Distinct oligoclonal band antibodies in multiple sclerosis recognize ubiquitous self-proteins

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Oligoclonal Ig bands (OCBs) of the cerebrospinal fluid are a hallmark of multiple sclerosis (MS), a disabling inflammatory disease of the central nervous system (CNS). OCBs are locally produced by clonally expanded antigen-experienced B cells and therefore are believed to hold an important clue to the pathogenesis. However, their target antigens have remained unknown, mainly because it was thus far not possible to isolate distinct OCBs against a background of polyclonal antibodies. To overcome this obstacle, we copurified disulfide-linked Ig heavy and light chains from distinct OCBs for concurrent analysis by mass spectrometry and aligned patient-specific peptides to corresponding transcriptome databases. This method revealed the full-length sequences of matching chains from distinct OCBs, allowing for antigen searches using recombinant OCB antibodies. As validation, we demonstrate that an OCB antibody from a patient with an infectious CNS disorder, neuroborreliosis, recognized a *Borrelia* protein. Next, we produced six recombinant antibodies from four MS patients and identified three different autoantigens. All of them are conformational epitopes of ubiquitous intracellular proteins not specific to brain tissue. Our findings indicate that the B-cell response in MS is heterogeneous and partly directed against intracellular autoantigens released during tissue destruction. In addition to helping elucidate the role of B cells in MS, our approach allows the identification of target antigens of OCB antibodies in other neuroinflammatory diseases and the production of therapeutic antibodies in infectious CNS diseases.

multiple sclerosis | oligoclonal bands | proteomics | transcriptomics | autoantigens

Multiple sclerosis (MS) is a severe inflammatory disease of the central nervous system (CNS) with a presumed autoimmune pathogenesis (1–4). Oligoclonal bands (OCBs) (5–7) of the cerebrospinal fluid (CSF) are the only established immunological biomarker of MS that has a diagnostic and prognostic relevance. OCBs are expanded Ig species that contain abundant somatic hypermutations, supposedly in response to sustained antigen stimulation (8–14). They are visualized by analytical immunoblotting on isoelectric focusing (IEF) gels, but so far, no particular target antigens could be assigned to distinct OCBs (15), because OCB quantities in diagnostic lumbar punctures are too low for direct antigen searches using biochemical techniques. Furthermore, OCBs are superimposed on a background of polyclonal antibodies, precluding differentiation between signals from OCB and background antibodies. Antibody cloning from single CSF B cells (16, 17) is an elegant approach for obtaining matching heavy (H) to light (L) chains of antibodies, but it is impossible to know whether a particular antibody relates to an OCB or to the polyclonal background. Candidate target antigens detected with unfractionated CSF or with recombinant antibodies cloned from single CSF-resident B cells included lipids, proteins, combinatorial peptides, and viral products (18–23). Of note, none of these studies assigned a defined antigen to a distinct OCB antibody.

We combined refined biochemical analysis, proteomics, and transcriptomics to characterize distinct OCB antibodies for recombinant expression and antigen searches. Although we could previously relate OCB proteomes and transcriptomes showing that CSF-resident B cells produce OCBs (24), we could not assign IgG-H to -L chains of distinct OCB antibodies. Here, we overcame this limitation by copurifying disulfide-linked IgG-H and -L chains from distinct OCBs and analyzing the matching chains together by mass spectrometry. This method allowed us to relate patient-specific Ig peptides, which contained unique amino acid substitutions introduced by somatic hypermutation and V(D)J

Significance

Oligoclonal bands (OCBs) of the cerebrospinal fluid (CSF) are a hallmark of multiple sclerosis (MS). They are expanded antibody species that are detectable in >95% of patients. Because several OCB and polyclonal antibodies are present in a CSF sample, it was for technical reasons thus far not possible to isolate distinct OCBs and identify their antigens. Here we combined refined biochemical analysis, proteomics, and transcriptomics to molecularly characterize distinct OCB antibodies. We produced six recombinant OCB antibodies and characterized three autoantigens. All of them were ubiquitous intracellular proteins, not specific to brain tissue. This finding indicates that in MS, part of the OCBs do not directly mediate tissue destruction, but rather, indicate a secondary immune response.

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Conflict of interest statement: B.O. is now an employee at Biogen, but was affiliated with the Ludwig-Maximilians-University at the time the study was performed; i.e., Biogen was not a sponsor of this study. J.B. is now an employee at Bayer, but was affiliated with the Ludwig-Maximilians-University at the time the study was performed; i.e., Bayer was not a sponsor of this study. M.S. has received honoraria for lecturing and/or travel expenses for attending meetings from Bayer and Biogen Idec. T.O. has received lecture and/or advisory honoraria, as well as unrestricted MS research grants from Biogen, Novartis, Genzyme, Merck, and TEVA, none of which has had any relation to this study. W.K. has received advisory honoraria, speaker's fees, or unrestricted travel grants from Biogen, Novartis, and Merck-Serono, none of which has had any relation to this study.

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Data deposition: The IgG sequences reported in this paper have been deposited in the BioProject database, www.ncbi.nlm.nih.gov/bioproject (BioProject ID PRJNA294639). The ProtoArray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE72789).

