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Antigen microarrays identify CNS-produced autoantibodies in RRMS

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ABSTRACT

Objective: Multiple sclerosis (MS) is characterized by the local production of antibodies in the CNS and the presence of oligoclonal bands in the CSF. Antigen arrays allow the study of antibody reactivity against a large number of antigens using small volumes of fluid with greater sensitivity than ELISA. We investigated whether there were unique autoantibodies in the CSF of patients with MS as measured by antigen arrays and whether these antibodies differed from those in serum.

Methods: We used antigen arrays to analyze the reactivity of antibodies in matched serum and CSF samples of 20 patients with untreated relapsing-remitting MS (RRMS), 26 methylprednisolone-treated patients with RRMS, and 20 control patients with other noninflammatory neurologic conditions (ONDs) against 334 different antigens including heat shock proteins, lipids, and myelin antigens.

Results: We found different antibody signatures in matched CSF and serum samples The targets of these antibodies included epitopes of the myelin antigens CNP, MBP, MOBP, MOG, and PLP (59%), HSP60 and HSP70 (38%), and the 68-kD neurofilament (3%). The antibody response in patients with MS was heterogeneous; CSF antibodies in individual patients reacted with different autoantigens. These autoantibodies were locally synthesized in the CNS and were of the immunoglobulin G class. Finally, we found that treatment with steroids decreased autoantibody reactivity, epitope spreading, and intrathecal autoantibody synthesis.

Conclusions: These studies provide a new avenue to investigate the local antibody response in the CNS, which may serve as a biomarker to monitor both disease progression and response to therapy in MS. ***Neurology*® 2012;78:532–539**

GLOSSARY

AI = antibody index; **HSP** = heat shock protein; **Ig** = immunoglobulin; **MS** = multiple sclerosis; **ODN** = other noninflammatory neurologic condition; **PCA** = principal component analysis; **RFU** = relative fluorescence units; **RRMS** = relapsing-remitting multiple sclerosis; **VZV** = varicella zoster virus.

Antibodies and B cells play an important role in the pathogenesis of multiple sclerosis (MS). Clonally expanded B cells are found in the lesions and the CSF of patients with MS,[1] and B-cell follicles have been described in the meninges of patients with secondary progressive MS.[2] These CNS-resident B cells have been linked to the production of intrathecal antibodies of restricted specificity, detectable as oligoclonal bands.[3,4]

Antigen microarrays allow the high-throughput characterization of the antibody response using limited amounts of sample[5,6] with greater sensitivity than ELISA.[6,7] We and others have used antigen arrays to characterize the immune response in MS[7–9] and its experimental model experimental autoimmune encephalomyelitis,[9–11] other autoimmune conditions,[5,12–15] tumors,[16] and healthy individuals.[17] In our studies on MS, we found patterns of serum antibody reactivity associated with different stages and pathologic subtypes of the disease.[7] Moreover, the characterization of the serum antibody response in patients with MS led us to identify the Toll-like receptor-2/

Editorial, page 522

Supplemental data at www.neurology.org

Supplemental Data

From the Center for Neurologic Diseases (F.J.Q., M.F.F., H.L.W.), Brigham and Women's Hospital, Harvard Medical School, Boston, MA; Molecular Biology Service (G.I., M.L.) and MS Unit (G.I.), University of Sevilla, Sevilla, Spain; and Department of Immunology (I.R.C.), The Weizmann Institute of Science, Rehovot, Israel.

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poly(ADP-ribose) polymerase-1 signaling pathway as important in the promotion of neuroinflammation.[18]

Antigen arrays have been recently used to investigate the antibody response to lipids in the CSF of patients with MS and to follow the response to treatment with DNA vaccines.[8,9,19,20] To date, antigen arrays have not been used to compare the autoantibody repertoire in matched CSF and serum samples and investigate the local production of autoantibodies in the CNS. Here, we used antigen arrays to investigate the antibody response in the CSF of patients with relapsing-remitting MS (RRMS) and the effect of treatment on the local CNS antibody response.

