Polyspecific, antiviral immune response distinguishes multiple sclerosis and neuromyelitis optica

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ABSTRACT

Background: A polyspecific, intrathecal humoral immune response against neurotropic viruses such as measles, rubella and varicella zoster virus (MRZ reaction, MRZR) is present in 80–100% of patients with multiple sclerosis (MS), but has not to date been evaluated in patients with neuromyelitis optica (NMO).

Aims: To evaluate whether MRZR distinguishes NMO and MS.

Methods: 20 patients with NMO and 42 with MS were included. The intrathecal synthesis of antibodies against measles, rubella and varicella zoster virus was detected by calculation of the respective antibody indices (AI).

Results: A positive MRZ reaction, as defined by a combination of at least two positive AIs, was found in 37/42 MS, but in only 1/20 NMO patients ($p<0.0001$). Median AI values differed significantly between the groups ($p<0.0005$).

Conclusions: The polyspecific antiviral humoral immune response characteristic for MS is widely missing in NMO, irrespective of the NMO-IgG status of the patients. Our findings further strengthen the case for NMO being pathologically distinct from MS.

Neuromyelitis optica (NMO, Devic syndrome) is a severe inflammatory disorder of the CNS of putative autoimmune aetiology, predominantly affecting the spinal cord and optic nerves.[1] Whether NMO is distinct from multiple sclerosis (MS) or rather a variant of MS is currently under discussion.[2 3] To further elucidate this question, we compared the antiviral, intrathecal antibody repertoire in MS and NMO. MS is well known to be characterised by a polyspecific, intrathecal B cell response against neurotropic viruses such as measles, rubella and varicella zoster virus (MRZ reaction, MRZR), which is detectable in 80–100% of patients with MS.[4–9] In this study, we investigated whether such a reaction is also present in NMO, and whether it might help to distinguish between NMO and MS.

PATIENTS AND METHODS

Twenty consecutive patients, with clinically definite NMO according to Wingerchuk's 2006 criteria,[10] from various university hospitals in Germany and Italy seen between 1999 and 2007 were included. All but one patient (with brainstem involvement at onset) also fulfilled Wingerchuk's 1999 criteria.[1] NMO-IgG was assessed by indirect immunofluorescence, as described previously,[11] in 18 patients with NMO and was positive in 11 (61%). Oligoclonal bands (OCBs) were found in 7/20 patients (35%). The sex distribution (men:women) was 1:3 overall and 1:4.5 in the NMO-IgG positive subgroup. Median age was 40 years (range 19–72). Longitudinally extensive spinal cord lesions extending ⩾3 vertebral segments were present in all patients. Except for severe vomiting caused by a bulbar lesion in one patient, no history of clinical disease outside the optic nerve or spinal cord was found. Extra-opticospinal MRI lesions (not fulfilling McDonald criteria for MS[12]) were detectable in 7/20 patients (35%; 4/7 NMO-IgG positive) during the course of disease. NMO followed a relapsing course in 18 patients and was monophasic in two. Median follow-up was 39 months (range 11–288). Serum and CSF samples were obtained 0–286 months from first myelitis (median 2 months; <6 months in nine patients; >24 months in four) and 0–282 months from first optic neuritis (median 11 months; <6 months in nine patients; >24 month in seven). As a control group, MRZR results from 42 consecutive patients with MS (28 with myelitis) from Germany were analysed, including 29 patients with relapsing–remitting MS (RRMS), four patients with secondary progressive MS (SPMS) and nine patients with a clinically isolated syndrome suggestive of MS (CIS) at the time of lumbar puncture. Diagnosis of MS was established according to McDonald and colleagues.[12] The sex distribution was 1:3.2 (10 men, 32 women). Median age was 34.5 years (range 18–62). Serum and CSF samples were collected 0–294 months from first relapse (median 13 months; <6 months in 17 patients; >24 months in 15). All lumbar punctures were performed for diagnostic purposes only.

Quantitative expressions of the intrathecal, humoral immune response were based on calculation of the CSF/serum ratios of specific antiviral IgG antibodies and total IgG ($Q_{IgG[spec]} = IgG_{spec[CSF]}/IgG_{spec[serum]}$, and $Q_{IgG[total]} = IgG_{total[CSF]}/IgG_{total[serum]}$). Antibody levels were determined using a commercially available enzyme linked immunosorbent assay (Dade Behring, Germany) according to the manufacturer's instructions. Total IgG and total albumin concentrations in serum and CSF were determined nephelometrically (BN ProSpec, Dade Behring, Germany). The intrathecal synthesis of antibodies to M, R and Z was detected by calculation of the corresponding antibody indices (AI):