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recombination, to the corresponding full-length Ig transcriptomes obtained from CSF B cells of the same patients (24). This strategy revealed full-length cDNA sequences of matching Ig chains and allowed us to produce recombinant OCB (rOCB) antibodies. As proof of concept, we show that an OCB antibody from a patient with neuroborreliosis (NB) binds to a protein from *Borrelia* (25). Then, we characterized six rOCB antibodies from four MS patients and identified three different OCB target autoantigens. Their common feature is that they are intracellular proteins that are expressed in many cell types, suggesting that part of the OCB antibodies in MS are directed to antigens released through secondary tissue destruction.

Results

Characterization of Distinct OCB Immunoglobulins. A key step in the improved technology used here (Fig. 1) is the copurification of disulfide-linked IgG-H and -L chains from single OCB spots by affinity chromatography and two-dimensional (2D) gel electrophoresis under nonreducing conditions. This method yields intact H_2L_2 complexes. Although the amount of distinct OCB antibodies obtained was by far too small for direct antigen searches, it was sufficient for identifying several peptides from both chains by mass spectrometry. These peptides do not need to cover the full-length IgG-H and -L sequences. It is sufficient that some of them contain highly specific mutations introduced by somatic hypermutation and V(D)J recombination (referred to as "characteristic peptides") (24, 26). We aligned characteristic peptides to patient-specific Ig transcriptomes from CSF-resident B cells, which contain unique nucleotide mutations that correspond to the amino acid mutations of the characteristic peptides. Thus, few peptides that covered only parts of the sequences permitted unequivocal reconstruction of full-length, matching IgG-H and -L chains. Recombinant expression then yielded sufficient amounts of antibodies representing distinct OCBs for antigen searches.

We characterized six distinct H_2L_2 complexes from four patients with MS and one from a patient with NB. From a nonreducing 2D gel (Fig. 2*A*), we isolated H_2L_2 complexes, in-gel digested the chains with trypsin, and subjected the fragments to MALDI-TOF/TOF mass spectrometry (Fig. 2*B*). The amino acid sequences of >50% of the peptides were verified by tandem mass spectrometry. We then aligned the masses and sequences of the identified peptides to patient-specific Ig-transcriptome databases, which we had generated in parallel from CSF cells (Fig. 2*C*). Characteristic peptides allowed for unambiguous assignments. We expressed rOCB antibodies from MS patients MS1 (rOCB-MS1-s2, -s8, and -s9), MS2 (rOCB-MS2-s5), MS3 (rOCB-MS3-s1), and MS4 (rOCB-MS4); rOCB-NB1-s13 from NB patient NB1; and the control antibody r8-18C5, which recognizes myelin oligodendrocyte glycoprotein (MOG) (27) as full-length humanized antibodies in HEK293EBNA cells (28) (Fig. S1). For purification and detection, the IgG-H chains were extended by His_6 and V5 tags. rOCB-MS2-s5 was toxic to HEK cells and was therefore expressed as single-chain Fv (scFv) fragment in *Escherichia coli* (rOCB-scFv-MS2-s5).

An OCB from a Patient with NB Recognizes a *Borrelia* Antigen. For proof of concept, we used rOCB-NB1-s13 from a patient with NB. As expected, rOCB-NB1-s13 recognized lysates from *Borrelia* in ELISA, whereas the control antibodies r8-18C5 and rOCB-MS3-s1 yielded background signals (Fig. 3*A*). The specific signal of rOCB-NB1-s13 is relatively low because the ELISA format used here is designed for clinical routine analysis, where unfractionated CSF samples from NB patients are used to screen crude *Borrelia* lysates. Such CSF samples contain many different *Borrelia*-specific antibodies, and the lysates contain many different *Borrelia* antigens, resulting in a high overall signal. By contrast, a monoclonal antibody as used here binds only to one antigen, which is diluted in the crude lysate and, therefore, expectedly yields a lower signal. Experiments using commercially available immunoblots with different *Borrelia* antigens indicated that rOCB-NB1-s13 recognizes an antigen termed p21 by the manufacturer, which corresponds to a 21-kDa fragment of the protein BBK53 (29) from *Borrelia burgdorferi* (Fig. 3 *B* and *C*).