METHODS

Patient samples. Paired CSF and serum samples were collected at the University Hospital, School of Medicine, University of Sevilla, from untreated patients with RRMS (n = 20) or patients with RRMS treated with methylprednisolone (n = 26) 2 months before sample collection. Note that both RRMS patient groups consist of different individual patients and do not include samples taken before and after treatment from the same patients. All patients with RRMS had intrathecal immunoglobulin (Ig) G secretion and IgG oligoclonal bands and did not have other autoimmune disorders. The clinical characteristics of the patients are listed in table e-1 on the *Neurology*® Web site at www.neurology.org. Control samples (n = 20) were taken from patients with other noninflammatory neurologic conditions (ONDs), including hydrocephalus, seizures, migraine, and depression. Note that the age in the control group is significantly higher than that in the RRMS group ($p < 0.05$). The mean IgG index of both the untreated and steroid-treated patients with RRMS was higher than 0.7 and higher than the IgG index in the OND group ($p < 0.05$), consistent with the production of significant amounts of IgG in the CNS that characterizes MS.

Standard protocol approvals, registrations, and patient consents. This study was approved by the institutional review board at Brigham and Women's Hospital. Written informed consent was obtained from all patients participating in the study.

Antigens. Peptides were synthesized at the Biopolymers Facility of the Department of Biological Chemistry and Molecular Pharmacology of Harvard Medical School (Boston, MA). Recombinant proteins and lipids were purchased from Sigma-Aldrich (St. Louis, MO), Abnova (Taipei City, Taiwan), Matreya LLC (Pleasant Gap, PA), Avanti Polar Lipids (Alabaster, AL USA), Calbiochem (San Diego, CA), Chemicon (Temecula, CA), GeneTex (San Antonio, TX), Novus Biologicals (Littleton, CO) Assay Designs (Ann Arbor, MI), ProSci Inc. (Poway, CA), EMD Biosciences (San Diego, CA), Cayman Chemical (Ann Arbor, MI), HyTest (Turku, Finland), Meridian Life Science (Memphis, TN), and Biodesign International (Saco, ME). The antigens used in the construction of antigen microarrays are listed in table e-2.

Antigen microarray production, development, and data analysis. The antigens listed in table e-2 were spotted in replicates of 6 on SuperEpoxy 2 slides (TeleChem, Sunnyvale, CA) as described previously.[5] The microarrays were blocked with 1% bovine serum albumin and incubated for 2 hours at 37°C with the test serum or CSF diluted 1/10 in blocking buffer. In preliminary experiments, we confirmed that the antigen arrays provide a linear response at this dilution, which can be inhibited by preincubation with increasing concentrations of soluble antigen (data not shown).[7] Similar observations have been made by other groups who have analyzed CSF antibody reactivities at a 1:10–1:20 dilution with antigen arrays,[9,20] or at a 1:1–1:15 dilution by ELISA.[21,22] In clinical laboratories, the determination of the intrathecal synthesis of antibodies directed against viral or bacterial antigens by ELISA is done using different dilutions to analyze CSF and serum samples to compensate for differences in the immunoglobulin content of these specimens. Future studies should investigate whether the use of antigen microarrays for the determination of the intrathecal production of antibodies in a clinical setup requires the use of different dilutions to analyze serum and CSF samples as well.

