Supplementary data

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

Birgit Obermeier, Laura Lovato, Reinhard Mentele, Wolfgang Brück, Ignasi Forne, Axel Imhof, Friedrich Lottspeich, Kathrin W. Turk, Simon N. Willis, Hartmut Wekerle, Reinhard Hohlfeld, David A. Hafler, Kevin C. O'Connor, & Klaus Dornmair



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



Supplementary Fig. 2

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

InC here

Δ	brain lesions	1							
A	CSF CSF	V-H		CDR	1	CDR2		CDR3	
	1213191917111	Iramity		UDI		ODIL		ODINO	
	x x	1-18/a 1-18/b	QVQLVQSGAEVKKPGASVKV	SCKASGYRFNSFGI	YWVRQAPGQGLEWLGWISA. YWVRQAPGQGLEWLGWISA.	. YNGETNYAERVOOR	VTMTTDTSTTTAYMELRSLRSDDTAVYYCARALDOSS VTMTTDTSTTTAYMELRSLR <mark>SDDTAVYYCAR</mark> ALDGSS	IPWGGF	DPWGQGTLVIVSSASTK
	×	1-18 <i>/</i> c	QVQLVQSGAEVKQPGASVKV	CRASGENFAG SGI	SWVHQAPGQGLEWMGWISA.	. YNGNTNYAQKFQDR	ITLTTDTSTSTAYHELRGLRSDDTAYYYCAR PAYYNS	PWEP	DYWGQGSLVTISSASTK
	x x	1-18/d	QVQLVQSGAEVKOPGASVKV	CPASGENEAG SGI	SWVRQAP GQGLEWMGWISA.	. YNGNTNYAQKFQDR	IT LTTDTSTSTAVHELR GLRSDDTAVYYCAK. PAYYNS	PWEPF	DYWGQGSLVTVSSASTK
		1-10/0	QUOLVORGENEVERARINA	PROCESSION AND AND AND AND AND AND AND AND AND AN	SWINGAL OVOLDWIOWI SA.	· month month of		advery vor	DUMOCOTOVICO CANANA
	Q 0	1-18/g	QVQLVQSGPEVKKPGASVKV:	SCHASGYTFTA YGI	SWVRQAPGQGLEWVGWISG.	. YDGKSHYTQKVRGR	VTVTLDTSRTTAYMEVRSLRSDDTAVYYCAR DNPWYY	GGHSSFYGM	DVWGQGTTVSVSSASTK
	× ×	3-07/a	EVQLVESGGGLVQFGGSLRL	BOSGFSFSR HWM	SWV ROPP GROLEWVANMINO.	. BOSERYYVDSVKGR	ETISRDNARNALYLOMEDLRAEDTAVYFOVRERATSI	EGVIAWGPKPRDNYYIM	DVWQQGTTVIVSAASTK
	x x	3-23/a	EVQLLESGGGLVLPGGSLRLS	TVVSGFTFSD YAM	NWVRQAPGKGLEWVSSINA.	GVGDNTDYPDSVKGR	FTISRDNSKNTLYLQMSSLRAEDTAVYECAKVQEDTY	HTDAF	DIMOQGTEVTVSSASTE