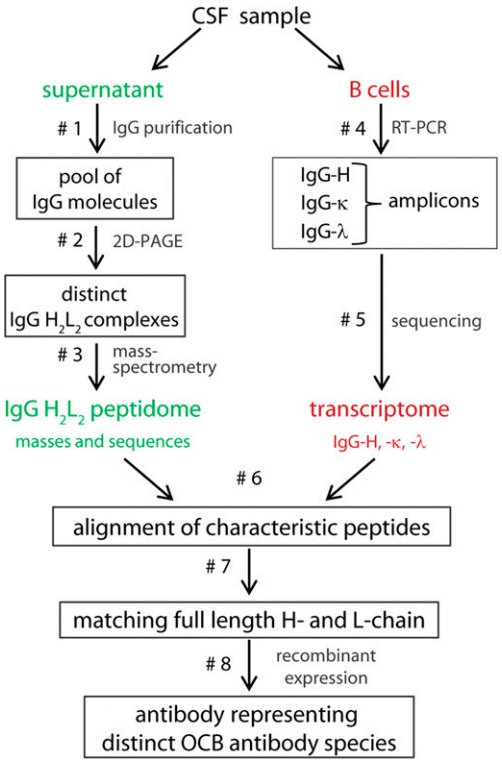

Fig. 1. Flowchart for the molecular characterization of distinct OCB antibody species. From a diagnostic CSF sample, the Ig proteome was revealed from the supernatant (*Left*; green) and the Ig transcriptome from the B cells in the pellet (*Right*; red). A pool of all IgG molecules was purified by protein G affinity chromatography and deglycosylated to yield a more homogeneous preparation (step 1). Next, a modified 2D gel electrophoresis was performed, comprising an improved IEF electrophoresis using polyacrylamide gels followed by SDS/PAGE (step 2). All steps were performed under nonreducing conditions to prevent disassembly of the IgG-H and -L chain heterodimers (H_2L_2) as far as possible. However, because of the high electrical field required for the polyacrylamide IEF gels, some disassembly of H_2L_2 complexes was still observed. H_2L_2 complexes were separated from complexes where one or more chains were dissociated (H_2L, HL, and H_2) by a second dimension of nonreducing SDS/PAGE. Spots that contained H_2L_2 complexes were excised from the stained 2D gels and in-gel digested with trypsin, and the tryptic peptides were analyzed by mass spectrometry (step 3). In parallel, the transcriptomes of IgG-H and -L chains were determined. To this end, B-lineage cells were collected, IgG-H and -L transcripts were amplified by RT-PCR (step 4), and either conventionally cloned and sequenced or subjected to next-generation sequencing (step 5). The characteristic peptides identified by mass spectrometry were then aligned to the specific Ig transcriptome database (step 6). Only when unambiguous alignments of characteristic peptides were possible (step 7), matching IgG-H and -L chains representing distinct OCB antibody species were expressed in a recombinant expression system (step 8).

Target Antigens of OCB from MS Patients. Several initial attempts to identify the target antigens of rOCB from MS patients were negative. rOCB or rFab fragments did not recognize suspected causative pathogens (Epstein–Barr, herpes simplex, measles, rubella, and zoster viruses and *Chlamydia*) or nuclear or neutrophil cytoplasmic antigens in clinical routine assays (EUROIMMUN). Similarly, tests for ssDNA, dsDNA, lipopolysaccharide, and insulin (30) and for lipid reactivity (21), Western blotting on denaturing 2D gels of human brain homogenates, and attempts to stain brain tissue by immunohistochemistry failed to yield consistent results. Owing to fixation, disulfide-reduction, and chaotropic agents, the above methods likely destroy protein secondary structures. Therefore, it may not be surprising, in retrospect, that these tests were negative, because we later found that at least rOCB-MS1-s2 and -s8 and rOCB-MS2-s5 recognize strictly conformational epitopes. Finally, we applied an unbiased searching strategy with antigen arrays