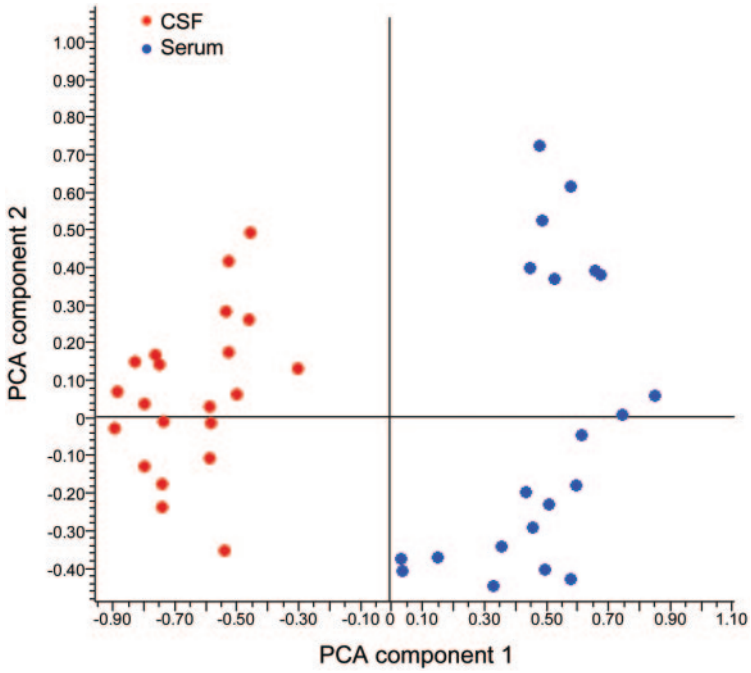
The arrays were then washed and incubated for 45 minutes with a 1:500 dilution of goat anti-human IgG Cy3-conjugated and goat anti-human IgM Cy5-conjugated detection antibodies (Jackson ImmunoResearch Laboratories). The arrays were scanned with a ScanArray 4000X scanner (GSI Luminomics, Billerica, MA). Raw data were normalized and analyzed using GeneSpring software (Silicon Genetics, Redwood City, CA). Antigen reactivity was defined by the mean intensity of binding to the replicates of that antigen on the microarray and is expressed as relative fluorescence units (RFU).

We have compared the sensitivity of the antigen-microarray technique with that of the standard ELISA technique using commercially available monoclonal and polyclonal antibodies directed against CNS, heat shock protein, and lipid antigens.[7] The antigen microarrays detect antigen reactivities at $\log_{10}$ dilutions that were 1–2 logs greater than the reactivities detected using the ELISA method.[7] These results are consistent with published reports from other groups[6,9] and demonstrate that the antigen microarray technique is more sensitive than a standard ELISA.

IgG antibodies reactive with varicella zoster virus (VZV) antigens are detectable in the CSF of patients with MS.[23] To validate the use of antigen arrays for the study of CSF antibodies, we performed a pilot experiment in which the IgG reactivity of CSF samples from RRMS and OND controls diluted 1/10 was analyzed using antigen arrays in which the ORF26 antigen from VZV was included. VZV-specific IgG positive reactivity was scored as 3 times the mean of background binding. VZV-specific IgG positive reactivity was found in 6 of 20 OND controls, compared with 10 of 11 RRMS samples ($p < 0.005$). The average IgG reactivity of RRMS CSF samples to VZV ORF26 protein was significantly higher than that of OND CSF samples ($p < 0.005$; figure e-1). These results are similar to those obtained when VZV-reactive IgG antibodies were measured in CSF by ELISA,[24] demonstrating that the antigen arrays and experimental conditions used in this study are appropriate to investigate CSF antibodies.

Calculation of IgG index and specific antibody index. The IgG index was calculated as described previously,[21] using the following formula: (IgG in CSF/IgG in serum)/(albumin in CSF/albumin in serum) = IgG index. The specific antibody index (AI) for each antigen was calculated based on the published method used to calculate the rMOG index,[21] using the following formula: (RFU in CSF/RFU in serum)/(albumin in CSF/albumin in serum) = AI. Note that the AI was calculated using a formula that does not include the IgG levels. Therefore,

Figure 1 **Different antibody repertoires are detected with antigen arrays in paired CSF and serum samples from patients with relapsing-remitting multiple sclerosis (RRMS)**



Principal component analysis (PCA) of antibodies in paired CSF and serum samples from patients with RRMS.

the AI provides antigen-specific information, distinct from that provided by total antigen unspecific IgG intrathecal production. To define a sample as positive for intrathecal antibody synthesis against a specific antigen we established a cutoff equal to the mean + 3 SD of the AI against that specific antigen measured in the OND control group.

