Serum autoantibodies to myelin peptides distinguish acute disseminated encephalomyelitis from relapsing-remitting multiple sclerosis

Keith Van Haren[1,2], **Beren H Tomooka**[3,4], **Brian A Kidd**[3], **Brenda Banwell**[5], **Amit Bar-Or**[6], **Tanuja Chitnis**[7], **Silvia N Tenembaum**[8], **Daniela Pohl**[9], **Kevin Rostasy**[10], **Russell C Dale**[11], **Kevin C O'Connor**[12], **David A Hafler**[12], **Lawrence Steinman**[1], and **William H Robinson**[3,4]

[1]Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Palo Alto, USA

[2]Division of Child Neurology, Lucile Packard Children's Hospital, Palo Alto, USA

[3]Division of Immunology and Rheumatology, Department of Medicine, Stanford University School of Medicine, Palo Alto, USA

[4]Geriatric Research Education and Clinical Center (GRECC), VA Palo Alto Health Care System, USA

[5]Department of Pediatrics, Division of Neurology, Children's Hospital of Philadelphia, USA

[6]Partners Pediatric MS Center, Department of Child Neurology, Massachusetts General Hospital, Boston, USA

[7]Montreal Neurological Institute and Hospital, McGill University, Montreal, Canada

[8]Department of Neurology, Dr Juan P Garrahan National Pediatric Hospital, Argentina

Corresponding author: William H Robinson MD PhD, wrobins@stanford.edu, Veterans Affairs Palo Alto Health Care System, Building 101, Room C4-185, 3801 Miranda Avenue, Palo Alto, CA 94304-1207.

[9]Division of Neurology, Children's Hospital of Eastern Ontario, University of Ottawa, Ontario, Canada

[10]Department of Pediatrics, Medical University Innsbruck, Innsbruck, Germany

[11]Department of Paediatrics & Child Health, University of Sydney, Australia

[12]Department of Neurology, Yale School of Medicine, New Haven, USA

Abstract

Background and objective—Acute disseminated encephalomyelitis (ADEM) and relapsing remitting multiple sclerosis share overlapping clinical, radiologic, and laboratory features at onset. Because autoantibodies may contribute to the pathogenesis of both diseases, we sought to identify autoantibody biomarkers capable of distinguishing them.

Methods—We used custom antigen arrays to profile anti-myelin-peptide autoantibodies in sera derived from individuals with pediatric ADEM ($n = 15$), pediatric multiple sclerosis ($n = 11$), and adult multiple sclerosis ($n = 15$). Using isotype-specific secondary antibodies, we profiled both IgG and IgM reactivities. We used Statistical Analysis of Microarrays to confirm differences in autoantibody reactivity profiles between ADEM and multiple sclerosis samples. We used Prediction Analysis of Microarrays to generate and validate prediction algorithms based on the autoantibody reactivity profiles.

Results—ADEM was characterized by IgG autoantibodies targeting epitopes derived from myelin basic protein, proteolipid protein, myelin-associated oligodendrocyte basic glycoprotein, and alpha-B-crystallin. In contrast, multiple sclerosis was characterized by IgM autoantibodies targeting myelin basic protein, proteolipid protein, myelin-associated oligodendrocyte basic glycoprotein, and oligodendrocyte specific protein. We generated and validated prediction algorithms that distinguish ADEM serum (sensitivity 62–86%; specificity 56–79%) from multiple sclerosis serum (sensitivity 40–87%; specificity 62–86%) on the basis of combined IgG and IgM anti-myelin autoantibody reactivity to a small number of myelin peptides.

Conclusions—Combined profiles of serum IgG and IgM autoantibodies identify myelin antigens that may be useful for distinguishing multiple sclerosis from ADEM. Further studies are required to establish clinical utility. Further biological assays are required to delineate the pathogenic potential of these antibodies.

Keywords

Acute disseminated encephalomyelitis (ADEM) and relapsing remitting multiple sclerosis (MS) are distinct forms of autoimmune-mediated demyelination, yet they may share polysymptomatic neurological deficits, multifocal white matter lesions and laboratory findings. This can render initial prediction of monophasic outcome (typical of ADEM) versus prediction of relapsing-remitting MS, difficult and observation over time is essential to define outcome. An improved understanding of the biological factors that distinguish transient immune targeting of the CNS from chronic CNS-directed immunity has both

diagnostic and potential therapeutic implications. Molecular biomarkers that can distinguish between MS and ADEM are therefore highly sought after.[1–3]