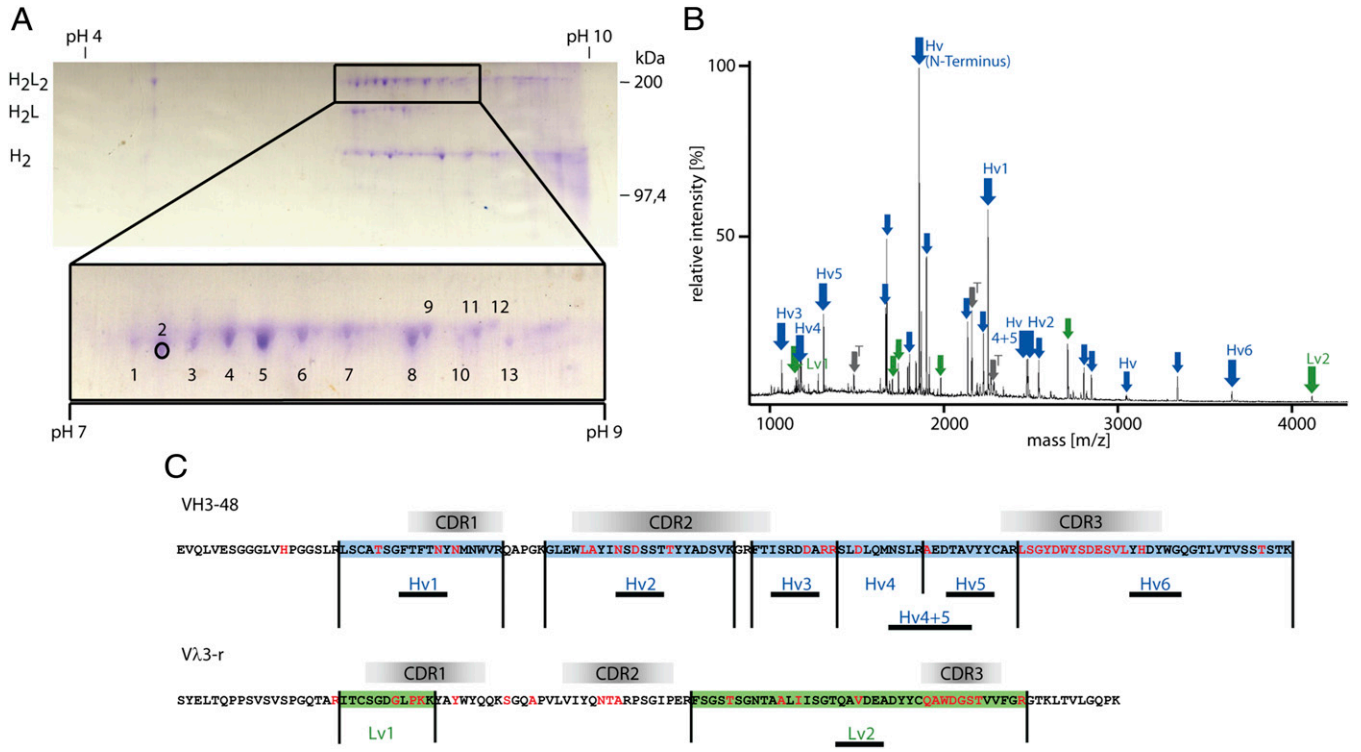

Fig. 2. Analysis of matching H and L chains of OCB from patient MS1 yielding rOCB-MS1-s2. (*A*) Nonreducing 2D gel electrophoresis of antibodies from a CSF sample. For the first dimension, an IEF gel was run, and for the second dimension, SDS/PAGE was performed. Most spots were detected between pH 7.0 and 9.0 (see detail). The positions of the H_2L_2 heterodimers, the H_2L, and H_2 complexes (left) and a mass scale (right) are indicated. Single spots of H_2L_2 heterodimers were excised, digested with trypsin, and analyzed by mass spectrometry. Spot 2 is indicated by a circle. (*B*) MALDI-TOF spectrum of spot 2 from *A*. The relative peak intensities are plotted as a function of the mass-to-charge ratio *m/z*. Peptides from the IgG-H (blue arrows) and IgG-L (green arrows) chains are indicated. Peaks representing peptides from VN(D)NJ-regions are termed "Hv" and "Lv" followed by a number. Peaks from the constant regions are indicated by arrows only. Peaks from trypsin are indicated in gray. (*C*) Deduced amino acid sequences of the H and L chains as obtained by cDNA sequencing. Peptides identified by mass spectrometry are numbered and highlighted in blue and green. Peptide sequences verified by tandem mass spectrometry are underlined. Amino acids introduced by somatic hypermutation or V(D)J recombination are highlighted in red letters. The putative positions of the complementarity determining regions CDR1, CDR2, and CDR3 regions are indicated.

as pioneered by Robinson et al. (31). We hybridized the rOCB to protein microarrays (ProtoArray), which display ~9,400 full-length recombinant human proteins produced in insect cells. The positive control antibody r8-18C5 hybridized to its cognate antigen MOG; rOCB-MS2-s5 hybridized to isoforms of MAP kinase-interacting serine/threonine kinase (MKNK1 and MKNK2); rOCB-MS1-s8 hybridized to family with sequence similarity 84 member A (FAM84A); and rOCB-MS1-s2 hybridized to A-kinase anchoring protein 17A (AKAP17A) (Fig. 4*A*). Characteristics of these proteins are given in the *SI Materials and Methods*. The three other rOCBs did not show consistent reactivity with any protein contained in the array.

Next, we validated the ProtoArray results by immunoprecipitation and ELISA—i.e., by methods that rely on high-affinity interaction—and used antigens that were produced in organisms different from insect cells. Thus, rOCB-MS1-s8 immunoprecipitated full-length FAM84A produced in HEK293 cells (Fig. 4*B*). In a parallel experiment, rOCB-MS1-s8 recognized FAM84A from *E. coli* in an ELISA in a concentration-dependent manner (Fig. 4*C*). The control antibody ab58330 yielded a much stronger signal than rOCB-MS1-s8, presumably because only clones with high avidity were selected for commercial production. None of the control antibodies rOCB-MS3-s1, rOCB-MS1-s2, or r8-18C5 recognized FAM84A by immunoprecipitation or ELISA.

In an earlier study (22), FAM84A was recognized just below significant levels by unfractionated CSF from MS patients. Therefore, we examined its recognition by CSF samples from patients with MS and inflammatory and noninflammatory CNS diseases (Fig. S2). Confirming the earlier results (22), MS1 was the only MS patient recognizing FAM84A. However, we detected higher levels of anti-FAM84A antibodies in patients with acute inflammatory CNS diseases compared with MS or noninflammatory diseases, concomitant to increased overall IgG levels. This finding is consistent with the presence of anti-FAM84A antibodies in patients with inflammatory bowel disease (32) and indicates that FAM84A is an intracellular antigen that might elicit strong secondary immune reactivity when released as cellular debris.

rOCB-scFv-MS2-s5 and the commercial anti-MKNK1 antibody PA5-13951, but no other rOCB-Fab fragments tested, immunoprecipitated MKNK1 produced in human HEK293 cells (Fig. 4*D*), confirming the specific recognition found by array hybridization. Titration of MKNK1 by rOCB-scFv-MS2-s5 and PA5-13951 revealed dose-dependent recognition (Fig. 4*E*).

