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Clinical significance of IgM and IgA class anti-NMDAR antibodies in herpes simplex encephalitis

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ABSTRACT

Background: Herpes simplex encephalitis (HSE) is a devastating disease, often leaving patients with severe disabilities. It has been shown that IgG anti-N-methyl-D-aspartate receptor (NMDAR) antibodies appear in approximately 25% of HSE patients and could be associated with impaired recovery of cognitive performance.
Objectives: To characterize the prevalence of IgM and IgA anti-NMDAR antibodies in HSE patients, in relation to subsequent development of IgG anti-NMDAR and correlation to cognitive performance.
Study design: A total of 48 subjects were included from a previously described cohort of patients with HSE verified by HSV-1 PCR. Cerebrospinal fluid (CSF) and serum samples drawn close to onset of disease, after 14–21 days of iv aciclovir treatment and after 90 days of follow-up, were analyzed for the presence of IgM and IgA anti-NMDAR, and related to IgG anti-NMDAR. Antibody levels were correlated to the recovery of cognitive performance, as estimated by the Mattis Dementia Rating Scale (MDRS), for a total of 24 months.
Results: In total, 27 of 48 (56%) study subjects were anti-NMDAR positive, defined as the presence of IgG (12/48, 25%), IgM (14/48, 29%) or IgA (13/48, 27%) antibodies in CSF and/or serum. IgM or IgA anti-NMDAR did not predict subsequent IgG autoimmunization and did not correlate to cognitive outcome. IgG anti-NMDAR serostatus, but not antibody titers, correlated to impaired recovery of cognitive performance.
Conclusions: A majority of HSE patients develop IgG, IgM or IgA anti-NMDAR antibodies. However, the predictive value and clinical relevance of non-IgG isotypes remains to be shown in this setting.

1. Background

Herpes simplex encephalitis (HSE) is a devastating disease, in adults almost exclusively caused by herpes simplex virus type 1 (HSV-1). In its natural course the mortality is approximately 70% and even with current state-of-the-art treatment, patients often suffer severe and permanent disabilities [1–4]. In addition to the cytopathic effect of HSV-1 infection in neuronal cells, the human immune response also causes cellular damage and death due to its necessary but imprecise attempts to control infection. Based on results from animal models and retrospective clinical studies suggesting a beneficial effect of immunotherapy, attempts has been made to further improve clinical outcome by adding corticosteroids to the antiviral treatment [5,6]. However, as there are currently no data available from prospective randomized clinical trials investigating the effect of immune modulators in HSE, there is a need for further studies.

In cases of encephalitis of previously unknown origin, a significant portion has now been identified as autoimmune reactions involving the *N*-methyl-D-aspartate receptor (NMDAR) [7]. NMDAR encephalitis is more common in young patients than in the elderly and was first described as a paraneoplastic syndrome, but in the majority of cases there is no known underlying disease [8–11]. Synaptic autoimmunity affecting the NMDAR has also previously been described in HSE patients with and without clinical relapse [12]. It has been suggested that the

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development of anti-NMDAR antibodies could explain a majority of the clinical relapses seen in the months after acute HSE [13]. This is consistent with previous studies of relapse where the cerebrospinal fluid (CSF) profile of cytokines and neural and glial cell biomarkers indicate an immune mediated process rather than direct viral cytotoxicity [14].

We have previously shown that IgG anti-NMDAR antibodies appear in approximately 25% of HSE patients within 90 days and could be associated with impaired recovery of cognitive performance [15]. Also, there are case reports of other types of neuronal autoantibodies post HSE [16]. However, varicella-zoster virus, Epstein-Barr virus and influenza A have also been associated with post-infectious NMDAR autoimmunity [17–20]. This suggests that the autoimmunization is not caused by HSV-related molecular mimicry but rather by exposed synaptic antigens during cytolytic viral infection. It is not known whether viral infections that affect brain regions with higher NMDAR expression have an increased risk of NMDAR autoimmunization.

In infectious disease, IgM antibodies usually occur in the early response against pathogens but are less antigen specific and of lower avidity than the IgG antibodies that are produced during seroconversion. However, in autoimmunity this is not necessarily true. IgM rheumatoid factor (RF) is the major RF isotype found in Caucasian rheumatoid arthritis patients, without any tendency to change in predominating isotype over time. IgA autoantibodies against tissue transglutaminase are the major diagnostic findings in celiac disease, again without any change in autoantibody isotype over time [21,22]. During the years predating RA diagnosis, IgG antibodies against citrullinated proteins/peptides (ACPA) appear first around ten years before diagnosis, closely followed by IgA ACPA and with IgM ACPA appearing during the last pre-diagnosis year [23]. Thus it is not evident that autoimmune serology appearing during infections will follow the same order of appearance for immunoglobulin isotypes as for antibodies directed against the pathogen *per se*.