The etiology of MS is multi-factorial, with a role for both genetic and environmental factors. The natural history of MS involves episodic exacerbations overlying a chronic and progressive decline, though life-long immunomodulatory therapy can moderately attenuate the disease. The current diagnosis of MS is based on clinical and radiologic confirmation of relapsing inflammatory demyelination that occurs in multiple CNS regions separated in both space and time.[4] In contrast, ADEM is a monophasic disorder in more than 90% of cases and affects primarily children. Many patients report recent upper respiratory illness, prompting consideration of an infectious trigger for the immune response. The criteria for diagnosing ADEM remain a subject of debate, though many clinicians apply the consensus criteria proposed by a 2007 international panel of experts who describe the disorder as a first episode of inflammatory demyelination with multi-focal CNS involvement and encephalopathy.[5]

In both ADEM and MS, autoantibodies to specific myelin proteins and lipids may contribute to disease pathogenesis[6–9] and activate complement in some patients.[10–12] We have developed antigen arrays for profiling autoantibodies in a variety of autoimmune diseases, including demyelinating disorders.[13–15] Preliminary studies suggest that the two diseases have distinct autoantibody profiles,[2, 16] including differences in immunoglobulin (Ig) subtype.[1, 8, 9, 12, 17–19] Based on the available literature, we hypothesized that IgG and IgM autoantibodies in ADEM and MS patients might offer disease-specific profiles. In this study, we use antigen arrays to identify serum anti-myelin autoantibody profiles that can distinguish between ADEM and MS.

Materials and methods

Patient samples

Archived serum samples from patients with pediatric ADEM (n=15), pediatric MS (n=11), or adult MS (n=15) were available for analysis. Demographic data are summarized in Table 1. The samples were collected at several sites under protocols approved by the local Institutional Review Board. Informed consent was obtained from all subjects. Samples were stored at −80°C until used. Neurological history, relapse features, neurological examination, MRI, and CSF findings were reviewed when available. Each clinical diagnosis was validated using the data from the clinical intake form or available records. Patients diagnosed with pediatric or adult MS met the 2001 McDonald criteria for lesion dissemination in time and space at the time blood was drawn.[20] Pediatric MS was defined as cases of MS with an onset of symptoms prior to 18 years of age. The definitions proposed by the International Pediatric MS Study Group were used to distinguish ADEM, and pediatric MS [9]. None of the pediatric or adult MS patients were taking disease modifying therapies at the time of sample acquisition. Treatment data was not available for two pediatric MS patients. Patients were diagnosed with ADEM if they experienced a polysymptomatic inflammatory demyelinating event accompanied by encephalopathy, seizure or coma without subsequent evidence of further inflammatory disease. ADEM patients were drawn from four international sites located in Argentina, Canada, Germany,

and the United Kingdom. Adult MS patients were diagnosed at a single center in the United States. Pediatric MS cases were drawn from the USA, Germany, Russia, and Canada. Samples were classified as acute, if collected within 90 days of an attack, and convalescent if >90 days after an attack.

Antigen arrays

We generated antigen arrays that contained overlapping peptide sequences derived from several myelin proteins, each selected based on previously published reports of possible antigenicity in demyelinating disorders: myelin basic protein (MBP)[12, 17, 21], proteolipid protein (PLP)[21, 22] myelin-associated oligodendrocyte basic protein (MOBP) [23], myelin oligodendrocyte glycoprotein (MOG)[2, 12], 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase)[24], oligodendrocyte specific protein (OSP)[25], and the heat shock protein alpha-B-crystallin (abCrys)[15]. Whole-protein human IgG, IgA, and IgM were included as positive controls, and influenza virus, mouse, bovine, and chicken proteins as negative controls. The full set of peptides and proteins are listed in Supplemental Table 2. In total, each array contained 125 unique peptide sequences, with each putative antigen printed twice. Thus, each serum sample was screened against a total of 264 features (Supplemental Table 1). The antigen arrays were custom ordered from JPT Peptide Technologies, Inc (Berlin, Germany), which uses short molecular strands to fasten and orient each individual peptide and protein to the glass array, a technique that ensures a high degree of uniformity in protein orientation and concentration.[26] Using these custom arrays, we profiled autoantibodies in patient sera as previously described.[13] Briefly, we blocked the arrays overnight at 4 °C in PBS with 0.5% Tween-20 and 3% FCS, probed them for 1.5 hours at 4 °C with 300uL of 1:150 dilutions of the patient sera, and labeled them for 1 hour at 4 °C with 300uL of a 1:1000 dilution of Cy3-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories, Inc. West Grove, PA, USA) and a 1:1500 dilution of Cy5-conjugated goat anti-human IgM (Jackson ImmunoResearch Laboratories, Inc. West Grove, PA, USA). Representative array results are shown in Supplemental Figure 1. The probed arrays were scanned with a GenePix 4000B scanner (MDS Analytical Technologies, Sunnyvale, CA, USA). Antibody reactivity was quantified in fluorescence units (FU) by using GenePix Pro 5.0 software. Mean values for antibody reactivity to each peptide or protein were calculated by averaging the median pixel intensity (median intensity of the feature minus the median intensity of background) from each feature, as measured at two distinct locations on each array. We used these antigen arrays to probe the serum of 41 patients in two array experiments on two separate occasions. From these data sets, we empirically selected non-overlapping patient cohorts (Supplemental Table 3) to generate and validate disease-specific prediction algorithms as described below.