AKAP17A from HEK293 EBNA cells was immunoprecipitated by rOCB-MS1-s2 and a commercial anti-AKAP17A antibody, but not by r8-18C5 and rOCB-MS3-s1. However, although rOCB-MS1-s8 recognized FAM84A, but not AKAP17A, on the microarray, it immunoprecipitated both proteins (Fig. 4*F*), whereas rOCB-MS1-s2 recognized only AKAP17A but not FAM84A (Fig. 4 *B* and *C*). To explain this cross-reactivity, we compared the sequences of AKAP17A and FAM84A and found two regions of high homology (Fig. 5*A*). We mutated individual amino acids in the putative epitopes of FAM84A to alanine and found that mutations of amino acids 188, 190, 191, and 267 did not change recognition by rOCB-MS1-s8 in ELISA, whereas exchanges of 263, 264, 268, and 271 considerably reduced recognition (Fig. 5*B*). This finding indicated that the homologous regions between amino acids 263–271 in FAM84A and 310–318 in AKAP17A are shared epitopes of rOCB-MS1-s8. Because rOCB-MS1-s8 did not recognize AKAP17A on the ProtoArray, we surmise that the epitope is conformation-dependent.

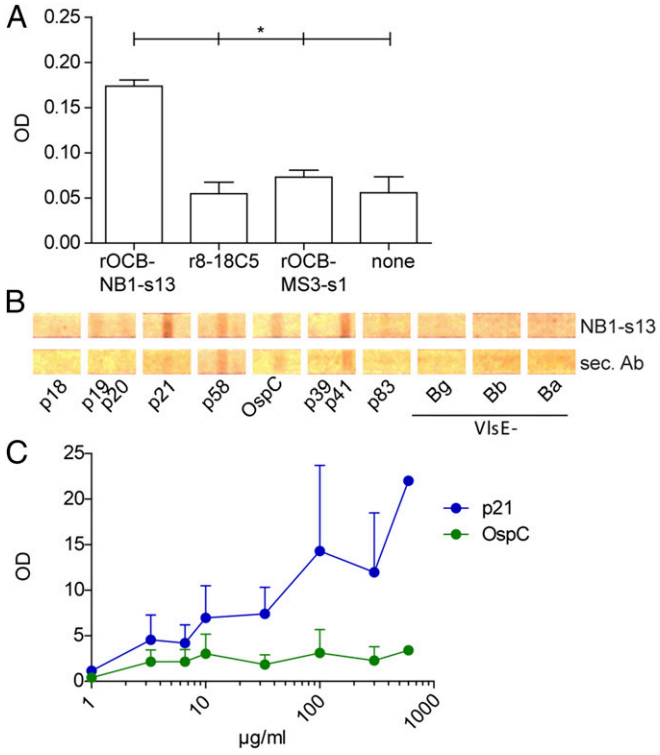

Fig. 3. rOCB-NB1-s13 isolated from CSF of a patient with NB recognizes a *Borrelia* antigen. (*A*) Lysates from *Borrelia* were tested in a clinical routine ELISA format. rOCB-NB1-s13, the control antibodies r8-18C5 and rOCB-MS3-s1, and no antibody (none) were diluted in CSF samples from four patients with NINDs. $*P \leq 0.05$ using the Mann–Whitney *u* test. Data represent mean signal of four individual experiments. (*B*) Representative immunoblot of 12 *Borrelia* protein antigens used in clinical routine. The purified antigens contain common *Borrelia* proteins p18-21, p58, outer surface protein C (OspC), p39, p41 (flagellin), p83, and the expressed variable major protein-like sequence (VlsE) specific for *garinii* (-Bg), *burgdorferi* (-Bb), and *afzelii* (-Ba) strains. Some antigens showed relatively high signals with the polyclonal secondary antibody alone. Only p21 showed a significantly higher signal with rOCB-NB1-s13 compared with the secondary antibody. The concentration of rNB1-s13 was 300 µg/mL. (*C*) Titration of rOCB-NB1-s13 with the candidate antigen p21 and the control OspC in the range from 1 to 600 µg/mL The intensities of the spots were determined by densitometry. Signals were normalized to the secondary antibody. The assays were performed in triplicate, except for the highest concentration. Error bars indicate SEM.

Discussion

OCBs have been regarded as a perennial conundrum in MS since their first description in 1959 (7, 33, 34). They are also observed in infectious CNS disorders, where it is known that the OCBs recognize the relevant infectious agent (25). In MS, however, no such infectious agent could be identified (35), but, nevertheless, >95% of MS patients have OCBs in their CSF. Assuming that the OCBs are directed against self-antigen(s), there are two main possibilities. First, the OCB might recognize a pathogenetically relevant, perhaps even unifying autoantigen—e.g., an "encephalitogenic" autoantigen identified in animal models (36). Second, the OCB response might occur as a secondary reaction to tissue injury. To address this question, we set out to isolate and produce antibodies from distinct OCBs.

In the past, mainly technical reasons precluded identification of OCB antigens from MS patients. Firstly, the small amount of CSF obtained during a diagnostic lumbar puncture precludes direct biochemical analysis of distinct OCBs. Secondly, the CSF contains not only OCB Ig, but also many polyclonal antibodies. Therefore, when whole CSF is used for analysis, it is not known whether a signal arises from this polyclonal background or from an OCB. Thirdly, when antibodies are cloned from single B cells and expressed recombinantly (16, 17), it will again not be known whether a particular B cell has produced an OCB or contributed to the polyclonal background.

