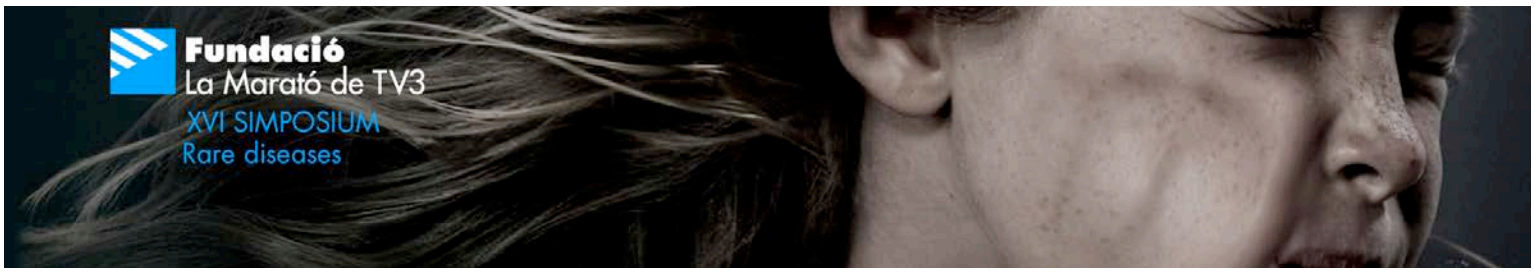

Analysis of new antigens in seronegative (NMO-IgG/AQP4) neuromyelitis optica (Devic disease)

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1. Summary

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease of the CNS characterized by selective involvement of the optic nerves and spinal cord. NMO has a worldwide distribution with an estimated prevalence of 0.4-4/100,000. The disease is more frequent in women, and the median age of onset is 39 years, but it also occurs in children and elderly people. Up to 90% of patients have a relapsing course with increased disability due to frequent and severe attacks. More than 50% of patients are blind in one or both eyes or need ambulatory help within 8.5-10 years of onset. NMO was considered a variant of MS for long time but the implication of the humoral immunity in pathological studies, and the identification of a specific serum autoantibody marker, NMO-IgG, which bound selectively to aquaporin4 (NMO-IgG/AQP4), a water channel highly expressed in the polarized foot processes of astrocytes, led to differentiation of both diseases.

The identification of NMO-IgG/AQP4 led to expansion of the clinical spectrum to limited forms of the disease, i.e. recurrent longitudinally extensive transverse myelitis (LETM) and optic neuritis (ON), to define a new set of diagnostic criteria in which patients with clinical or brain MRI evidence beyond the optic nerve and spinal cord were not excluded, to help to make an early and accurate diagnosis, and in making therapeutic decisions. However, around 20-35% of patients clinically diagnosed with NMO are NMO-IgG/AQP4 seronegative, and the percentage increases when limited forms of the disease (LETM, and ON) are included in the analysis. The pathogenic role of NMO-IgG/AQP4 has been suggested in recent studies on in vitro and animal models. However, the physiopathology involved in seronegative NMO-IgG/AQP4 is still unknown. Currently, therapeutic strategies are aimed at minimizing the damage associated with the relapse, and at preventing the recurrences. Hence, the importance of early identification of patients.

Objectives

To identify new antibodies that could be involved in the pathogenic humoral immune response in seronegative (NMO-IgG/AQP-4 antibodies) NMO patients.

2. To characterize the antigens identified in the previous objective.

3. To evaluate their effect in an animal model by intra-cerebral injections of the purified IgG.

2. Results

1. ***We have expanded the available tools for NMO-IgG/AQP4 detection and improved the sensitivity, reducing the frequency of seronegative NMO cases.*** Coded serum from 103 patients with NMO and 122 inflammatory controls was studied by an optimized immunohistochemistry (IHC-o), and the results were compared with those of two cell-based assays: one in-house (CBA-ih) with cells transfected with the M23 isoform of the AQP4, and a commercial CBA-c. The sensitivity of the IHC-o was similar to that of the CBA-ih and better than the CBA-c, and all of them had a specificity of 100%. Our previous reported sensitivity of 65%, using a conventional IHC and a CBA with the M1 isoform of the AQP4 increased to 77% (Reference 1).