RESULTS **Different antibody repertoires are present in paired CSF and serum samples of patients with MS.** The production of intrathecal IgG antibodies is a hallmark of MS. To characterize the antibody response in the CNS, we analyzed paired CSF and serum samples from 20 untreated patients with RRMS using antigen arrays. The antigen arrays used in these studies included 334 myelin and inflammation-related CNS antigens commonly associated with MS, CNS antigens associated with other neurologic diseases, and heat shock proteins (HSPs) (table e-2). As a first step in investigating the antibody reactivity in paired CSF and serum samples, we performed a principal component analysis (PCA) to study the relatedness of the immune response in paired CSF and serum samples from untreated patients with RRMS. In this type of analysis, the antibody reactivity in each sample (serum or CSF) is represented as a single point in a bidimensional space. The PCA of the antibody reactivity detected with antigen arrays revealed major differences in CSF and serum (figure 1). We found that the antibody reactivity in a given CSF sample was more similar to that of other CSF samples than to the reactivity of serum isolated from the same individual. These findings demonstrate the presence of a local antibody response in the CNS of patients with MS as measured by antigen arrays.

CSF antibodies in patients with MS recognize CNS antigens and HSPs. To investigate the specificity of the antibody response in the CSF of patients with MS as measured by antigen arrays, we compared the reactivity of CSF antibodies in 20 untreated patients with RRMS with the reactivity in 20 CSF samples taken from patients with ONDs. Antibody binding to an antigen in the antigen microarray was scored as positive when the RFU was equal to or higher than the mean intensity + 3 SD of the fluorescent signal directed against the same antigen detected in the CSF of OND samples. Using this approach, we found 115 antigens that were recognized by IgG or IgM antibodies in the CSF of at least one of the RRMS samples that we analyzed (table e-3, figure e-2). The distribution of these antibody reactivities was heterogeneous: all patients with RRMS had CSF antibodies that reacted with at least one antigen in the microarray, but whereas CSF samples from some patients reacted with only a few antigens in the array, others had antibodies that reacted with up to 64 antigens (table e-3, figure e-2). We found that, on average, CSF antibodies in untreated patients with RRMS reacted with 26.4 ± 4.2 antigens (mean ± SEM).

We then analyzed the frequency of the CSF antibodies to the self-antigens listed in table e-3 in RRMS and OND samples. We found that of 115 antigens recognized by CSF antibodies in at least one patient with RRMS (table e-3, figure e-2), antibodies directed against 29 antigens showed a significant increase in frequency in patients with MS vs OND controls (table 1). The targets of these antibodies were epitopes of the myelin antigens CNP, MBP, MOBP, MOG, and PLP (59%), HSP60 and HSP70 (38%), and the 68-kD neurofilament (3%).

CSF autoantibodies found in patients with RRMS are produced in the CNS. The antibodies in the CSF of patients with MS can result from the passage of serum antibodies or can also be produced by B cells in the inflamed CNS. Indeed, B-cell activation and differentiation into antibody-producing plasma cells has been described in the CNS of patients with MS, and the intrathecal production of antibodies is a hallmark of MS. To analyze the intrathecal antibody production in patients with RRMS, we calculated a specific AI[21] for the antibodies listed in table 1. The AI was calculated as the ratio between the RFU for a specific antigen in CSF and serum, corrected by the ratio between albumin levels in the CSF and serum:

Table 1 Mean RFU and frequency of CSF antibodies significantly associated with MS[a]

