Supplementary data

Related B cell clones that populate the CSF and CNS of patients with multiple sclerosis produce CSF immunoglobulin

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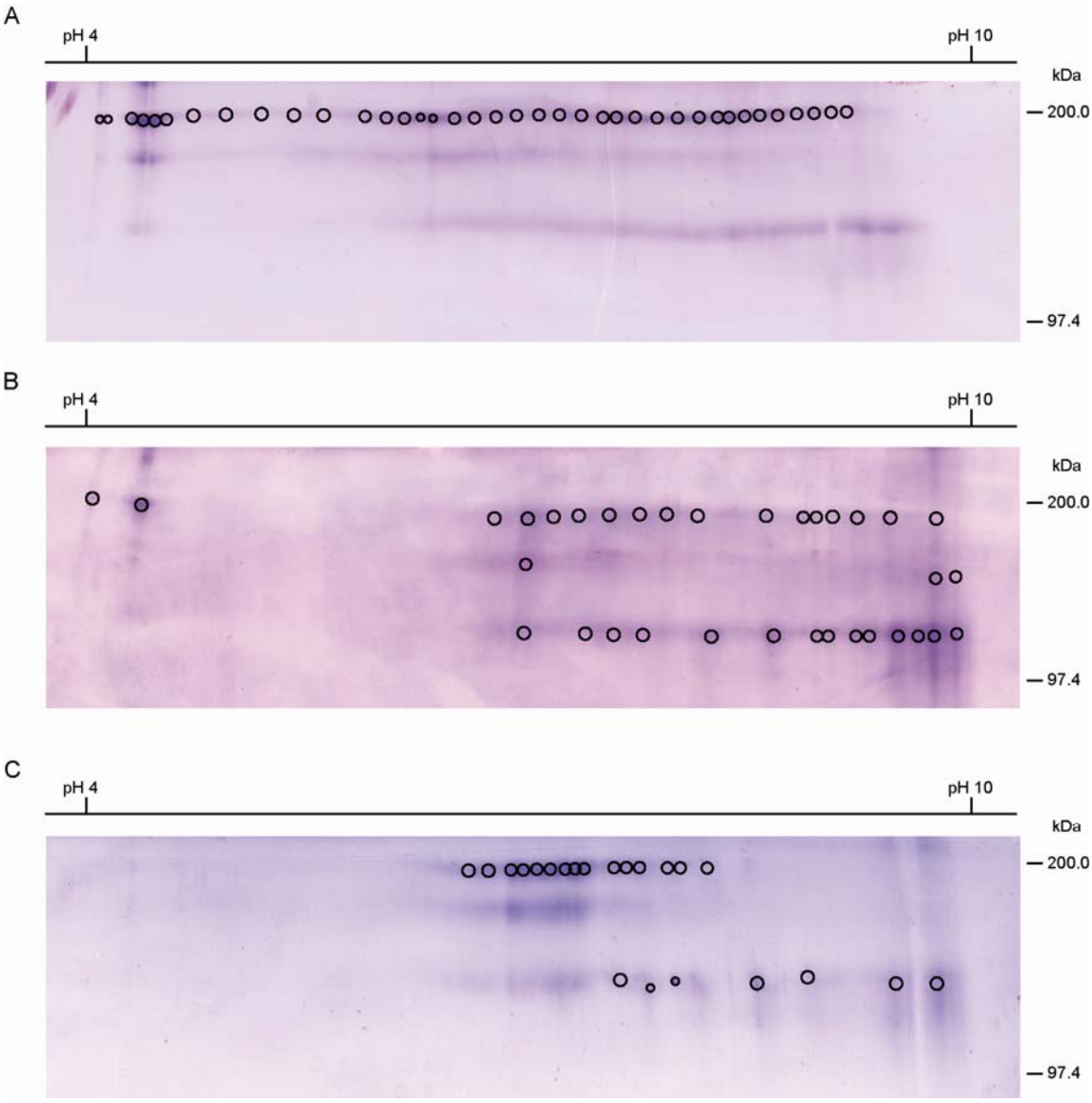

Supplementary Fig. 1. Purification of IgG-molecules from CSF samples by 2-d gel electrophoresis. At the positions indicated by circles, spots were cut from the gel, the IgG-molecules were digested by trypsin, and the peptides analyzed by MALDI or ESI mass spectrometry. **(A)** patient MS-4; **(B)** patient MS-B2A; **(C)** patient L-296.