Data analysis

We used Significance Analysis of Microarrays (SAM) software (version 3.09, http://www-stat.stanford.edu/~tibs/SAM/) and the statistical package R (version 3.10) to identify antigens whose autoantibody reactivity differed between patient groups, with statistical accuracy presented as false discovery rate ("q"). We arranged the results of the SAM analysis into relationships by using the hierarchical clustering software Cluster® (http://rana.lbl.gov/EisenSoftware.htm), and then displayed the results of the Cluster analysis as

autoantigen heatmaps by using TreeView® software (http://rana.lbl.gov/EisenSoftware.htm).

To identify a subset of serum autoantibody specificities that can correctly determine whether the tested serum sample is from a patient with ADEM or from a patient with MS (i.e. "diagnostic class prediction"), we used Prediction Analysis of Microarrays (PAM) software (version 2.1 http://www-stat.stanford.edu/~tibs/PAM/), as previously described.[27] Briefly, after completing two large array experiments (Supplemental Table 3), we divided our ADEM and adult MS patients into two non-overlapping cohorts (Figure 2 and Supplemental Table 3). This allowed us to create completely independent "training" and "validation" groups such that our validation cohorts never contained patients from our training cohorts. Moreover, to replicate real-world interassay variability, our training and validation cohorts were drawn from array experiments performed on separate dates. To allow for a larger validation cohort, we analyzed the pediatric MS samples only in the validation experiments. The allotment of patients to Cohort 1 and Cohort 2 was determined empirically before beginning the analysis. We next applied the PAM algorithm to the autoantibody profiles of the "training" sample set, allowing PAM to establish mean levels of autoantibody reactivity to each protein or peptide in each group of patients with a given diagnosis (i.e. class). We narrowed the hierarchical list of antigens generated by PAM to the final list of classifying antigens by using the cross-validation results (Supplemental Figure 2) to select the detection threshold that offered the highest sensitivity and specificity while using the smallest number of classifying antigens. We then applied this final algorithm to our independent patient cohorts to determine whether our PAM analysis of autoantibody reactivity to this subset of differentiating antigens could enable "class prediction" (ADEM vs MS and ADEM vs Ped MS) (Figure 2). To validate further, we used the same process in reverse, training with the original validation cohorts and validating with the original training cohorts (Figure 2 and Supplemental Table 3).

Results

ADEM and MS serum have distinct anti-myelin autoantibody profiles

To compare serum autoantibody profiles in ADEM and MS, we used antigen arrays to profile IgG and IgM autoantibodies in sera from individuals with pediatric ADEM, pediatric MS, or adult MS. We performed two large independent array experiments (Supplemental Table 3). SAM analysis of the autoantibody profiles comparing ADEM vs adult MS and ADEM vs pediatric MS showed that several antigens were differentially targeted by autoantibodies in ADEM sera compared to those in MS sera (Figure 1; Supplemental Figure 3). SAM analysis comparing the adult and pediatric MS cohorts did not yield any significant differences (data not shown). In Cohort 1, six antigens were differentially targeted by IgG antibodies (q<8.8%) and 15 by IgM antibodies (q<23.1%) in ADEM sera compared to adult MS sera (Figures 1A & 1B). The IgG-targeted antigens comprised 3 MBP peptides (amino-acid sequences 81–100; 150–171; 180–198), 2 MOBP peptides (1–20; 171–183), and 1 abCrys peptide (110–131). The IgM-targeted antigens comprised 6 OSP peptides (81–100; 91–110; 111–130; 121–140; 161–180), 3 MOBP peptides (11–30; 101–120; 111–130), 3 PLP peptides (21–40; 151-–170; 231–250), MOG peptide (N-terminus), 1 abCrys peptide

(81–100), and 1 CNPase peptide (356–375). Analysis of the antibody profiles obtained from a second, confirmatory experiment (Cohort 2) yielded similar results (Supplemental Figure 3). Thirteen antigens (q<16.7%) were differentially targeted by IgG antibodies in ADEM sera compared to pediatric MS sera: 7 MBP peptides (1–20; 51–70; 81–100; 111–129; 140–161; 150–171; 180–198), 3 MOBP peptides (1–20; 71–90; 161–180), 2 abCrys peptides (110–131; 161–176), and 1 CNPase peptide (343–362) (Supplemental Figure 3A). Six antigens (q<33.3%) were differentially targeted by IgM antibodies: 3 OSP peptides (91–110; 111–130; and 161–180), 1 PLP peptide (151–170), 1 MOBP peptide (171–183), and 1 abCrys peptide (81–100) (Supplemental Figure 3B).