	Mean RFU			Frequency, %		
Antigen	OND	MS	*p* Value	OND	MS	*p* Value
HSP60 166-185	2 ± 1	59 ± 21	0.0100	0	45	0.0012
HSP60 210-229	0 ± 0	25 ± 10	0.0179	0	30	0.0202
HSP60 255-275	0 ± 0	123 ± 49	0.0162	0	30	0.0202
HSP60 286-305	17 ± 14	300 ± 73	0.0005	5	45	0.0084
HSP60 511-530	0 ± 0	48 ± 20	0.0250	0	30	0.0202
HSP60 556-575	5 ± 2	29 ± 7	0.0021	5	40	0.0197
HSP70 61-80	42 ± 30	381 ± 117	0.0079	5	35	0.0436
HSP70 166-185	6 ± 5	108 ± 41	0.0182	5	40	0.0197
HSP70 286-305	4 ± 4	68 ± 26	0.0202	5	40	0.0197
HSP70 376-395	0 ± 0	20 ± 10	0.0419	0	25	0.0471
HSP70 616-635	2 ± 2	46 ± 19	0.0254	5	50	0.0033
CNP 16-35	52 ± 23	359 ± 66	0.0001	5	50	0.0033
CNP 61-80	7 ± 3	39 ± 11	0.0058	0	30	0.0202
CNPm 376-395	5 ± 4	671 ± 152	0.0001	5	65	0.0001
MBP 166-185	258 ± 89	911 ± 162	0.0012	0	25	0.0471
MBP 301-320	57 ± 30	437 ± 106	0.0014	0	35	0.0083
MOBP 76-95	20 ± 7	132 ± 23	0.0000	0	60	<0.0001
MOBP 106-125	0 ± 0	21 ± 8	0.0221	5	35	0.0436
MOBP 151-170	1 ± 0	30 ± 12	0.0198	5	35	0.0436
MOG 16-35	0 ± 0	187 ± 59	0.0033	0	45	0.0012
MOG 35-55	0 ± 0	42 ± 11	0.0005	5	50	0.0033
MOG 181-199	164 ± 58	696 ± 156	0.0030	0	25	0.0471
Neurofilament 68 kD	0 ± 0	43 ± 20	0.0396	0	25	0.0471
PLP 1-20	25 ± 19	412 ± 116	0.0023	5	45	0.0084
PLP 16-35	10 ± 10	262 ± 73	0.0015	5	45	0.0084
PLP 31-50	2 ± 2	129 ± 48	0.0127	5	45	0.0084
PLP 106-125	0 ± 0	94 ± 29	0.0027	0	55	0.0001
PLP 225-244	0 ± 0	43 ± 19	0.0269	0	30	0.0202
PLP 391-410	68 ± 30	500 ± 100	0.0002	0	55	0.0001

Abbreviations: MS = multiple sclerosis; OND = other noninflammatory neurologic condition; RFU = relative fluorescence units.
[a] Mean RFU ± SEM and frequency of CSF antibodies that show a significant increase in frequency in MS vs OND controls.

(RFU in CSF/RFU in serum)/(albumin in CSF/albumin in serum). As shown in table 2, the 29 CSF autoantibodies that had an increased frequency in patients with RRMS had an AI higher than 200 and also showed AI values significantly higher that those measured in OND samples, suggesting that they were locally synthesized in the CNS (table 2). We found no correlation between the antibody reactivity in CSF and matched serum samples (data not shown). Thus, these data demonstrate that the CSF antibodies detected in patients with RRMS with antigen arrays are produced intrathecally.

Effect of methylprednisolone on the CNS antibody response. An important question is whether these CSF antibodies change in response to treatment. Antigen arrays have been recently used to demonstrate that myelin-reactive antibodies change after treatment of patients with RRMS with a DNA vaccine coding for MBP.[8] IV administered methylprednisolone is commonly used to treat MS exacerbations. Thus, we asked whether treatment with steroids, a standard anti-inflammatory treatment given to patients with MS, was associated with changes in the CSF antibody response. We studied the intrathecal antibody response detected with antigen arrays in untreated patients with RRMS and in samples from patients with RRMS treated with methylprednisolone 2 months before sample collection. Note that both RRMS patient groups consist of different individual patients and do not include samples taken before and

Table 2 Mean AI and frequency of intrathecal antibody production in MS and OND[a]