Supplementary Fig. 2

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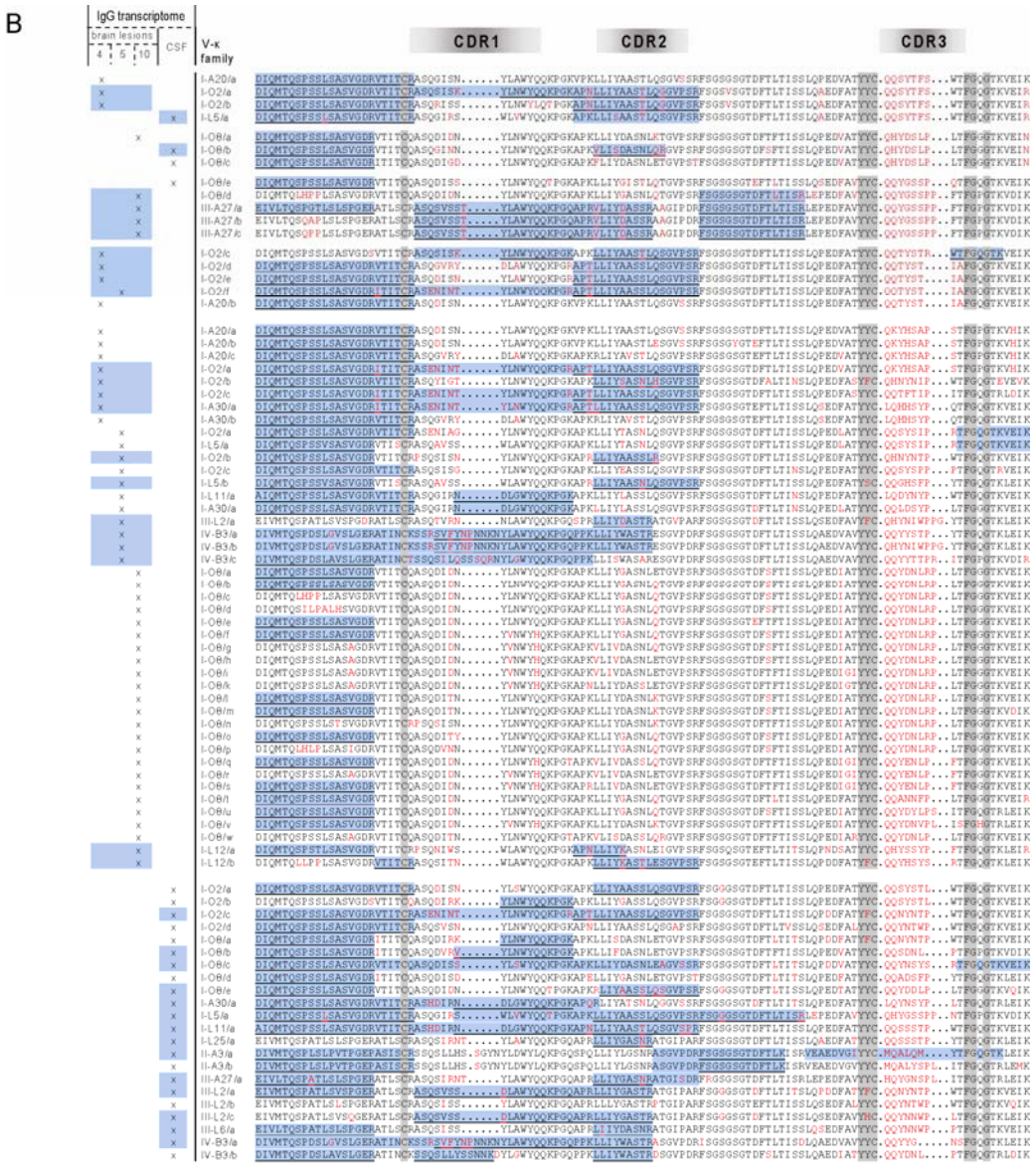
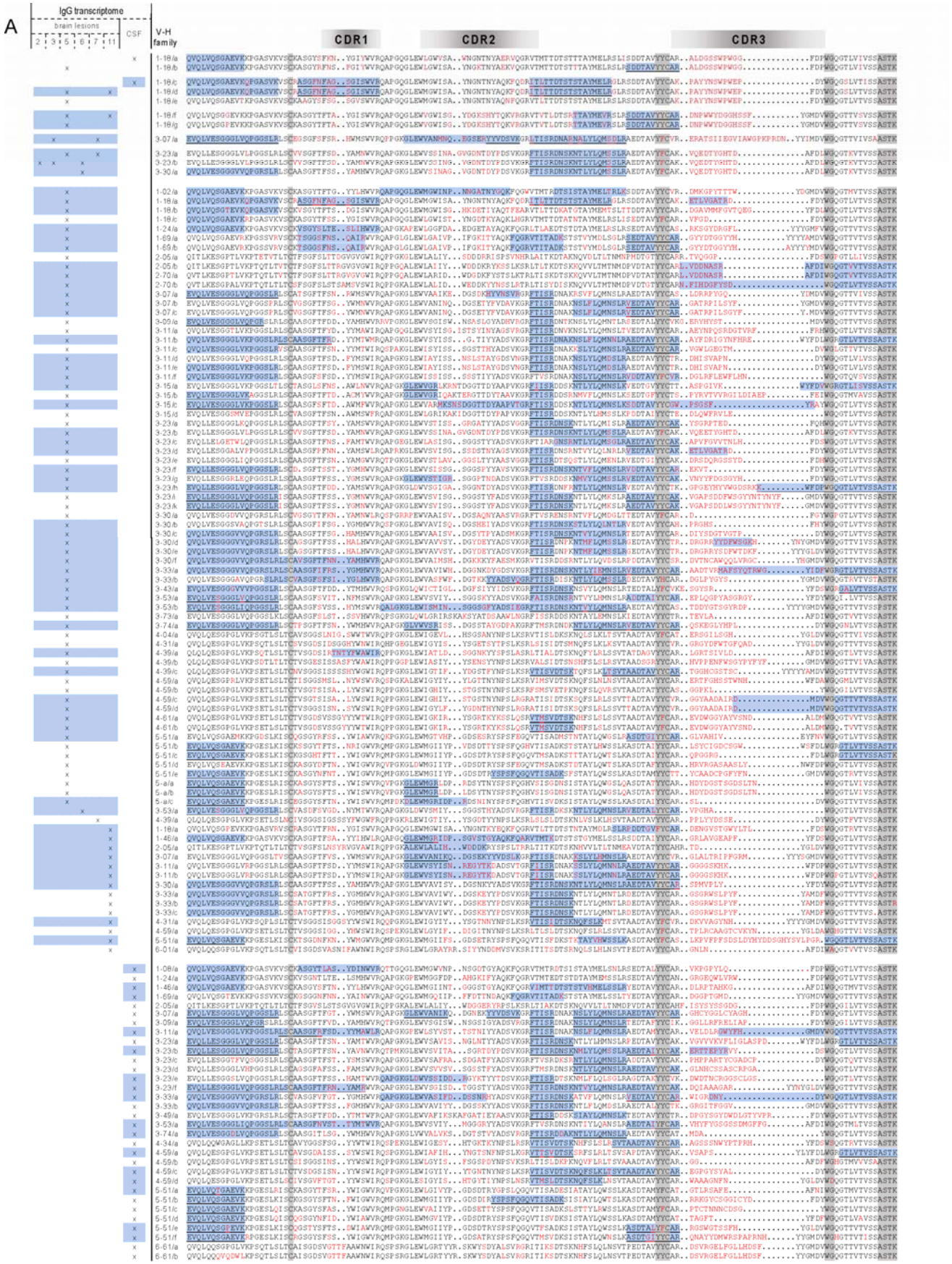