Together, these findings suggest that ADEM and MS have distinct profiles of autoantibody reactivity to myelin peptides. Notably, we found that nearly all of the differentiating IgG-targeted antigens showed higher autoantibody reactivity among ADEM patients (Figures 1A & 1C and Supplemental Figure 3A), whereas nearly all of the differentiating IgM-targeted antigens showed higher autoantibody reactivity among MS patients (Figures 1B & 1D and Supplemental Figure 3B). We had noted a similar pattern in our preliminary studies in which we found higher myelin-specific IgG reactivity among ADEM patients (Supplemental Figures 4A & 4D) compared to MS. Analysis of these reactivity profiles using PAM yielded prediction algorithms (Supplemental Figures 4B & 4E) with greater sensitivity for ADEM than MS patients (Supplemental Figures 4C & 4F). These preliminary results (Supplemental Figure 4A–F), together with previous observations of elevated IgM levels in the CSF and serum of patients with acquired demyelinating syndromes,[8, 9, 12, 18, 28, 29] provided our original impetus for profiling IgM antibodies in addition to IgG antibodies in ADEM and MS with the goal of achieving greater sensitivity for MS autoantibody reactivity profiles.

Distinct autoantibody profiles can be used to distinguish ADEM from MS

We used the prediction software PAM to determine whether the autoantibody profiles we identified could be used to reliably classify a patient's clinical diagnosis as ADEM or MS. Using samples from 7 adult MS patients and 7 ADEM patients as the "training" cohort, PAM generated a prediction algorithm, comprising 5 IgG-targeted antigens (MBP 150–171; PLP 201–220; MOG 51–70; abCrys 1–20; abCrys 110–131) and 3 IgM-targeted antigens (MOBP 11–30; MBP 31–50; MOBP 61–80) (Figure 2). We then validated this algorithm using an independent set of 27 patient samples from Cohort 1. Our algorithm correctly classified 5/8 (62%) ADEM patients, 7/8 (87%) adult MS patients, and 8/11 (73%) pediatric MS patients (Figure 2, Prediction on Cohort 1). The sensitivity (SN) and specificity (SP) for each patient group in this analysis were as follows: ADEM SN 62%, SP 79%; adult MS SN 87%, SP 62%; pediatric MS SN 73%, SP 62%.

Conversely, when we performed this prediction in reverse, by using samples from Cohort 1 as the training group, we generated a classification algorithm comprising 1 IgG-specific (abCrys 110–131) antigen and 3 IgM-specific (PLP 231–250; OSP 161–180; MOBP 11–30) antigens (q<1.6) (Figure 2). We then validated this algorithm using an independent set of 25 patient samples from Cohort #2. This algorithm correctly classified 6/7 (86%) ADEM patients, 5/7 (71%) adult MS patients, and 4/10 (40%) pediatric MS patients (Figure 2, Prediction on Cohort #2). The sensitivity and specificity for each patient group in this

prediction analysis were as follows: ADEM SN 86%, SP 56%; adult MS SN 71%, SP 86%; pediatric MS SN 40%, SP 86%. A comparison of the prediction algorithms derived from Cohort 1 and Cohort 2 suggests that IgG and IgM antibodies targeting portions of PLP, abCrys, and MOBP provide the greatest diagnostic value in our sample (Figure 2; Supplemental Table 4).

In total, our SAM and PAM analyses from Cohorts 1 and 2 resulted in 8 independently generated lists of antigens (6 lists from SAM analyses and 2 lists from PAM training analyses). Several of these antigens appeared in more than one statistical analysis (Supplemental Table 4). Two antigens appeared in both prediction matrices: IgM-targeted MOBP 11–30 and IgG-targeted abCrys 110–131. Both of these antigens had higher antibody reactivity with ADEM sera than MS sera.

Discussion

Using antigen arrays, we identified distinct profiles of anti-myelin autoantibody reactivity in a trial population of ADEM and MS patients. We used these profiles to generate prediction algorithms based on a small number of antigens capable of distinguishing our ADEM and MS patients with reasonable accuracy and reproducibility within this trial population. Several myelin autoantigens were validated in multiple analyses and should be considered for use in future studies seeking to develop diagnostic tools for classifying acquired demyelinating syndromes. This represents, to our knowledge, the first use of autoantibody array technology to distinguish between clinically distinct demyelinating syndromes.