Prüss et al. have previously shown that IgM and IgA anti-NMDAR could be detected in HSE patients, but the clinical relevance of non-IgG isotypes remains to be clarified [12]. In this study, using CSF and serum samples from 48 HSE patients, we have retrospectively analyzed the temporal development of anti-NMDAR IgM, IgA and IgG isotypes, and the correlation to cognitive performance.

2. Objectives

The objective of this study was to investigate the clinical relevance of IgM or IgA autoantibodies and whether subsequent IgG NMDAR autoimmunization can be predicted, possibly giving an opportunity for preventive treatment.

3. Study design

3.1. Study subjects

A total of 48 subjects with sufficient amounts of CSF remaining were included from a previously described cohort of HSV-1 PCR-verified HSE patients during 2001–2009 at five study sites in Sweden [24]. All participants were previously included in the NCT00031486 clinical trial and hence randomized to receive oral valaciclovir or placebo for a 90 day follow-up period [24]. Adjuvant corticosteroid treatment (betamethasone, dexamethasone or methylprednisolone) was given at the discretion of the investigators. CSF and serum samples were drawn close to onset of disease (onset), after 14–21 days of intravenous (iv) aciclovir treatment when clinical follow-up was started (FU start) and after 90 days of follow-up (FU 3M). Cognitive performance was measured using the Mattis Dementia Rating Scale (MDRS) at intervals during a total of 24 months [25]. Informed consent was obtained from all study participants. The study was approved for all study sites by the Regional Ethical Review Board at Karolinska Institutet, Sweden.

3.2. Anti-NMDAR assay

Anti-NMDAR antibodies were detected by indirect immune fluorescence using HEK293 fibroblasts that had been transfected with the NMDR R1 type glutamate receptor (Euroimmun, Lübeck, Germany). As negative controls, cells transfected with empty plasmids were investigated for each sample. Serum samples were diluted 1:10 and CSF samples were used undiluted, in agreement with previous studies [12,15]. Thirty µl sample was applied to each reaction field for 30 min at room temperature before application of fluorescein isothiocyanate labelled goat anti-human IgM or IgA antibodies (ready to use, Euroimmun). The staining technique has previously been described in detail [15]. Two samples positive for IgA and IgM anti-NMDAR and one negative sample were included in all experiments. All stainings were independently evaluated by two analysts (AS and JR) and thereafter all reactions were viewed and discussed together at the microscope. Discrepant results, especially concerning IgM anti-NMDAR, were resolved by consensus.

Initially 51 healthy blood donor controls were investigated for IgA and IgM anti-NMDAR. None of the controls had detectable IgA anti-NMDAR, whereas two controls showed a weak/intermediate (2 +) IgM anti-NMDAR reactivity.

3.3. Statistical analyses

The primary statistical analyses, specified *a priori*, were the sensitivity and specificity of anti-NMDAR IgM and IgA in samples drawn at FU start to predict development of anti-NMDAR IgG. Secondary analyses were comparison of MDRS total score between anti-NMDAR IgM and IgA seropositive and negative groups using the Mann-Whitney *U* test, and the correlation between antibody titers and MDRS total score using Spearman's rank correlation. All analyses were performed using R version 3.4.1 (R Core Team, Vienna, Austria). P-values below 0.05 were regarded as significant.

4. Results

4.1. Anti-NMDAR isotype serostatus and subject characteristics

In total, 27 of 48 (56%) study subjects were anti-NMDAR positive, defined as the presence of IgG (12/48, 25%), IgM (14/48, 29%) and/or IgA (13/48, 27%) class antibodies in CSF or serum. IgG were predominantly found in CSF (12/12) rather than serum (2/12), while IgM (CSF 7/14, serum 11/14) and IgA (CSF 10/13, serum 6/13) were present in both compartments. There were no clear differences between groups according to IgM, IgA and total anti-NMDAR (IgG/M/A) serostatus in relation to subject characteristics, clinical presentation, radiologic findings or interventions such as acute phase adjuvant treatment with corticosteroids or prolonged antiviral treatment with valaciclovir (Table 1).

4.2. Prediction of IgG anti-NMDAR seroconversion

Of 48 subjects, 12 (25%) were IgG anti-NMDAR positive at or before FU 3M. Of these, 6/12 were IgM positive (5/12 in CSF, 3/12 in serum) but only 3/12 (3/12 in CSF, 2/12 in serum) prior to FU 3M. With regards to IgA, 5/12 were positive (5/12 in CSF, 2/12 in serum) in total but only 1/12 (both CSF and serum) were positive prior to FU 3M. In total, only 4/12 (33%) of the IgG positive subjects (4/12 in CSF, 3/12 in serum) could be predicted by the development of IgM, IgA or IgG anti-NMDAR in samples drawn at FU start.

To evaluate the specificity of a negative IgM or IgA anti-NMDAR result in relation to IgG development, all subjects with complete data at FU start and FU 3M were analyzed. Of 36 IgG negative subjects, 29 had such data. Of these, 24 (27 in CSF, 24 in serum) were IgM negative in both compartments at FU start, indicating that most (83%) IgG anti-