Supplementary Fig. 2. Overlap of CSF-proteome with transcriptomes from CSF and three morphologically distinct CNS lesions of patient MS-4. Data from the IgG-transcriptome and IgG-proteome analysis for the IgG-H **(A)** and Ig-κ chains **(B)**, respectively. A fraction of the heavy chain data is also included in the study of Lovato et al. (Lovato et al., in press). A total of 108 IgG-H and 82 Ig-κ chains from CSF and three CNS lesions were analyzed. On the left panel we indicate by "X" in which of the three brain lesions or of the CSF samples a particular transcript was found. When a characteristic peptide could be found

in a particular transcript, we consider this clone as a match of the peptide sequence to the transcript. Such clones are highlighted in blue in the first column. When identical transcript sequences were identified in CSF and CNS, we highlighted the clones in red. The second column lists the germline V-families of the chains. If several clones of the same family carried different somatic mutations (intraclonal variants), they are distinguished by an additional letter as suffix. Column 3 lists the deduced amino acid sequences of the IgG-H and Ig-κ chains obtained by cDNA cloning. Germline coded amino acids are shown in black and amino acids, which comprise the CDR3 regions or were introduced by SHM are shown in red. The sequences are aligned to the conserved cysteine residues of the V regions, the tryptophan-glycine residues of the J-H, and the phenylalanine-glycine-X-glycine residues of the J-κ elements, which are indicated by grey backgrounds. The positions of putative CDR1, 2, and 3 regions are indicated. The transcripts are grouped according to their CDR3 regions. Very similar transcripts, which often differed only in single somatically mutated amino acids were detected independently in CSF and in brain lesions. The peptides identified by mass spectrometry are highlighted in blue. Peptides whose sequence could be confirmed by tandem mass spectrometry are underlined.