	X X X	3-23/b 3-30/a	EVQLLESGGGLVLPGG5LRL: OVOLVESGGGVVOPGRSLRL:	SCVVSGFTFSDYAM SCAASGFTFSSYAM	NWVRQAPGKGLEWV55ING. HWVROAPGKGLEWV55INAG.	. VGDNTDYPDSVKGR	FTISRDNSKNTLYLOMSSLRAEDTAYYFCAK VQEDTY FTISRDNSKNTLYLOMSSLRAEDTAYYFCAK VOEDTY	HTDAF	DLWGQGTEVTV55ASTK
	^	0.0010	T.F. D. HO. COLLET. CLOSEL	_					
	×	1-02/a 1-18/a	QVQLVQSGAEVKKPGASVKVS OVOLVOSGAEVKOPGASVKVS	SCKASGYTFTGYYL SCRASGFNFAGSGI	HWV RQAP GQGLEWMGWINP. SWVROAP GOGLEWMGWISA.	. NNGATNYGQKFQGW . YNGNTNYAOKFODR	VTMTRDTSISTAYMELTRLKSDDTAVYYCVRDMKGPY ITLTTDTSTSTAYMELRGLRSDDTAVYYCAK.ETLVGA	TTW. YOGM	DVWGQGTHVTVSSASTK DYWGOGTLVTVSSASTK
	x	1-18/b	QVQLVQSGTEVKQPGASVKV	SCKVSGYTFTSDGI	SWVRQAPGQGLEWMGWISG.	HKDETIYAQTFEAR	VTLTTDKATGTAYMEMTSLTTDDTAVYYCARDGAVMM	GVTQEGYF	DLWGQGTLVTVSSASTK
	×	1-18/c 1-24/a	QVQLVQSGAEVKRPGASVKV QVQLVQSGAEVKKPGASVKV	SCRASGYTFSSYGI SCRVSGYSLTESLI	SWVRQAPGQGLEWLGWIST. HWVRQAPGKAPEWLGGFDA.	. YNGDTKYAQKLHGR .EDGETAYAOKFOGR	VTMTTDTATNTAYMELRSLISEDTAVYFCARVFGD. LTLAEDTSTDTAYMELRSLRSEDTAVYYCASRKSGYD	GFLYYYGM	DYWGQGTLVTVSSASTK FVWGQGTTVTVSSASTK
	×	1-69/a	QVQLVQSGAEVRKPGSSVRV:	CKTSGGSFNSQA1	RWVRQAPGLGLEWLGAIVP.	.IFGKITYAQKFQGR	VTITADKSTSTVYMDLSGLR <mark>SEDTAVYYCAR</mark> GYYDTG	YYHYYYYAM	DVWGQGTTVTVSSASTK
	×	1-69/b 2-05/a	QVQLVQSGAEVRKPGSSVRV: QITLKESGPTLVKPTETVTL1	CTFSGFSLTTDGVGV	EWVRQAPGLGLEWLGAIVP. GWIRQPPGKGLEWLALIY	. SDDDRRI SPSVNHR	VTITADKSTSTVYMDLSGLRSEDTAVIYCARGYYDTG LAITKDTAKNQVDLTLTNMNPMDTGTYYCARR.TVQGGP	TTHF	DSWGPGTLLIVSSASTK
	×	2-05/b	QITLKESGPTLVKPTQTLTV1	CTFSGFSLTTRGVGV	GWIRQPPGQALEWLAIIY	. WDDDKRYKSSLKSR	LTLTKDTSKKQVVLTMTNMDPVDTATYYCARL.VDDNAS	AF	DIWGQGTVVTVSSASTK
	× ×	2-70/b	QVT LKESGPALVKPTQTLTL1	CSFSGFSLST SAMSV	SWIRQPPGKALEWLALID	.WEDDKYYNSSLRTR	LTISYDISKNOVVLTMTNMDPVDTGTYYCARN.FIHDGF	(SD	WGQGTLVTVSSASTK
	×	3-07/a 3-07/b	EVOLVESGGGLVOPGGSLRLS EVOLVESGGGLVOPGGSPRLS	SCATSGETESN WM SCVGSGETESG VWM	SWVRQAPGKGLEWVAAIKE. NWVROAPGKGLEWVANINO.	DGSDKHYVNSVRGR	FTISEDNAKRSVFLQMNSLEAEDTAVYYCAS. LDRRPY FTISEDNAKNSLELOMNSLEVEDTAVYYCAE. GATEPT	QYDM	DVWGQGTTVIVSSASTK
