}$ and HERV-W

env-su$_{248–262}$. Furthermore, NMOSD patients were divided by age ($\leq$50 or >50). Fourteen out of 16 (87.5%) patients showed positivity toward AQP4 antibodies and were more than 50 years of age, while 11 out 20 (55%) patients were younger than 50 years of age and showed positivity toward AQP4 antibodies. No difference has been observed regarding Ab positivity against HERV-W env-su$_{248–262}$ peptide with respect to age.

Discussion

This study is the first to evaluate the humoral response against selected peptides of HERV-W env proteins in a cohort of patients with NMOSD. The key result of the study is that NMOSD and RRMS show a differential Ab reactivity to HERV-W env-su glycoprotein antigens detectable in serum. Indeed, none of the peptides evaluated was significantly recognized by NMOSD patients, regardless of age at initial symptom onset. Notably, only HERV-W env-su $_{93–108}$ and HERV-W env-su$_{248–262}$ were able to show a significant immune response in MS patients when compared to HCs, as previously observed by Mameli et al.[20]

Interestingly, although our data are in line with the previous study by Mameli et al., in this study we found a significantly higher percentage of MS patients with specific Ab titers (31.25% vs 78% and 15% vs 64% for HERV-W env-su$_{93–108}$ and HERV-W env-su$_{248–262}$, respectively). This difference may be due, at least in part, to the more heterogeneous cohort studied by Mameli et al., which also included both primary and secondary progressive MS patients. Moreover, all of our patients with RRMS presented with CSF-restricted oligoclonal bands, which, although no longer required for MS diagnosis,[4] are useful for characterization of a homogeneous sample of patients. In our study, Ab response against HERV-W env-su$_{93–108}$ was able to better discriminate between MS and NMOSD (78% vs 8%, $p < 0.0001$), although this response was also observed in a significant proportion of HCs (47%). Why NMOSD patients have a lower prevalence of these HERV-W antibodies compared to HCs is unclear. Previous studies might support the hypothesis that these autoantibodies (in HCs) may also serve a protective function.[22] By contrast, no response against HERV-W env-su$_{248–262}$ was observed in HCs (0%). Of note, this response was statistically significantly different between MS and NMOSD patients also (64% vs 19%, $p = 0.0001$). Moreover, none but one of the 11 AQP4-IgG-negative NMOSD patients expressed antibodies against HERV-W env-su$_{93–108}$ and HERV-W env-su$_{248–262}$. This finding further supports the usefulness of the Wingerchuck criteria for distinguishing AQP4-IgG-seronegative NMOSD from MS.[9]

Thus, the present data suggest that two of the four selected HERV-W peptides can discriminate between MS and NMOSD. The fact that they provide significantly decreased Ab reactivity in NMOSD, while discriminating an increased Ab response in MS vs HCs (HERV-Wenv-su$_{93–108}$), is by itself indicative that this could be a useful biomarker for a differential diagnosis. Moreover, with examples of AQP4-negative cases, this decreased Ab reactivity could reveal complementary to AQP4-IgG detection for NMOSD diagnosis when the latter is negative. The same data may also suggest that, if MS may be associated with HERV-W pathogenic expression, NMOSD would not be. Nonetheless, immunoreactivity to peptides Wenv$_{248–262}$ also shows that a significant Ab response can be detected in NMOSD. Thus, HERV-W may also be associated with a subgroup of NMOSD patients for whom known bioclinical parameters could be compared to "negative" others. As Ab responses vary in time, this may conversely reflect physiopathological variations without relation to particular clinical subtype. Regardless, a major question arises since the Ab response to given epitopes from an antigenic protein is driven by individual major histocompatibility complex (MHC) class II phenotypes. Thus, considering a global involvement of HERV-W, the "negative" response to the two peptides and the lower response to a third one, when compared to MS, may reflect different human leukocyte antigen (HLA)-DR/DQ phenotypes between MS and NMOSD patients. Future studies will clarify the eventual contribution of MHC in this differential reactivity to different selected antigenic peptides/epitopes.

This is a pilot study with some limitations, in particular the lack of follow-up and the relatively small sample size (also considering the relative rarity of NMOSD). Furthermore, we have not evaluated Ab response in CSF because it was not available for all NMOSD patients. The MS patients of this study were not routinely tested for AQP4-IgG and MOG-IgG at the time of diagnosis. However, all MS patients included in the study presented with CSF-restricted oligoclonal bands that are commonly absent both in NMOSD- and MOG-IgG–positive patients, thus reducing the possibility of misdiagnosis.

In conclusion, our results show that the humoral Ab response against HERV-W env peptides may be a potential valuable biomarker in differentiating two chronic inflammatory demyelinating diseases of the CNS such as NMOSD and RRMS, especially in patients in whom AQP4 antibodies are not detectable. Similarly, the study of human endogenous retroviruses in NMOSD and other autoimmune non-MS demyelinating conditions of the CNS may help to better understand the controversial role of these HERVs in MS pathogenesis. For this purpose, MOG-IgG are emerging as useful in clinical practice in delineating a distinct autoimmune oligodendrocytopathy different from MS.[22-24]

Future research is needed to reproduce these results in different cohorts including MOG-IgG–positive patients and those with other non-MS demyelinating disorders, in order to clarify if the Ab response against HERV-W env antigenic peptides may be used as a useful biomarker for MS in clinical practice.

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Authors' contributions include the following: GA, ES and GPS conceived and designed the study. GA performed the experiments. SM, AF, CM, DA, SF, AG, RC and SM, tested NMOSD patients for antibodies to AQP4 and MOG. LAS coordinated the ELISA studies. GA, ES, GAD, EC and GM performed data analysis. GA, ES and GPS wrote the manuscript. All authors contributed to the interpretation of the data, participated in drafting, reviewing and editing the paper, and approved the final manuscript.

Conflicts of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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