Supplementary Fig. 3



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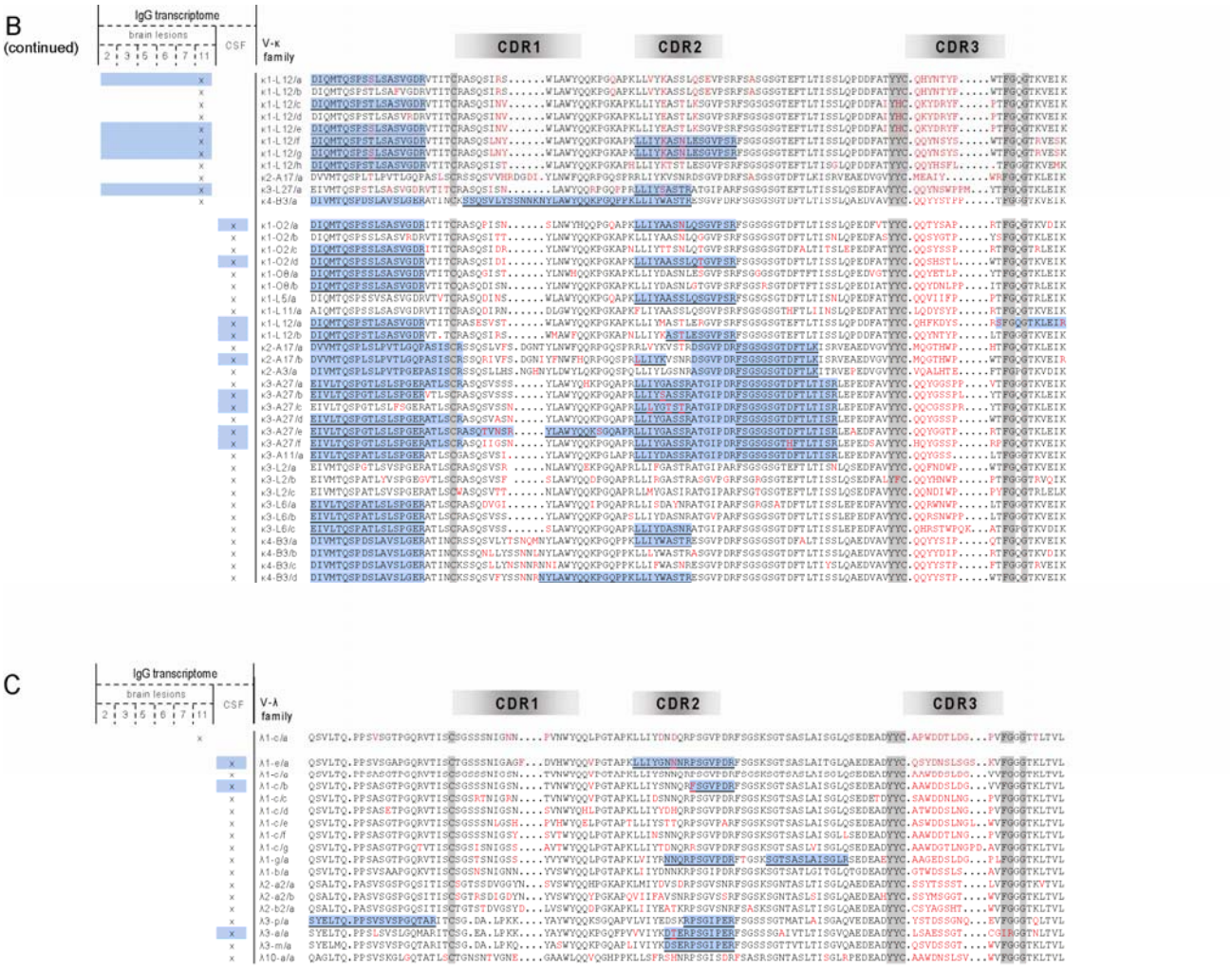
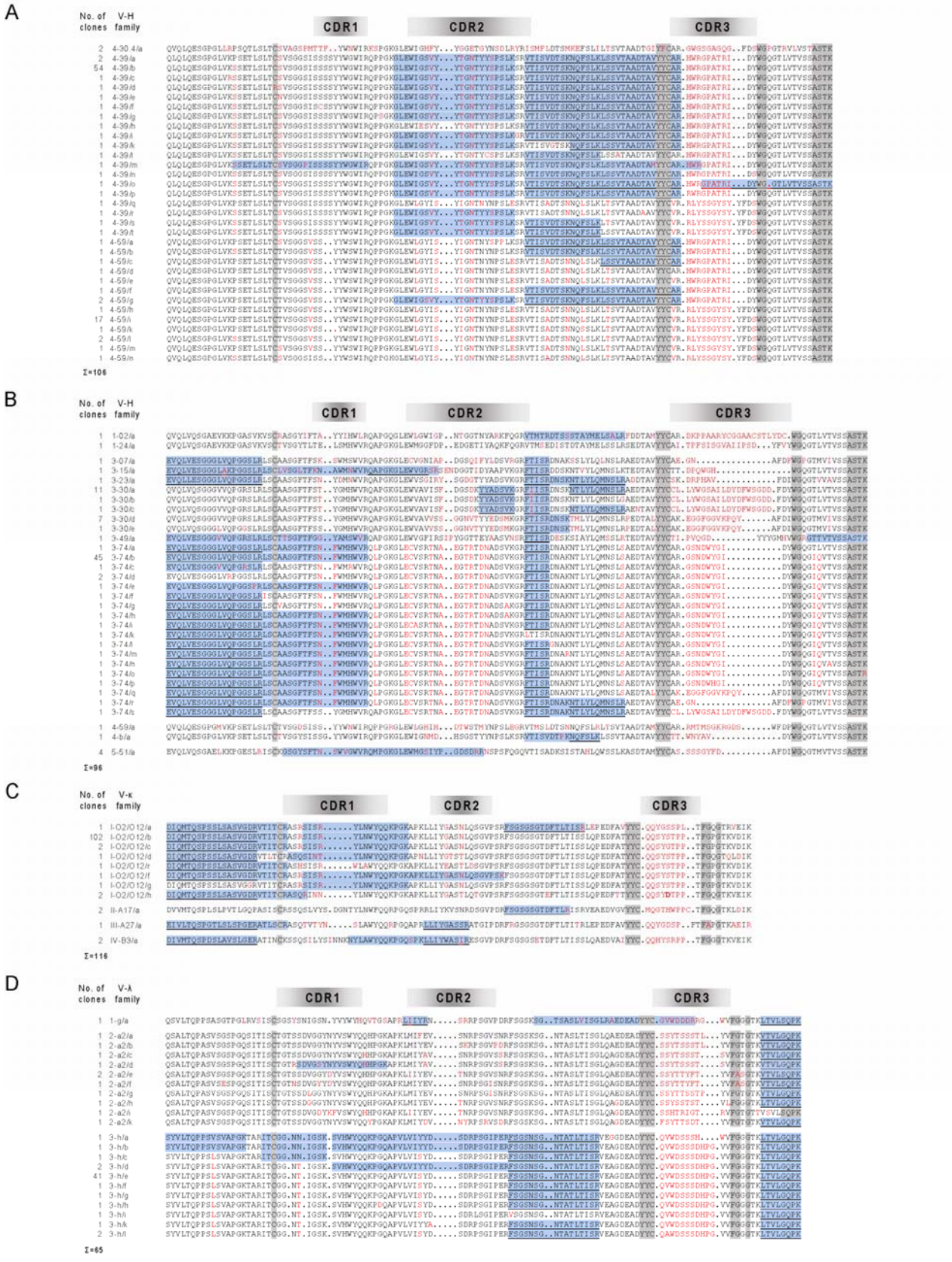