We have overcome these technical challenges by combining refined biochemical analysis, proteomics, and transcriptomics. As a crucial initial step, we copurified disulfide-linked IgG-H and -L chains from distinct OCBs for concurrent mass spectrometry, revealing characteristic patient- and OCB-specific peptides (24). From these "fingerprints," the full-length sequences of matching chains can be deduced by aligning the peptides to Ig transcriptomes. This method allowed us to express distinct OCB antibodies recombinantly and to search for their target antigens.

We could produce six recombinant antibodies from distinct OCBs of four MS patients and characterize three different target autoantigens, whereas the other three OCB antibodies did not yield reproducible signals. The three antigens are ubiquitous intracellular proteins that are not specific to brain tissue. The observed diversity and heterogeneity of the OCB response and the intracellular localization of their target autoantigens indicate that, in MS, part of the OCB response represents a secondary reaction to cellular debris, as postulated by Grabar decades ago as a general concept (37). Such reactivity might not be specific to MS, but could be a more general feature of (neurological) autoimmune and inflammatory diseases. As shown by immunoprecipitation and site-directed mutagenesis, one OCB recognized an epitope shared by two different intracellular autoantigens. Such cross-reactivity would seem consistent with the "debris hypothesis." In future studies, it will be interesting to see whether "public" antigens can be detected in informative groups of patients. Furthermore, it will be important to assess the frequencies of antigen-specific B cells in CSF and blood and, ideally, to investigate the CSF B-cell response over time.

It should be noted that none of our OCBs showed the classic "polyreactivity" pattern described by Wardemann et al. (30). Although we did not observe any consistent antilipid or antiviral reactivity of our OCB antibodies, we cannot rule out the possibility that some OCBs in MS recognize nonself or nonprotein antigens (18, 21, 23, 38, 39) based on the small sample of OCBs that we could investigate in such detailed manner. For the same reason, we cannot exclude the possibility that some OCB antibodies recognize antigens exposed at the surface of brain-resident cells and, therefore, might contribute to the pathogenesis. Thus, OCB antigens may be quite heterogeneous, not only in their structure and function, but also regarding their possible pathogenic role. Moreover, cross-reactivity between autoantigens and microbial antigens—i.e., molecular mimicry—cannot be excluded (reviewed in ref. 15).

Beyond MS, our approach may allow the identification of a priori unknown target antigens from diagnostic CSF samples, other body fluids, or tissues, where expanded antibodies are detectable along with B cells. For future therapeutic purposes, it will be especially helpful that such antibodies are directly "produced" by human patients who have already mounted an immune response to fight pathogens. This aspect may be important in infections where our strategy allows identification and, eventually, large-scale production of human antibodies against infectious agents such as bacteria, viruses, or parasites. One such example is the anti-*Borrelia* antibody rOCB-NB1-s13 described here, which indeed reacts to a surface antigen of the pathogenic intruder. Such antibodies, which were selected in vivo for optimal reactivity against the most relevant antigenic epitopes, could be produced as prophylactic or therapeutic agents for "passive immunization."

Materials and Methods

Clinical Samples. CSF from patient NB1 with NB and patients MS1, MS2, MS3, and MS4 with clinically defined relapsing–remitting MS (40) were used for analysis of OCBs. Clinical data are listed in Table S1. For testing reactivity to FAM84A by ELISA, we used CSF samples from patients with MS ($n = 21$), NB ($n = 13$), other infections of the CNS ($n = 7$), cranial nerve palsy ($n = 9$), and noninflammatory neurological diseases (NINDs; $n = 17$). Informed consent was