Our results indicate that MS is characterized by the presence of serum autoantibodies of the IgM isotype targeting PLP, MOBP, and OSP, whereas ADEM is characterized by serum autoantibodies of the IgG isotype targeting MBP and MOBP. Because IgM is a non-class-switched isotype, our findings indicate that the autoantibody response to MBP in MS has not undergone the class-switching that is typically observed in antigen-driven immune responses, consistent with previous findings that many of the autoantibodies observed in MS are of the IgM isotype.[8, 9, 12, 18, 19, 28] In contrast, that ADEM is characterized by class-switched IgG responses to MBP and MOBP supports the notion that the immune response is primed during the prodromal illness that often precedes the onset of demyelination in ADEM. These findings are consistent with recent work demonstrating that levels of clonal IgG are higher in the serum of ADEM patients than in that of MS patients.[3] Several of us (BB, AB, DP, KR) have previously described higher levels of IgG reactivity in monophasic ADEM patients that disappear in the months following event resolution, yet persist in a small subset of MS patients.[30, 31] In the present study, both ADEM and pediatric MS patients had blood collected within the same proximity to a demyelinating event (3 months or less), yet only the ADEM patients demonstrated the tendency toward elevated IgG reactivity. This suggests that the higher IgG reactivity observed in our ADEM cohorts is not simply an artifact of disease acuity.

Identifying biologically relevant antigens is a critical early step in developing autoantibody-based biomarker tests. In the current study, several myelin antigens were implicated in multiple analyses (Supplemental Table 4). Of the MBP peptides that were selectively

targeted in our arrays, MBP 81–100 has the greatest precedence given the well-characterized encephalitogenic potential.[32] The presence of peptides outside this region (MBP peptides 51–70 and 150–171) is consistent with previous reports.[18, 29] The presence of diverse antibody reactivity in these regions may be related to the complementary roles that T-cells and B-cells, which function synergistically to recognize a variety of epitopes from both intact and damaged proteins.[33] While T-cells recognize only linear epitopes, B-cells and their antibodies recognize both linear and conformational epitopes, which do not always overlap precisely with T-cell targeted regions.[34] It is also possible that the sera of ADEM patients (and EAE mice) more accurately reflect the autoantibodies present in the CSF[3] when compared to MS patients.[29] This distinction may provide advantages when using serum autoantigen arrays for discriminating between these disorders.

IgM-targeted MOBP 11–30 and IgG-targeted abCrys 110–131 were validated in two independently generated prediction algorithms. Both of these antigens reacted more strongly with antibodies in ADEM sera than with those in MS sera. Although MBP has been previously implicated in ADEM,[1, 21, 35] this is, to our knowledge, the first report of selective targeting of MOBP in ADEM sera. Here we see MOBP peptides differentially targeted by both IgG (1–20; Figure 1A) and IgM (11–30; Figure 1B) isotype antibodies in ADEM sera, with the latter playing a prominent role in our prediction algorithms (Figure 2). Reactivity to abCrys also differed between the ADEM and MS groups. Recently, we demonstrated that the heat-shock protein abCrys may bind immunoglobulins rather than the other way around.[36] It is possible that this activity could be responsible for the binding observed at the abCrys features on the array, and thus, abCrys may not represent an "autoantigen" biomarker but rather represents a feature of heat shock protein binding.

Anti-MOG autoantibodies have been previously associated with ADEM and, to a lesser extent, MS [18, 29, 30, 37–40]. The relative paucity of MOG peptide reactivity among the patient profiles presented here is best explained by the conformation-specific nature of autoantibody reactivity to MOG epitopes[1, 37, 41], which is not preserved by the linear peptides employed on our array. In fact, Supplemental Figure 4 demonstrates increased reactivity to the whole MOG protein (cbMOG) among ADEM patients without strong reactivity to the MOG peptides on the same array, all of which is in keeping with previous studies. It is plausible that this conformational specificity may be relevant to other myelin proteins as well.[19] Similarly, post-translational modification (e.g. acetylation) can also play an important role in antigen specificity[42]. Although our array performed well with only linear epitopes, the inclusion of conformational myelin epitopes with or without post-translational modifications, might benefit the development of future prediction arrays.

The strengths of our study include our inclusion of patients with both pediatric and adult MS, our use of an innovative prediction tool, and our generation of diagnostic prediction algorithms using relatively few antigens with reasonably accurate results. Our primary study limitation was our modest sample size. It is interesting to note that while our adult MS cohort differed in temporal proximity to an acute demyelinating event compared with our pediatric ADEM and MS cohorts, we found no significant differences between our pediatric and adult MS cohorts on SAM analyses, suggesting that neither the age of the MS patient

nor the proximity to an acute event had a significant impact on autoantibody profiles in our cohorts.