Supplementary Fig. 3. Overlap of CSF-proteome with transcriptomes from CSF and six morphologically distinct CNS lesions of patient MS-B2A. We show IgG-H **(A)**, Ig-κ **(B)**, and Ig-λ chains **(C)**. A total of 132 IgG-H, 164 Ig-κ, and 18 Ig-λ chains from CSF and three CNS lesions were analyzed.

Supplementary Fig. 4



Supplementary Fig. 4. Overlap of CSF-proteome with transcriptomes from CSF and CNS of patient L-296. We show IgG1-H **(A),** IgG2-H **(B),** Ig-κ chains **(C)** and Ig-λ chains **(D)**. A total of 106 IgG1-H, 96 IgG2-H, 116 Ig-κ, and 65 Ig-λ chains were analyzed.

Supplementary Table 1. Primer sequences for PCR analysis of formaldehyde-fixed brain tissue from patient L-296. The primers were designed to fulfill the following criteria: first, the amplicon must be kept as short as possible, second, the amplicon had to comprise a unique sequence i.e. containing somatic hypermutations or spanning the CDR3 region, and third, the Taq polymerase should start with a mutated nucleotide, if possible. For each chain we designed and tested two independent primer sets covering different positions of the same chain.

VH4-59 CDR2 for	5'-AGGGAAGGGACTGGAGTGGC
VH4-59 CDR2 rev-out	5'-TGATATGGTGACTCGACTCTC
VH4-59 CDR2 rev-in	5'-GGAGGGGTTGTAGTTGGTGT
VH4-59 CDR3 for	5'-CGTGTATTATTGTGTGAGACGA
VH4-59 CDR3 rev-out	5'-AGGCTGAGGAGACGGTGAC
VH4-59 CDR3 rev-in	5'-GGTTCCCTGGCCCCAGGA
VK1-O2 CDR1 for	5'-TCACTTGCCGGGCAAGTCG
VK1-O2 CDR1 rev-out	5'-ATGCACCATAGATCAGGAGC
VK1-O2 CDR1 rev-in	5'-TTAGGGGCTTTCCCTGGTTTT
VK1-O2 CDR3 for	5'-CTGAAGATTTTGCAACTTACTAT
VK1-O2 CDR3 rev-out	5'-AGATGGTGCAGCCACAGTTC
VK1-O2 CDR3 rev-in	5'-GCAGCCACAGTTCGTTTGATA

Supplementary Methods

1. Clinical and pathological details of the patients

The investigated MS lesions derived from two autopsy cases (MS-4 and MS-B2A) and one brain biopsy (case L-296).

Patient L-296 was a 42 year old female with highly active remitting MS. The brain biopsy showed an inflammatory demyelinating process consistent with MS. The demyelinated lesion was infiltrated by numerous T cells and macrophages that were found perivascularly and within the brain parenchyma. Axons and oligodendrocytes were largely preserved within the lesion and there were early signs of remyelination detectable.

Patient MS-B2A was a 34 year old female with severe relapsing course of demyelinating disease with progressive gate disturbance despite intense immunosuppressive therapy with different agents incl. cyclophosphamide. The patient died from cardiovascular complications. Neuropathological evaluation showed multifocal demyelinating disease involving the brain, spinal cord (transverse myelitis) and optic nerves (bilateral optic neuritis).

Patient MS-4 was a 39 years old female with relapsing course of MS and progressive accumulation of deficit including cognitive impairment despite consecutive treatment with IFN-β and copaxone. The patient died with severe disability of massive pulmonary embolism. Neuropathological evaluation revealed multiple plaques in the periventricular white matter extending into the corpus callosum, as well as in the subcortical white matter, cerebellum, and pons.