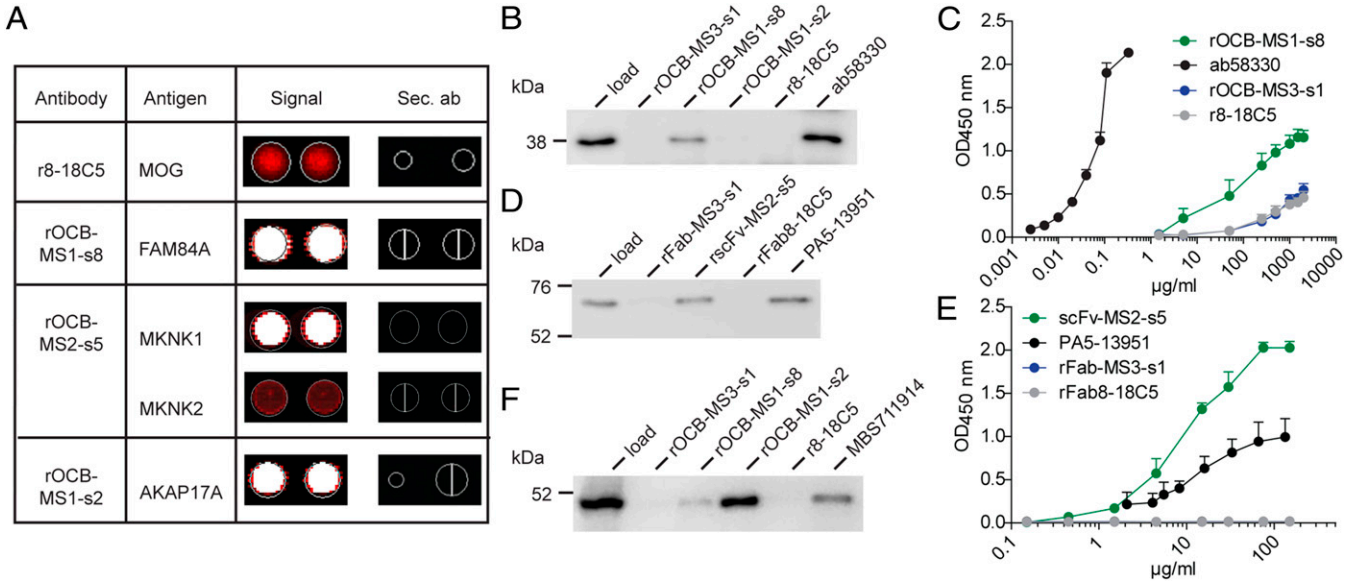

Fig. 4. Identification and validation of antigens of OCB antibodies from patients with MS. (*A*) Hybridization of the control antibody r8-18C5 and three rOCB antibodies to ProtoArrays. Spots are shown in duplicate. The color code ranges from black (no reactivity) to red (medium reactivity) and white (strong reactivity). Compilation of rOCBs (left column), their antigens (second column), the signals from array hybridization (third column), and the corresponding signals from hybridization of secondary antibodies alone (fourth column) is shown. The anti-MOG antibody r8-18C5 showed an intermediate, but specific, signal with MOG (second row); rOCB-MS1-s8 showed strong signal with FAM84A (third row); rOCB-MS2-s5 showed a strong signal with MKNK1 and an intermediate signal with the highly homologous isoform MKNK2 (fourth and fifth rows); and rOCB-MS1-s2 showed strong signals with AKAP17A (sixth row). All secondary antibodies alone (sec. Ab) showed no reactivity (fourth column). (*B*) Validation of FAM84A recognition by rOCB-MS1-s8 by immunoprecipitation. Recombinant FAM84A produced in HEK293 cells was specifically precipitated by rOCB-MS1-s8 and the commercial anti-FAM84A antibody ab58330, but not by control antibodies rOCB-MS3-s1, rOCB-MS1-s2, and r8-18C5. Lane 1 shows the loading control. The blot is representative of three independent experiments. (*C*) Titration of FAM84A recognition by rOCB-MS1-s8 analyzed by ELISA. Recombinant FAM84A produced in *E. coli* was specifically recognized by rOCB-MS1-s8 in a dose-dependent manner. The commercial monoclonal anti-FAM84A antibody ab58330 recognized FAM84A at considerably lower concentrations, indicating higher avidity. The negative control antibodies rOCB-MS3-s8 and r8-18C5 bound only very weakly at very high concentrations. The secondary antibody alone yielded background signal. Error bars indicate SD of the mean. Data are representative of four independent experiments. (*D*) Validation of MKNK1 recognition by rOCB-MS2-s5 by immunoprecipitation. Recombinant MKNK1 produced in HEK293 cells was specifically precipitated by rOCB-MS2-s5 and the commercial anti-MKNK1 antibody PA5-13951, but not by control antibodies rOCB-MS3-s1 and r8-18C5. Lane 1 shows the loading control. The blot is representative of three independent experiments. (*E*) Titration of MKNK1 recognition by rscFv-MS2-s5 analyzed by ELISA. Recombinant MKNK1 produced in HEK293 cells was specifically recognized by rscFv-MS2-s5 and with a slightly higher avidity by the commercial anti-MKNK antibody PA5-13951, but not by control Fab fragments rFab-MS3-s1 and rFab-8-18C5. Error bars indicate SD of the mean. Data are representative of four independent experiments. (*F*) Validation of AKAP17A recognition by rOCB-MS1-s2 by immunoprecipitation. AKAP17A produced in HEK293 EBNA cells was precipitated by rOCB-MS1-s2 and -s8 and the commercial anti-AKAP17A antibody MBS711914, but not by the control antibodies rOCB-MS3-s1 and r8-18C5. Lane 1 shows the loading control. The blot is representative of five independent experiments.

obtained from all patients. The study was approved by the Institutional Review Boards of the Ludwig-Maximilians-University, Karolinska Hospital, and the University of Ulm.

Analysis of CSF Ig Transcriptomes. We generated IgG-H and -L chain transcriptomes from CSF B cells as described (24) with few exceptions detailed in *SI Materials and Methods*.