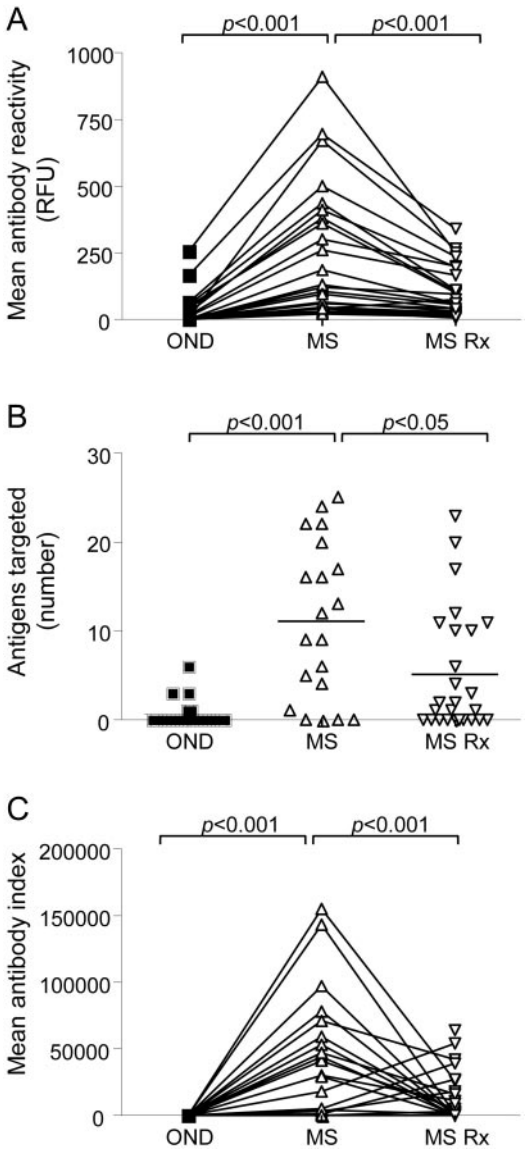
	Mean AI			Frequency, %		
Antigen	OND	MS	*p* Value	OND	MS	*p* Value
HSP60 166-185	13 ± 5	7,555 ± 3,507	0.0380	5	55	0.0012
HSP60 210-229	3 ± 0	4,489 ± 1,832	0.0191	0	80	<0.0001
HSP60 255-275	3 ± 0	22,819 ± 10,730	0.0401	0	75	<0.0001
HSP60 286-305	74 ± 59	67,547 ± 21,425	0.0032	5	45	0.0084
HSP60 511-530	3 ± 0	6,986 ± 5,407	0.2042	0	80	<0.0001
HSP60 556-575	18 ± 8	5,065 ± 1,558	0.0025	5	70	<0.0001
HSP70 61-85	49 ± 43	92,917 ± 28,329	0.0023	5	55	0.0012
HSP70 166-185	2 ± 0	11,591 ± 6,053	0.0631	0	65	<0.0001
HSP70 286-305	18 ± 16	4,084 ± 2,091	0.0593	5	45	0.0084
HSP70 376-395	2 ± 1	237 ± 162	0.1553	0	50	0.0004
HSP70 616-635	8 ± 6	2,992 ± 1,087	0.0092	5	70	<0.0001
CNP 16-35	208 ± 99	92,252 ± 18,898	0.0000	5	65	0.0001
CNP 61-80	21 ± 8	4,497 ± 1,675	0.0111	0	70	<0.0001
CNP 376-395	23 ± 18	181,606 ± 54,209	0.0019	5	70	<0.0001
MBP 166-185	813 ± 334	241,256 ± 54,202	0.0001	0	70	<0.0001
MBP 301-320	228 ± 122	120,421 ± 30,400	0.0003	5	65	<0.0001
MOBP 76-95	46 ± 22	23,328 ± 8,144	0.0069	5	50	0.0033
MOBP 106-125	4 ± 1	2,632 ± 1,894	0.1733	5	85	<0.0001
MOBP 151-170	2 ± 0	5,810 ± 3,116	0.0701	0	80	<0.0001
MOG 16-35	4 ± 0	47,832 ± 17,989	0.0115	0	75	<0.0001
MOG 35-55	3 ± 2	3,957 ± 2,102	0.0676	5	65	0.0001
MOG 181-199	554 ± 229	108,559 ± 38,940	0.0086	5	60	0.0004
Neurofilament 68 kD	2 ± 1	8,159 ± 5,763	0.1650	0	50	0.0004
PLP 1-20	116 ± 82	80,654 ± 30,537	0.0121	5	45	0.0084
PLP 16-35	2 ± 1	47,713 ± 16,890	0.0075	0	70	<0.0001
PLP 31-50	12 ± 10	24,725 ± 10,542	0.0245	5	70	<0.0001
PLP 106-125	3 ± 1	17,096 ± 7,551	0.0294	0	80	<0.0001
PLP 225-244	3 ± 0	9,905 ± 3,935	0.0163	0	70	<0.0001
PLP 391-410	257 ± 116	144,472 ± 38,644	0.0006	5	65	0.0001