$AI = Q_{IgG[spec]}/Q_{IgG[total]}$, if $Q_{IgG[total]} < Q_{lim}$, and $AI = Q_{IgG[spec]}/Q_{lim}$, if $Q_{IgG[total]} > Q_{lim}$.[13]

The upper reference range of $Q_{IgG[total]}$, Q_{lim}, was calculated according to Reiber's formula.[13]. AI values >1.5 were considered to be indicative of intrathecal IgG production against the respective pathogen.[13]

Table 1 Demographic and laboratory findings in 62 patients with neuromyelitis optica (NMO) and multiple sclerosis (MS)

	NMO	MS	p Value
No of patients	20	42	–
Age (years) (medium (range))	40 (19–72)	34.5 (18–62)	–
Sex (men:women)	1:3	1:3.2	–
Positive MRZ reaction	1/20 (5%)	37/42 (88%)	<0.0001*
AI M+R+Z+	0/20 (0%)	20/42 (48%)	<0.0001*
AI M+R+ or M+R+ or M+Z+	1/20 (5%)	17/42 (40%)	<0.001*
AI measles (AU) (median)	1.18	3.9	<0.0005†
AI rubella (AU) (median)	1.03	3.1	<0.0005†
AI zoster (AU) (median)	1.02	2.6	<0.0005†
OCBs	7/20 (35%)	39/42 (93%)	<0.0001*
CSF cell count >5/μl	14/20 (70%)	35/42 (83%)	NS*
Cells/μl (median (range))	7.5 (1–96)	11.5 (0–94)	NS†
QAlb, elevated	10/20 (50%)	8/42 (19%)	<0.02*

+, positive antibody index; –, negative antibody index; AI, antibody indices; AU, arbitrary units; M, measles; OCB, CSF restricted oligoclonal bands; QAlb, albumin CSF/serum ratio (age dependent upper reference range = 4+age/15 according to Reiber and colleagues[13]); R, rubella; Z, zoster.
*Fisher's exact test (two sided); †Mann Whitney U test (two tailed).

RESULTS

A positive MRZ reaction, as defined by a combination of at least two positive AIs, was present in 37/42 patients with MS (88%) but in only 1/20 patients with NMO (5%) (p<0.0001, Fisher exact test) (table 1), corresponding to a positive and negative likelihood ratio of 17.62 (95% confidence interval (CI) 2.6 to 119.4) and 0.1 (95% CI 0.05 to 0.29), respectively.

MRZR was positive in patients with RRMS (24/29) as well as in patients with SPMS (4/4) and, notably, also in 9/9 patients with CIS, indicating that MRZR is present early in the disease course. A trispecific reaction, as defined by an increased AI to all three pathogens studied, was found in 14/29 RRMS and 6/9 CIS patients. A bispecific reaction was present in 10/29 RRMS, 4/4 SPMS and 3/9 CIS patients. Notably, immunosuppressive treatment seemed not to affect MRZR positivity. MRZR was positive (4×bispecific, 4×trispecific) in 8/8 patients treated with methylprednisolone, azathioprine or mitoxantrone (6×RRMS, 1×SPMS), or a combination of mitoxantrone, methylprednisolone, plasma exchange and intravenous immunoglobulin (1×SPMS), whereas all MRZR negative MS patients were untreated at the time of lumbar puncture.

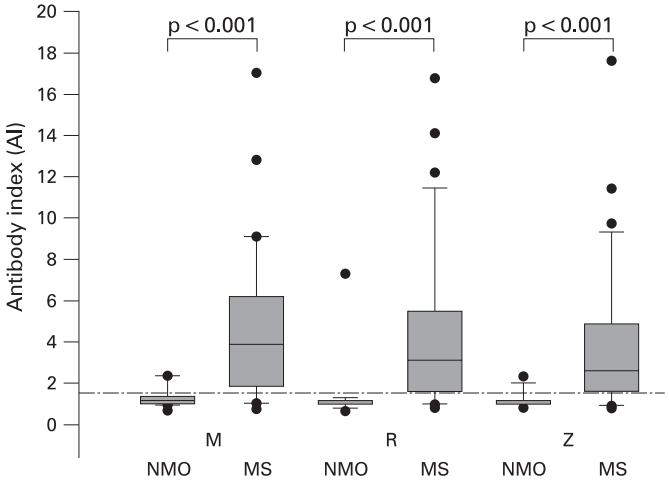

Figure 1 Box blot graph with antibody indices (AI) for measles (M), rubella (R) and zoster (Z) in patients with neuromyelitis optica (NMO) and multiple sclerosis (MS). The boundary of the box closest to 0 indicates the 25th percentile, the line within the box marks the median and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. In addition, outliers (•) are shown. The broken horizontal line indicates the upper reference limit (AI = 1.5).