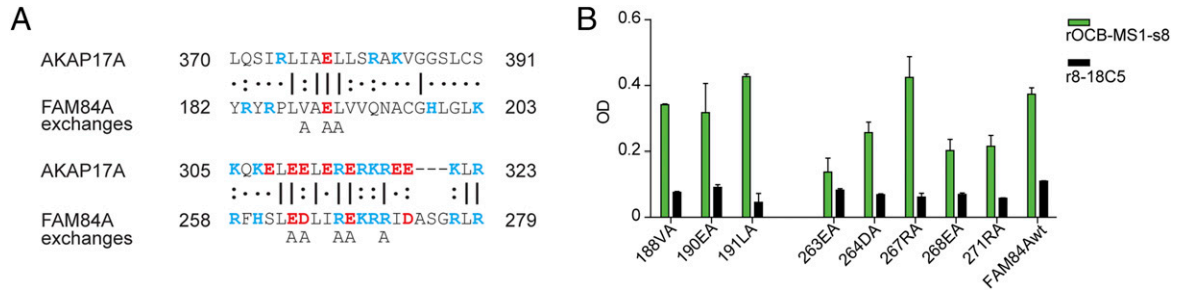

Fig. 5. Cross-reactivity of rOCB-MS1-s8 between highly homologous epitopes of FAM84A and AKAP17A. (*A*) Comparison of amino acid sequences of 370–391 (*Upper*) and 305–323 (*Lower*) of AKAP17A (upper lines) and 182–203 and 258–279 of FAM84A (middle lines). Identical amino acids are indicated by a dash, highly homologous amino acids by a colon, and similar amino acids by a dot. Positively and negatively charged amino acids are depicted in blue and red letters, respectively. The lowest line indicates which of the FAM84A amino acids were individually replaced by alanine. (*B*) Recognition of wild-type (FAM84wt) and the eight mutated FAM84A molecules by rOCB-MS1-s8 and the control antibody r8-18C5 as measured by ELISA. Recognition of FAM84A with exchanges of amino acids V188, E190, L191, and R267 to alanine was comparable to the wild-type protein, but considerably reduced by exchanges of amino acids E263, D264, E268, and R271. Data are representative of four independent experiments. Error bars indicate SD.

Purification, Separation by 2D Gel Electrophoresis, and Mass Spectrometry of CSF IgG Molecules. IgG antibodies were purified from CSF supernatant by Protein G Dynabeads, deglycosylated by *N*-glycosidase F, and subjected to IEF gel electrophoresis using 24-cm polyacrylamide gels and SDS/PAGE. All steps were performed under nonreducing conditions. Spots that contained the H_2L_2 heterodimer were excised, in gel digested by trypsin, and subjected to MALDI-TOF/TOF mass spectrometry. Peptides were aligned to patient-specific transcriptome databases by using MASCOT (24). Matching H and L chains could only be identified in some of the spots, where only one dominant H and one dominant L chain were unambiguously detected. This is not always the case, because a distinctly visible OCB need not be monoclonal, but may contain several comigrating other antibody chains. Details of the procedures are given in *SI Materials and Methods*.

Cloning, Expression, and Characterization of rOCB, rOCB Fragments, and Target Antigens. We cloned full-length recombinant antibodies of the H_2L_2-chain pairs of OCBs MS1-s2, MS1-s8, MS1-s9, NB1-s13, MS3-s1, and MS4. As a control, we expressed the MOG-specific antibody 8-18C5. For the constant regions, we used the human IgG1- and κ-regions. The IgG-H chains were extended with either His_6 and V5 tags or with a His_6 tag only. In parallel, we generated recombinant Fab fragments, which were extended by a His_6 tag. The IgG-H and -L chain pair of MS2-s5 was expressed as scFv in inclusion bodies in *E. coli* and refolded in vitro. All rOCB or rFabs were expressed in HEK293EBNA cells, purified by immobilized metal affinity chromatography, and characterized by SDS/PAGE, Western blotting, mass spectrometry, circular dichroism spectroscopy, and flow cytometry (r8-18C5 only) (Fig. S3). MKNK1, FAM84A, and AKAP17A were produced in HEK293 cells. In addition, soluble FAM84A was produced in *E. coli*. Individual amino acids in regions homologous to AKAP17A were exchanged to alanine by site-directed mutagenesis. Recombinant target proteins were characterized as rOCBs. Details are given in *SI Materials and Methods*.

Detection and Validation of OCB Antigens. For detection of *Borrelia*-specific antigens by rOCB-NB1-s13, the *Borrelia afzelii* + VlsE IgG ELISA Testkit (Virotech) and anti-*Borrelia*-EUROLINE-RN-AT immunoblot (EUROIMMUN) were used. To identify candidate antigens of the OCB antibodies from MS patients, ProtoArrays (Version 5.0; Human Protein Microarrays, Invitrogen) were used. Each array contained ∼9,400 human proteins produced in insect cells. Data were analyzed by using the ProtoArray Prospector program (Version 5.2.1; Life Technologies). Positive signals were defined as duplicate signals five times higher than the mean signal of the array and three times higher than the signal produced by the secondary antibody and an isotype control antibody (e.g., r8-18C5). Positive signals were sorted by signal intensity. All signals were visually confirmed. All candidate antigens were validated by independent experiments using recombinant proteins produced in either human HEK293 cells (FAM84A, AKAP17A, and MKNK1) or *E. coli* (FAM84A). FAM84A, AKAP17A, and MKNK1 recognition by rOCB-MS1-s8 and -s2 and rscFv-MS2-s5, respectively, were validated by immunoprecipitation. MKNK1 and FAM84A recognition by rscFv-MS2-s5 and rOCB-MS1-s8, respectively, were validated by ELISA. Antigens were detected by rOCB antibodies in transfected HEK293 cells (Fig. S4). All experimental details and the control antibodies are described in *SI Materials and Methods*.

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