	x	3-07 /c	EVQLVESGGGLVQPGGSPRL	CVGSGFTFSGYWM	NWVRQAPGKGLEWVANINQ.	. DGSETYFVDAVKGR	FTISRDNAKNSLFLOMNSLRVEDTAVYYCARGATRPI	SGYFY.M	DVWGQGTTVTVSSASTK
	×	3-09/a 3-11/a	EVQLVESGGLVQPGRSLRLS QVQLVESGGTLVKPGGSLRLS	SCAASGFTFDDYAH SCAASGFTFYDYYM	HWVRRVPGKGLEWVSGISW. AWIRQAPGQGLEWVSYISG.	.NSASLGYADHVRGR ISTSYINYAGSVRGR	FTISRDNTKNSLYLQMNTLRSEDTALYYCVKG.ERYHYS FTISRDNVKNSLYLQMNSLTVEDTAVYYCAR.AEYNPO:	RDGTVRF	DVWGQGTTVTVSSASTK RHWGQGTLVTVSSASTK
	×	3-11/b	QVQLVESGGGLVKPGGSLRL	CAASGFTFRDYYM	TWMRQAPGKGLEWVSYISS.	G.TIYYADSVKGR	FTISRDNAKNSLFLOMNNLRAEDTAVYYCAR. AYFDRI	YNFHREWYF	DLWGRGTLVTVSSASTK
	×	3-11/d	QVQLVESGGGLVQPGGSLRLS	SCAASGFTFSDFYM	SWIRQAPGKGLEWIAYISS.	.NSLSTAYGDSVNGR	FTISRONAKNSLYLOMDSLRAEDTAVIICAR VGWDGD	N	DYWGQGTLVLVSSASTK
	×	3-11/e	QVQLVESGGGLVKPGGSLRL:	CAASGFTFSDFYM	SWIRQAPGKGLEWIAYISS.	.NSLSTAYGDSVNGR	FTISEDNAKNSLYLOMDSLEAEDTAVYCOTE. DHI SVA	N	DYWGQGTLVLVSSASTK
	×	3-15/a	EVQLVESGGGLVKPGGSLTL	CTASGLSFNSAWL	NWV RQAP GK <mark>GLEWVGR</mark> LKRN	TDGGTTDYAAPVKGR	FIISRDDSKNTLYLOMNSLKVEDTGVYYCTT. ASPGIV	WYF	DVWGRGTLI SVSSASTK
	×	3-15/b 3-15/b	EVQLVESGGGLVKAGGSLRLS EVOLVESGGGLVKPGGSLRLS	SCAASGFTFTDACM	YWV RQAP GKGLEWVGRIQAK FWV ROAP GKGLEWVARMKSN	TERGTTDYTAAVKGR	FTISRDDSRNMVFLQMNSLKSEDTGFYYCTSPYRVTV FTISRDDSKNTLVLOMNSLKSDDTAVYYCOWPSGSF	RGILDIAEPF	LIWGQGTHVAVSSASTK
	x	3-15/d	EVQLVESGGSMVEPGGSLRL	SCVASGFTFSNAWM	SWFRQAPGKGLEWLGRIKAK	IDGGTTDYPAPVKGR	FTISR DDSKNTLYLHMSSLKPDDTAIYYCTE. DLQWFR	LE	WGQGTLVTVSSASTK
	×	3-23/a 3-23/b	EVQLLESGGGLKQPGGSLRLS EVOLLESGGGLVLPGGSLRLS	SCAASGFTF <mark>SY</mark> YAM SC VV SGFTFS D YAM	SWVRQAPGKGLEWVSTISS. NWVROAPGKGLEWVSSINA.	. GRGATYYADSVGGR GVGDNTDYPDSVKGR	FTISRDNSKNTLYLOMISLRAEDTAVYYCAK. YSGRPTI FTISRDNSKNTLYLOMSSLRAEDTAVYFCAK. VOEETY	5DF	2HMGQGTLVTVSSASTK DLMGOGTKVTVSSASTK