Our findings support previous work[2, 3, 18] suggesting that serum autoantibody arrays may eventually provide tools capable of reliably distinguishing a diverse set of inflammatory brain diseases. Follow-up studies using antibody arrays in demyelinating disorders might proceed in several potentially complementary directions. Correlation of clinical outcomes with autoantibody profiles might be applied to the study of prognostic tools perhaps capable of predicting risk of relapse following a first demyelinating event. The delineation of biologically distinct subgroups of autoimmune demyelinating disorders, particularly if augmented with emerging biomarkers such as IL-17[43, 44], which associates with a pathological phenotype in a mouse model of MS that is induced specifically with MOG-antigen[45]. Further development and validation of autoantibody arrays may eventually produce tools capable of reliably distinguishing a diverse set of inflammatory brain diseases.

The goal of this study was to test the feasibility of using myelin autoantibody array platforms to differentiate ADEM and RRMS as distinct clinical entities. Our favorable results in a small cohort of individuals suggest these array platforms may be suitable for further development as diagnostic assays. We acknowledge, however, that clinical exam findings and MRI already set a high bar for diagnostic sensitivity and specificity in distinguishing these two disorders.[46, 47] In this context, the autoantibody prediction paradigm outlined here might also be applied toward the study of the pathophysiologic origins of ADEM and MS. While a deep exploration of ADEM and MS pathophysiology was not within the intended scope of this project, our observations implicate differential IgG and IgM targeting of several myelin peptides as potentially distinct features of these disorders. Further validation and exploration of these findings in future investigations could expand our understanding of the antigenic triggers of ADEM and MS, perhaps eventually shedding insight on the very different natural histories associated with these two disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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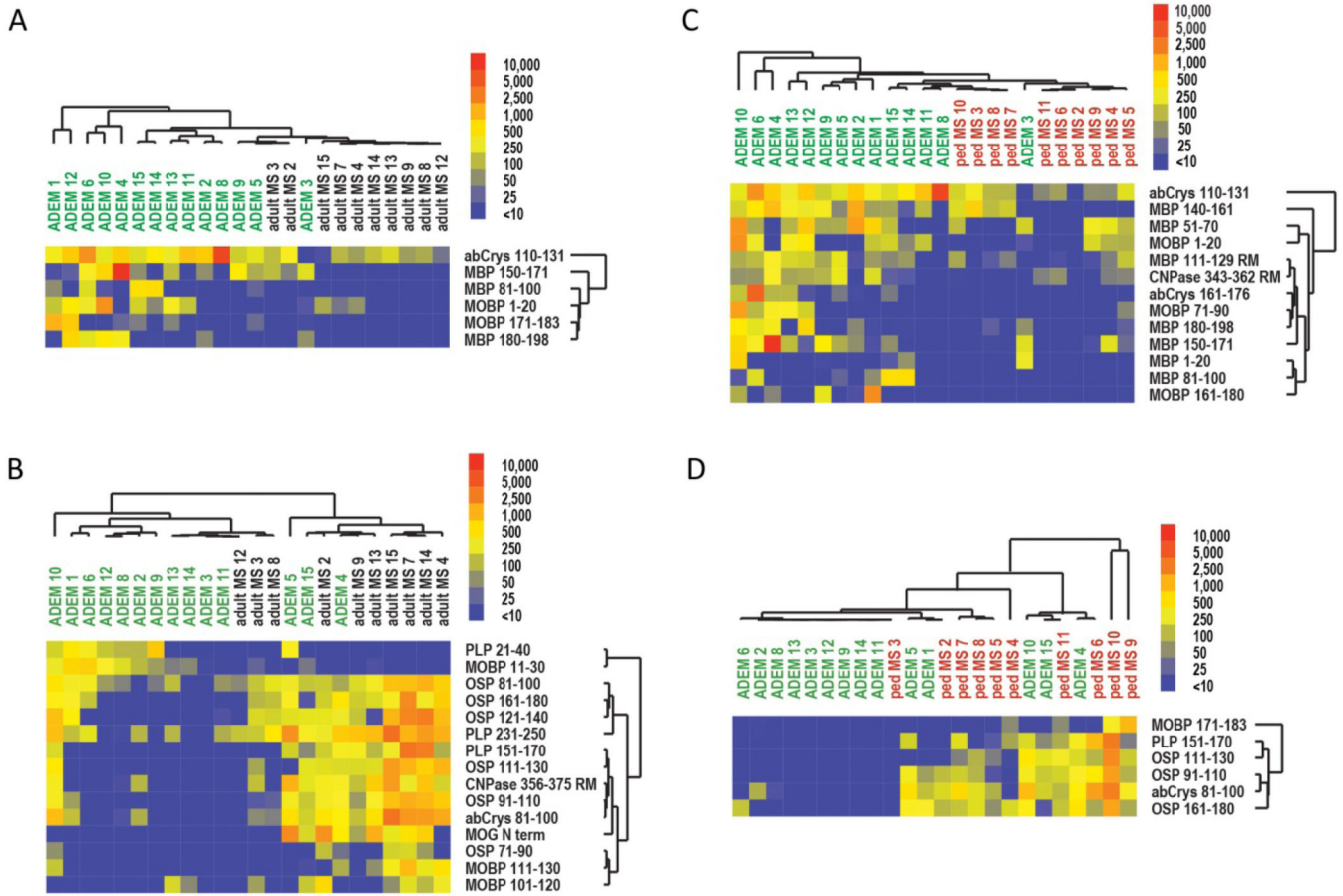
Figure 1. Differences in IgG and IgM autoantibody reactivity differentiate between ADEM and MS