2. ***We have identified that 15% of NMO-IgG/AQP4 seronegative patients have antibodies to myelin oligodendrocyte glycoprotein (MO-IgG), and its presence is associated with a better prognosis.*** Samples from 174 adult patients with NMO and associated spectrum (LETM, ON) were examined for NMO-IgG/AQP4 and MOG-IgG using CBA. We found MOG-IgG in 15% of patients without NMO-IgG/AQP4. MOG-IgG were more frequently associated with ON, NMO-IgG/AQP4 with NMO phenotype, and double seronegativity with LETM. Patients with MOG-IgG, compared to those with NMO-IgG/AQP4), were younger and without female predominance (50%), the clinical course was more frequently monophasic, and

although the severity of the disease at onset was similar they had a better outcome (Reference 2).

3. ***The HLA-DRB1 allelic distribution in Caucasian NMO patients is different from that found in MS patients. DRB1*03 allele seems to contribute to NMO seropositivity***, ***but patients with seronegative NMO are not different from those who are seropositive.*** We studied a cohort of 22 NMO patients, 16 (73%) of whom were NMO-IgG/AQP4 positive, and the results were compared to those of 228 MS patients and 225 healthy controls. NMO was associated with a higher frequency of DRB1*10 allele compared with MS. To increase the statistical power we pooled our results with those found in a French study. We found that NMO was associated with a higher frequency of DRB1*03 allele and was related to presence of NMO-IgG/AQP4 (Reference 3).

4***. Inflammatory longitudinally extensive transverse myelitis (LETM) is mostly idiopathic with a good outcome. It includes a relatively homogenous group of patients with an overrepresentation of the HLA-DRB1*13 genotype.*** We prospectively studied 23 adult patients who presented with isolated LETM. Most (74%) were female with a median age of 44.5 years and moderate-severe disability at nadir. NMO-IgG/AQP4 were found in 2 (9%) patients, antinuclear antibodies in 16 (70%), and genotype DRB1*13 in 13 (57%). The frequency of DRB1*13 genotype was higher compared with controls, MS, and NMO patients. At the end of the 32-month follow-up two patients converted to NMO, one to MS, and 20 remained as idiopathic with recurrences in four (20%). Disability at nadir was associated with the final outcome and extension of the spinal cord lesion with risk of recurrence (Reference 4).

5. ***We have identified that 45% of patients with unilateral or bilateral, severe, or recurrent isolated optic neuritis (ON) had antibodies. Those with NMO-Ig/AQP4 had the poorest visual outcomes, whereas patients with MOG-IgG had a better outcome similar to those with seronegative findings.*** We studied 51 patients with

isolated ON and 142 controls. The presence of NMO-IgG/AQP4, MOG-IgG and antibodies to the glycine receptor α1 subunit (GlyR-IgG) was determined by CBA. Antibodies were identified in 23 (45%) patients, including MOG-IgG in 10 patients, NMO-IgG/AQP4 in 6, and GlyR-IgG in 7 (concurrent with MOG in 3 and with AQP4 in 1). At the end of the follow-up, 35% of the patients who remained with the diagnosis of idiopathic ON had an antibody (MOG, GlyR or both) that suggest the immunomediated origin of the ON. We confirm that the presence of NMO-IgG/AQP4 associates with a poor final visual outcome and MOG-IgG with a good prognosis. The presence of GlyR-IgG seems to be a bystander effect because they are also found in MS and other neurological disorders (Reference 5).

6. ***We have identified that patients with anti-NMDAR encephalitis may develop concurrent or separated episodes of demyelinating disorders, and conversely patients with NMO or demyelinating disorders with atypical symptoms may have anti-NMDAR encephalitis.*** From a cohort of 691 cases with anti-NMDAR encephalitis we analyzed the 23 patients who presented with a demyelinating disorder. NMO-IgG/AQP4 and MOG-IgG were determined by CBA. Two groups of patients were identified: 1) patients who developed anti-NMDAR encephalitis preceded or followed by episodes of NMO spectrum disorder or brainstem or multifocal (12 patients, 4 with NMO-IgG/AQP4 and 7 with MOG-IgG); and 2) patients with simultaneous clinical and MRI features of demyelination (11 patients, 5 of them with NMO-IgG/AQP4 and 2 with MOG-IgG). Although most patients improved with immunotherapy, the demyelinating episodes require more intensive therapy and resulted in more residual deficits (Reference 6).