2. Analysis of the CSF IgG proteome

IgG antibodies from CSF supernatants were purified by Protein G Dynabeads (Invitrogen, Karlsruhe, Germany) and eluted in 1% (w/v) SDS after 2 minutes incubation at 37 °C. Then, we deglycosylated the IgG-molecules by heating the eluates to 95 °C for 1 minute and incubating for 3 hours at 37 °C in the presence of 0.5 % MEGA-10 (w/v) at pH 7.2 with 100 U/ml N-Glycosidase F recombinant (Roche, Mannheim, Germany). In order to remove SDS we dialyzed the samples against 6 M urea in a "D-tube Dialyzer Mini MWCO 12-14 kDa" (Novagen, Darmstadt, Germany), for 2 hours on a stirrer at room temperature, and then for 15 minutes at 50 V in a flat bed gel electrophoresis chamber.

First dimension of protein separation was performed in a 3100 OFFGEL Fractionator (Agilent, Böblingen, Germany) with 24 cm Immobiline DryStrip pH 3-10 isoelectric focusing gels (GE Healthcare Freiburg, Germany). We added 50 µl 6 M urea, 2 M thiourea, 10% glycerol (v/v) and bromphenolblue to the dialysed eluates and loaded them onto rehydrated strips by placing a loading cup (8 x 2 mm, conical) at position pH=4.5. The default in-gel focusing method was modified to a slower voltage-increase and an extended duration as follows: for the first 30 minutes the voltage was limited to 500 V and then for 30 minutes to 1000 V. During the electrophoresis we allowed 8000 V until 120 kVh were reached.

After isoelectric focusing we further separated the IgG-molecules by non-reducing SDS-PAGE. IEF strips were equilibrated for 20 minutes on a slow shaker in 6 M urea, 4% SDS (w/v), 50 mM Tris and 30% glycerol (w/v) and then placed onto 9% acrylamidgel. Electrophoresis was performed for 1 hour at 10 mA and then overnight at 25 mA using a cooled chamber. Gels were stained in 0.1% (w/v) Coomassie Brilliant Blue R-250.

We excised 38 spots for patient MS-4, 34 spots for patient MS-B2A and 22 spots for patient L-296. Half of the spots each were analyzed using a Proteomics Analyzer 4700 (MALDI-TOF/TOF) spectrometer (Applied Biosystems, Carlsbad, CA, USA) as described

(Obermeier et al., 2008). The other spots were analyzed by LC-ESI-MS. In-gel digested samples were resuspended in 10 µl 0.1% formic acid and half of the volume was injected in an Ultimate 3000 HPLC system (LC Packings Dionex, Idstein, Germany). Samples were desalted on-line in a C18 micro column (75 µm i.d. x 15 cm, packed with C18 PepMap™, 3 µm, 100 Å by LC Packings) and then separated in a analytical C18 micro column (75 µm i.d. x 15 cm packed with C18 PepMap™, 3 µm, 100 Å by LC Packings) with a gradient from 5 to 60% acetonitrile in 0.1% formic acid within 40 minutes. The effluent from the HPLC was directly electrosprayed into the LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, Schwerte, Germany). Survey full scan MS spectra were acquired from m/z 300 – 2000. Then, the 6 most intense peptide ions with charge states between 2 and 4 were fragmented in the linear ion trap by collision induced dissociation (CID) and fragment ion spectra were recorded in the LTQ part of the instrument. Peptides from constant regions of human immunoglobulins, from human keratins and from trypsin were excluded from this procedure.

Patient-specific Ig-transcriptomes obtained by cDNA cloning served as databases for the identification of peptide masses acquired by both MALDI- and ESI-MS using the program MASCOT (Matrix Science).

References

Lovato, L., Willis, S.N., Rodig, S.J., Caron, T., Almendinger, S.E., Howell, O., Reynolds, R., O'Connor, K.C., Hafler, D.A. 2010. Related B cell clones populate the Meninges and Parenchyma of Patients with Multiple Sclerosis. *Brain*, in press.

Obermeier, B., Mentele, R., Malotka, J., Kellermann, J., Wekerle, H., Lottspeich, F., Hohlfeld, R., & Dornmair, K. 2008. Matching of oligoclonal Ig transcriptomes and proteomes of cerebrospinal fluid in multiple sclerosis. *Nature Medicine*, 14, (6) 688-693