SAM-generated heatmaps highlight differences between IgM- and IgG-specific autoantibody reactivity between serum samples from patients with pediatric ADEM, adult MS, or pediatric MS. Individual patients are listed above the heat map and the individual antigens are listed to the right of the heat map. Autoantibody reactivities are conveyed with blue, yellow, and red hues representing low, medium, and high reactivity respectively. Differences in serum IgG (A) and IgM (B) reactivity between patients with ADEM and patients with adult MS. Differences in serum IgG (C) and IgM (D) reactivity between patients with ADEM and patients with pediatric MS. Q values for individual SAM analyses are 8.8% in A, 23.1% in B, 16.7% in C, and 33.3% in D. MOBP, myelin-associated oligodendrocyte basic protein; abCrys, alpha-B-crystallin; PLP, proteolipid protein; OSP, oligodendrocyte specific protein; MBP, myelin basic protein; CNPase, 2',3' cyclic nucleotide phosphodiesterase.

Prediction on Cohort #1

Prediction Accuracy on Cohort #2		
ADEM	**adult RRMS**	**pediatric RRMS**
86% (6/7)	71% (5/7)	40% (4/10)

Autoantibody Reactivity Profiles from Cohort #2
ADEM (N=7); adult RRMS (N=7); pediatric RRMS (N=10, testing only)

PAM Training

Validation

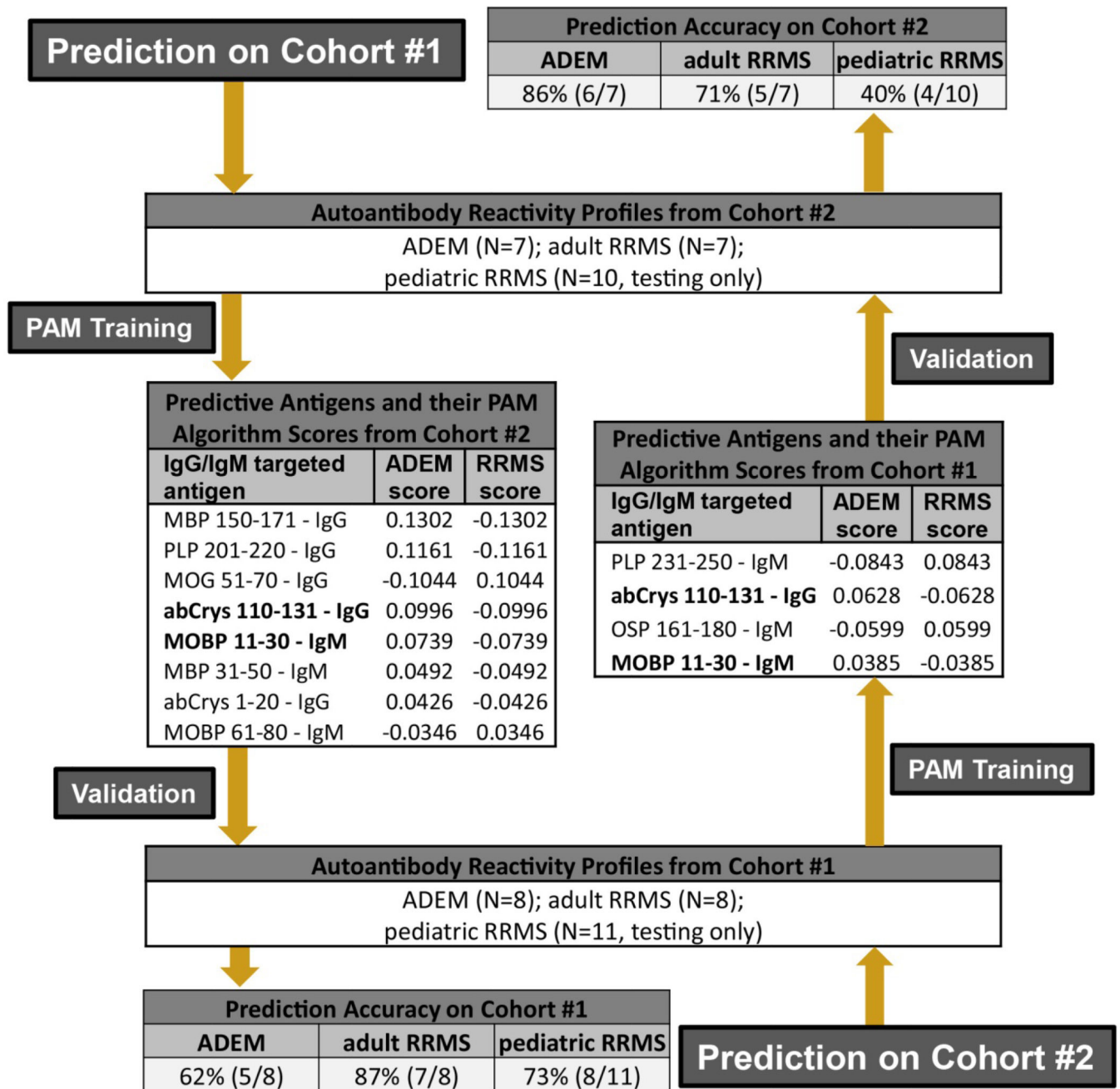| Predictive Antigens and their PAM Algorithm Scores from Cohort #2		
IgG/IgM targeted antigen	**ADEM score**	**RRMS score**
MBP 150-171 - IgG	0.1302	-0.1302
PLP 201-220 - IgG	0.1161	-0.1161
MOG 51-70 - IgG	-0.1044	0.1044
abCrys 110-131 - IgG	0.0996	-0.0996
MOBP 11-30 - IgM	0.0739	-0.0739
MBP 31-50 - IgM	0.0492	-0.0492
abCrys 1-20 - IgG	0.0426	-0.0426
MOBP 61-80 - IgM	-0.0346	0.0346