	×	3-23 <i>l</i> c	EVQLLELGETWLQFGGSLRL:	SCAASGFTFNSFAM	TWVRQAPGEGLEWLASISG.	.SGGSTYYADSVKGR	FT IARGNSRNTLYLOMSGLRAEDTAVYYCAK APVFGV	TNLHF	D SWG QGTLVTVSSASTK
	×	3-23/d 3-23/e	EVQLLESGGGLIQPGGSLRLS	SCAASGFFFFNNYAM	GWVRQTPGKGLEWVSVISG.	.GGSLTYYAASVGGR	FTISRDNSRNTVYDQUNRLRVEDTAVYYCAKBTLVGA FTISRDTSQSTLYLQMENLRVEDTGIYYCAKDRSDQR	SSYDF	DHWGQGTLVTVSSASTK
	×	3-23/1	EVQLLESGGGLVQPGGSLRL:	SCD.SGFTSSTYGM	HWVRQAPGKGLEWVSISSG.	SGGSPYYAVSVKGR	FTISRDNSKNTVFLOMNSLRVDDTAVYYCAR. EKVT.	F F	DYWGQGTLVTVS SASTK
	×	3-23/h	EVQLLESGGGLVQPGGSLRL	CAASGFTFDD YAM	SWVRQAPGKGLDWVSGIGA.	. SGGNTDYADSVKGR	FTISRDNFRNTLYLCMNSLRVEDTAVYYCTKGPGEYH	WWGDSRXKWF	DPWGQGTLVTVS SASTK
	×	3-23/	EVQLLESGGGLVQPGGSLRI EVOLLESGGGLVOPGGSLRI	SCAASGFTFSSYOM SCAASGFTFSSYOM	NWVRQAPGKGLEWVSVISG. NWVROAPGKGLEWVSVISG.	. SGGST YFADS VKGR . SGGST YFADS VKGR	FTISRDNSKNTLYLOMKSLRAEDTAVYYCAK. VGAPSD	FWSGYYNTYNYFGM	DVWGQGTTVTVSSASTK
	x	3-30/a	QVQLVESGGDVVQPGRSLRL	CVGSGYTFKNYAM	NWLRQSPGRRPEWVAAISV.	.DGSAQNYAASVRGR	FTVSRENSRNTVFLQMDGLTTEDTAVYFCARD	F	GYWGPGTLVTVSSASTK
	×	3-30/6 3-30/c	QVQLVESGGSVAQPGTSLRLS QVQLVESGGGVVQPGRSLRLS	SCAASGFIFSGYGM SCAASGFTFSGHAM	HWVRQSPGKGLEWVAVISQ. HWVRQAPGKGLEWVAAISV.	. DGSHEIYADSVKGR . DGSYTPYADSMKGR	FTISRDNSKSTLYLQLNTLRVEDTAVYYCAHPRGHS. FTISRDNSKNTVYLCMNSLRTEDTAVYYCARDIYSDG	VGTPF	AYWGQGTLVTVSSASTK DYWGQGTLVTVSSASTK
	×	3-30/d	QVQLVESGGGVVQPGRSLRL	SCAGSGFTFSSHAL	HWVRQAPGKGLEWVAVVSY.	. DGYDEYYADSVKGR	FTISEDNPKNTHFLOMSSLEGEDTAVYYCTR. DRGRRY	DEWSGKHYNYGM	DVWGQGTTVIVSSASTK
	×	3-30/f	QVQLVESGGGVVQPGRSLRL	SCAVSGFT FNN YAM	HWVRQAPGKGLEWVAIMSH.	. DGKKKYFAESMKGR	VTISRDDSRNTLYLQVDSLRPDDTAVYYCSR. DVTNCA	QQLVRGCYHGM	DVWGQGTTVTVSSASTK
	×	3-33/a 3-33/b	QVQLVESGGGVVQPGRSLRL: OVOLVESGGGAVOPGRSLRL:	SCAASGFIFRSYGM SCVASGFSFSIYGL	HWVRQAPGKGLEWVAVIWH. HWVROAPGKGLEWVASFWF.	. DGGNKDYGDSVKGR DGTKKYYADSVOGR	FTISRDNSKNTLYIEMNSLRVEDTAVYYCAR. AADTVR FTISRDISKNTLYLOMSSLRDEDTAVYNCAR. DGLPYG	AFSYQTRWGYI	DFWGRGTLVTVSSASTK