7. ***The passive experimental autoimmune encephalomyelitis model in C57BL/6 with MOG is useful to evaluate the role of B cells in disease mediated by antibodies.*** A comparative study between active experimental autoimmune encephalomyelitis (a-EAE) and passive transfer of activated T cells (at-EAE) in C57BL/6, showed that the development of EAE was similar, but the onset of symptoms started before and the severity was higher in the at-EAE model. Spinal

cord histological examination revealed an increased glial activation and more extensive demyelinating areas in the at-EAE model. Although the inflammatory infiltrates, macrophages and T-lymphocytes, were found in both models, B lymphocytes were significantly increased in the at-EAE model. The colocalization of these B cells with IgG and their predominant distribution in areas of demyelination would suggest that IgG-secreting B cells are involved in the neurodegenerative processes associated with at-EAE (Reference 7).

8. ***Evaluation of the effect of the IgG from seropositive and seronegative NMO patients.*** After the characterization of the EAE in vivo model we developed the LPS model in C57BL/6 mice, which develop a systemic inflammatory process involving the CNS and disrupting the blood-brain barrier. Animals were injected intraperitoneally with purified IgG from seropositive NMO-IgG/AQP4 patients (n=3), seronegative NMO-IgG/AQP4 (n=3), multiple sclerosis (n=3), and healthy controls (n=3). Twenty-four hours after treatment, mice were sacrificed and the brains and spinal cord were removed. The following procedures were performed: RT-PCR analysis (assessment of the inflammatory and glial genetic pattern); western-blot (expression of inflammatory and glial proteins); and histological analysis (analysis of GFAP and AQP4). In addition, cell cultures of primary astroglial-enriched cells from neonatal spinal cord, (40% of oligodendrocytes, 35% of astrocytes, 10% of microglia, and 5% of glial progenitors) were treated with the purified IgG from the different groups of patients and controls. The following analyses were performed with these cell cultures: assessment of glial markers (GFAP, Iba-1, and CNPase); proinflammatory markers (NOS2 and COS2), and analysis of nitric oxide; RT-PCR: assessment of expression of glial genes (GFAP, Iba-1, and CNPase); proinflammatories (NOS2, COX2, C/EBPβ, IL1β, IL6, TNFα, and IFNγ), and anti-inflammatory (TGFβII, IL4 and C/EBPα); and western-blot: expression of the corresponding proteins. The results of this study were presented in the meeting of the Federation of European Neuroscience Societies (Reference 8). However, the results have not been published because after improving our methods of detection of AQP4 and the discovery of MOG as an antigen (references 1 and 2), we

found that one of the samples used as seronegative NMO actually had MOG-IgG at high titres, and another low titres of NMO-IgG/AQP4.

3. Relevance and possible implications

1. We have expanded the available tools for NMO-IgG/AQP4 detection and improved the sensitivity reducing the frequency of seronegative NMO cases. These improvements facilitate the diagnosis and the early instauration of preventive therapy.

2. We have identified a new antibody, MOG-IgG, which is present in a subgroup of seronegative NMO-IgG/AQP4 patients. The identification is important because these patients have a different clinical and prognostic profile and its recognition has implications at the time of making a therapeutic decision.

3. We have characterized the clinical and prognostic profile of patients with longitudinally extensive transverse myelitis. The main prognostic factor is the severity of the impairment at onset, and the main associated marker a specific HLA-DRB1 genotype. In patients with severe or recurrent optic neuritis, the presence of NMO-IgG/AQP4 associates with poor visual outcome. Thirty-five per cent of the cases considered as idiopathic have an antibody that suggests the immunomediated origin of the disorder.

4. Patients with anti-NMDAR encephalitis may develop concurrent or separate episodes of demyelination disorders; the recognition of this association is important because treatment and outcome vary for each disorder.
5. The passive experimental autoimmune encephalomyelitis model in C57BL/6 with MOG is useful to evaluate the role of B cells in diseases mediated by antibodies, such as NMO.

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