In MS, median AIs were 3.9 for M, 3.1 for R and 2.60 for Z (mean AI 5.15, 4.60 and 4.54, respectively). Median AI in NMO was 1.18 for M, 1.03 for R and 1.02 for Z (mean 1.25, 1.37 and 1.15, respectively) (p<0.0005, Mann–Whitney U test) (fig 1). The only MRZR positive patient with NMO had an unusual presentation with onset at age 72 years and no further relapses (follow-up 39 months).

DISCUSSION

MRZR has been demonstrated to be positive in 80–100% of patients with MS.[4–8] Its specificity for MS, however, has never been systematically evaluated in appropriate controls. In this study, we assessed MRZR, for the first time, in patients with NMO. We found a positive MRZ reaction, as defined by at least two positive AIs, in 88% of our patients with MS. This is in good agreement with earlier reports.[4–8] In contrast, MRZR was negative in 19/20 patients with NMO. Our results strengthen the case of NMO being pathophysiologically distinct from MS[2 3] and, in addition, improve the differentiation of classical MS and NMO by routine CSF analysis.

The reason for this apparently deranged intrathecal B cell response in MS remains elusive. As simultaneous infection with several neurotropic viruses is unlikely and PCR for measles, rubella and varicella zoster virus has been shown to be negative in MRZR positive patients with MS, MRZR is thought to represent non-specific activation (so called "bystander" activation) of B cells within the CNS in the absence of viral replication, whereas in MRZR negative patients, a more targeted immune response is discussed.[4 9 13] This would be well in line with the recent proposal of a specific antigen in some NMO patients (ie, aquaporin-4).[14 15] Consistently, MRZR is also negative in patients with other CNS disorders characterised by well defined antibody targets such as paraneoplastic neurological syndromes associated with antibodies against Hu, Ri, Yo, Ma, Ta or amphiphysin (personal observation in 34 patients, SJ, RV), viral encephalitis or myelitis,[6 8] and neuroborreliosis.[16] The hypothesis of differential ways of B cell involvement is further supported by the finding that MRZR was also negative in OCB positive NMO patients.

Notably, MRZR testing discriminated well between MS and NMO, irrespective of the NMO-IgG serostatus of the patient or brain involvement. MRZR might thus be helpful in the differential diagnostic workup of seronegative patients with NMO, especially in those with brain lesions which often pose a differential diagnostic challenge. Future studies should especially address the question of whether MRZR testing allows us to predict conversion to NMO or MS in NMO-IgG negative patients with syndromes considered to indicate "high risk"[17] of NMO (recurrent optic neuritis (ON), recurrent transverse myelitis, ON with myelitis not extending three segments) and brain involvement. In a previous study, some of us have already shown that MRZR in patients with a first attack of ON and two or more cerebral lesions predicts progression to definite MS (positive predictive value 86% after 4 years).[18] Well in line with these findings, MRZR was positive in 9/9 CIS patients in our current series. Early discrimination between MS and NMO, however, is important as management for the two diseases differs. Whereas immunomodulating drugs such as interferon β or copaxone have been demonstrated to be effective in MS, immunosuppressants such as azathioprine and rituximab seem to be favourable in NMO[19–21]; patients with NMO may also be more eligible for plasma exchange than those with MS.[22] Moreover, early diagnosis and treatment has been demonstrated to influence the long term clinical outcome in MS significantly, and the same is probably true for NMO.[23 24]

It should be noted as a caveat that the rate of MRZR positivity in a given population seems to depend on the natural prevalence of the three viral antigens tested for, as well as on the local vaccination coverage, and the frequency of MRZR in MS thus might not be as high in tropical or subtropical regions as in Europe.[25]

In conclusion, MRZR discriminated well between MS and NMO in our study (n = 62), with a remarkably high sensitivity (88%), specificity (95%) and positive likelihood ratio (17.1; 95% CI 2.6 to 119.4), compared with a reported sensitivity of 73%, specificity of 91% and positive likelihood ratio of 8.07 (95% CI 2.13 to 30.6) for NMO-IgG in the largest study thus far (n = 67).[17] More importantly, by demonstrating differential ways of B cell involvement, our findings add further evidence to the hypothesis that NMO and MS are pathologically distinct disorders.[2 3] While the humoral immune response in MS is clonally stable, deranged towards polyspecific B cell activation in the CSF and reminiscent of interactions with neurotropic viruses, it is differently oriented towards the production of a specific serum antibody (ie, NMO-IgG) with probable pathogenetic relevance and useful diagnostic meaning in NMO. Larger studies are warranted to evaluate whether MRZR testing should be included in the multidimensional approach to the differential diagnosis of MS and NMO or its formes frustes.

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