Abbreviations: AI = antibody index; MS = multiple sclerosis; OND = other noninflammatory neurologic condition.
[a] Mean AI ± SEM and frequency of intrathecal antibody production in MS and OND.

after treatment from the same patients. We found that patients with RRMS treated with methylprednisolone showed reduced mean antibody reactivity to those antigens that were preferentially recognized by untreated patients with RRMS (figure 2A). We also found a significantly reduced number of antigens targeted by antibodies (figure 2B) and lower AI (figure 2C) in CSF samples from methylprednisolone-treated patients with RRMS.

DISCUSSION Increased IgG levels in the CSF and oligoclonal bands are classic immune features of MS, but the reactivity of CSF antibodies and their response to treatment are not well understood. The specificity of the intrathecal antibody response in patients with MS and in particular its reactivity with myelin antigens is a matter of debate. Myelin-reactive antibodies have been detected in the CSF of patients with MS,[25] but the identity of their target antigens differs, depending on the study. CSF antibodies have been reported to react with MOG,[26] MBP,[27] and PLP.[28] In addition, CSF B cells reactive with MBP,[29] MAG,[30] and MOG[31] have been found in patients with MS. However, it has also been reported that CSF antibodies do not react with MOG, MBP, or PLP.[25,32] We used antigen arrays, a high-throughput method that shows high sensitivity,[6,7] to analyze paired CSF and serum samples and investigate the local autoantibody response in the CNS. We found a CSF-specific antibody response in patients with RRMS directed against CNS antigens and

Figure 2 **Treatment with methylprednisolone reduces the intrathecal antibody response to CNS antigens**



Mean antibody reactivity (A), number of antigens targeted (B), and mean antibody index (C) in the CSF of other noninflammatory neurologic conditions (OND), untreated patients with RRMS (MS), and patients with RRMS treated with methylprednisolone (MS Rx) directed against the antigens listed in tables 1 and 2. RFU = relative fluorescence units

HSPs. Using a similar approach, antibodies to myelin antigens and lipids have been recently investigated in the CSF of patients with MS.[8,9,19,20] Collectively, these data demonstrate the existence of autoantibodies in the CSF that react with peptides derived from myelin and nonmyelin CNS antigens. It remains to be determined whether these epitopes are targeted by antibodies in vivo during the course of MS. However, antibodies reacting with linear epitopes (peptides) from MOG and MBP have been detected in MS brain lesions[33]; thus, it is possible that the antibodies detected with antigen arrays react with CNS-derived peptides in the lesions present in patients with MS.

Because of their increased sensitivity,[6,7] antigen arrays are likely to detect a higher number of self-reactive antibodies in CSF than those detected with other methods. However, the self-reactivity detected in CSF antibodies using antigen arrays is likely to underestimate the total autoreactivity of CNS antibodies in patients with RRMS, because CNS autoantibodies might be retained in the parenchyma as a result of antigen-specific or other interactions.[26] It is also possible that CSF antibodies recognize CNS antigens that were not included in our arrays.