Predictive Antigens and their PAM Algorithm Scores from Cohort #1		
IgG/IgM targeted antigen	**ADEM score**	**RRMS score**
PLP 231-250 - IgM	-0.0843	0.0843
abCrys 110-131 - IgG	0.0628	-0.0628
OSP 161-180 - IgM	-0.0599	0.0599
MOBP 11-30 - IgM	0.0385	-0.0385

Validation

PAM Training

Autoantibody Reactivity Profiles from Cohort #1
ADEM (N=8); adult RRMS (N=8); pediatric RRMS (N=11, testing only)

Prediction Accuracy on Cohort #1		
ADEM	**adult RRMS**	**pediatric RRMS**
62% (5/8)	87% (7/8)	73% (8/11)

Prediction on Cohort #2

Figure 2. Performance of serum autoantibody profiles in the differential diagnosis of ADEM and MS

We used PAM to generate and validate prediction algorithms based on autoantibody reactivity profiles. For prediction on Cohort 1 (starting top left), we generated an algorithm (antigens and scores as listed) by 'training' PAM using the reactivity profiles from Cohort 2. We validated this algorithm using the autoantibody reactivity profiles from Cohort 1. The prediction accuracy of the algorithm is listed at the bottom left. To further test the validity of our autoantibody prediction paradigm, we reversed the order of training and validation (starting bottom right) such that the training analysis was done using reactivity profiles from

Cohort 1 and validated using serum samples from Cohort 2. Shown in bold are the IgG-targeted antigens that differentiated between ADEM and MS in both prediction trials. There were no overlapping patients in Cohorts 1 & 2. SN, sensitivity; SP, specificity; MOBP, myelin-associated oligodendrocyte basic protein; abCrys, alpha-B-crystallin; PLP, proteolipid protein; OSP, oligodendrocyte specific protein; MBP, myelin basic protein; CNPase, 2',3' cyclic nucleotide phosphodiesterase, MOG, myelin oligodendrocyte glycoprotein.

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Table I

Patient demographics.

	Adult MS		Ped MS			ADEM	
	Cohort #1 (*n=7*)	Cohort #2 (*n=8*)	Cohort #1 (*n=10*)	Cohort #2 (*n=11*)	Cohort #1 (*n=7*)	Cohort #2 (*n=8*)
Median age, years (range)	41(29,62)	40(18,74)	14(6,15)	14(6,15)	5(2,15)	4(1,10)
Female, %	71.4%	75%	50%	45.5%	42.9%	62.5%
Patients within 3 months of acute demyelinating event, %	14.3%	0%	80%	81.8%	85.7%	88%
Median EDSS at blood draw (range)	1(0,2.5)	2(0,4)	1(0,3.5)	1(0,3.5)	Not specified	Not specified
Median disease duration at time of blood draw, months (range)	16(1,101)	120(36,516)	10(1,36)	12(1,36)	1(0,2)	0(0,4)
Median total duration of clinical follow-up, months (range)	16(1,101)	120(36,516)	17.5(4,50)	15(4,50)	9(5,66)	31(19,61)

ADEM: acute disseminated encep halo myelitis; EDSS: Expanded Disability Status Scale; MS: multiple sclerosis; Ped MS: pediatric MS.