Polyreactivity has been described for B cells and T cells in MS and other autoimmune disorders.[34] For example, monoclonal antibodies derived from the CSF of patients with MS have been reported to cross-react with CNS antigens that bear no sequence similarity. The analysis of the reactivity of Fab fragments generated from B cells clonally expanded in the CSF of patients with MS revealed that 2 of 9 MBP-reactive Fabs were also reactive to glial fibrillary acidic protein and 2′,3′-cyclic nucleotide 3′-phosphodiesterase, suggesting that a significant proportion of the self-reactive antibodies in the CSF are polyreactive.[35] Considering that most of the RRMS CSF samples analyzed in this study showed antibody reactivity to more than one antigen, it is possible that a fraction of the CNS-reactive CSF antibodies detected with antigen arrays are polyreactive.

We found that the CSF antibody response detected with antigen arrays was heterogeneous, i.e., different antigens were recognized by CSF antibodies in different patients. The CSF antibody response has also been found to be extremely heterogeneous when CSF samples from patients with RRMS were analyzed using 2-dimensional electrophoresis[36] or phage-displayed mimotopes.[37] Possibly, the intrathecal autoantibody response in RRMS results from the release of self-antigens during the T cell–mediated attack on the CNS. Alternatively, these autoantibodies could be a by-product of the local reactivation of Epstein-Barr virus or of B-cell activation by factors in the inflamed CNS (reviewed in Meinl et al.[38]).

The role played by CSF autoantibodies in MS is unknown. CNS-reactive antibodies have been described as pathogenic or reparative[39,40] or might be an epiphenomenon. However, because of its dependency on T helper cells, the production of IgG autoantibodies is likely to reflect the activity of the T-cell autoimmune response that contributes to the devel-

opment of MS. Indeed, it has been recently reported that myelin-reactive antibodies change after modulation of the immune response in patients with RRMS with a DNA vaccine coding for MBP.[8] We found that steroid treatment was linked to a decrease in antibody reactivity, epitope spreading, and local synthesis of IgG in the CNS. Thus, these studies provide a new avenue to investigate the local immune response in the CNS and may identify biomarkers to monitor both disease progression and response to therapy in MS.

AUTHOR CONTRIBUTIONS

Dr. Quintana wrote the manuscript, designed the study, contributed vital reagents/tools and analyzed the data. Dr. Farez designed the study and acquired and analyzed the data. Dr. Izquierdo designed the study and contributed vital reagents/tools. Dr. Lucas designed the study and contributed vital reagents/tools. Dr. Cohen designed the study. Dr. Weiner wrote the manuscript, designed the study, contributed vital reagents/tools, and analyzed the data.

DISCLOSURE

Dr. Quintana receives research support from EMD Serono, Inc., the National MS Society, and the NIH. Dr. Farez reports no disclosures. Dr. Izquierdo serves on scientific advisory boards for Biogen Idec, Bayer Schering Pharma, sanofi-aventis, Novartis, Merck Serono, and Teva Pharmaceutical Industries Ltd. Dr. Lucas reports no disclosures. Dr. Cohen serves on a scientific advisory board for Immune Array. Dr. Weiner serves as a consultant for Nasvax, Biogen Idec, EMD Serono, Inc., Genzyme Corporation, Teva Pharmaceutical Industries Ltd., Novartis, and GlaxoSmithKline; has received funding for travel or speaker honoraria from Nasvax, GlaxoSmithKline, Merck Serono, and The Guthy Jackson Charitable Foundation; serves on the editorial boards of *Multiple Sclerosis*, the *Journal of Immunology*, and the *Journal of Autoimmunity*; and receives research support from EMD Serono Inc., the NIH (NINDS, NIAID), and the National Multiple Sclerosis Society.

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