	x	3-43/a	EVQLVESGGGVVVPGGSLRL	SCAASGFTFDDYTM	HWVRQSPGKGLQWICTIGL.	DGFSIYYIDSVKGR	FTISRDNSKNSVYLQMNSLRTEDTALYFCVKE.SGYSR.	YF	DSWGRGALVTVSSASTK
	×	3-53/a 3-53/b	EVQLVESGGGLVQPGGSLRL: EVQLVESGGGLIQPGGSLRL:	SCAASGFSVITNFM SCAASGFSVSSHYM	SWVRQAPGKGLEWVSVIY SWVRQALGKGLEWISMIN	.SGGSKYYADSVKGR .SGGSGFYADSIEGR	FAISRDNSRNTVYLHMNSLRADDTAIYYCAR EPLQGP FTISRDNSKNTVYLOMNSLRAEDTAVYYCAS TDDYGT	FASGRGYYF SGYRDPYYYYGM	DYWGQI.LVTVSSASTK DVWGQGTTVTVSSPSTK
	×	3-73/a	EVQLVESGGGLVQPGGSLKL	SCAASGFSLSTSSV	HWVRQPSGKGLQWLGRIRSK	AKSYATDSAAWLNGR	FISSRDDSKNTAYLEMNSLKIEDTAVYYCTRTEVLA.	AF	DIWGQGTVVTVSSASTK
	×	3-74/a 4-04/a	QVQLQESGPGLVKPSGTLSL	CAVSGGSLNIG.SWW	TWVRQPPGKGLEWIGEVL	.HSGSANYNPSLKSK	VTISLDKSKNQLSLKLTSVTAADTAVYFCATERSGIL	GHYGL	DVWGQGTTVIVSSASTK
	X	4-31/a	QVQLQESGPGLVKPSQTLSL	CSVSGDSIDSGGHYW	NWIRQHPGEGLEWIGYIS	.YSGETSYTPSLKSR	LSISIDTSMNQFSLKLKSVTAADTAVYYCVR. GRGYDF	TGFQADYYGL	DVWGQGTTVTVSSASTK
	×	4-39/b	QLQLQESGPGLVKPSDTLTL	TCTVSGESISSASFYW	AWIRQPPGGPLEWIASIY	.YSENSYYNPSLKSR	VALSIDTSNSHFSLSLRSVTAADSGRYYCAR HVPPEN	WSGYPYFYFGM	DVWGQGTTVI VASASTK
	×	4-39/c 4-59/a	QLQLQESGPGLVKPSETLSL OVOLOESGPRLVKPSETLSL	CONVSGGSISSSSHYW DCTISGGSMSLNYW	GWIRQPPGKGLEWIGTIF SWVROSPGKALEWIGYIY	.YDGSTFYNPSLKSR .YLGTTNYNPSLKSR	VTISVDTSKTOFSLKLTSVTAADTAVYYCARTGGHCG VIMSVDTSKNOFSLNLNSVTAADTAVYYCARERTFGH	STSCYYYYYAM	DVWGQGTTVTVSSASTK DAWGOGMLVTVSSASTK
	x	4-59/b	QVQLQESGPGLVKPSETLSL	TCTVSGTSISALYW	SWIRQPPGKGLEWIGHIY	.STGSTNYNPSLKSR	FSMSVETPKNQFSLQVRSVTAADTATYYCASGGPKL.		YWGQGILVTVSSASTK
	×	4-59/c 4-59/d	QVQLQESGPGLVKPSETLSL QVQLQESGPGLVKPSETLSL	TCTVSGGSISNSYW	SWIRQPPGKGLEWIGHIY SWIRQPPGKGLEWIGYLF	.YGDNTHYNPSLRGR	ATISIDTSKSOFSLELSSVTTADTAVTYGVRGGTAAD ATISIDTSKSOFSLELSSVTTADTAVTYGVRGGYAAD	AIRDM	DVWGQGTTVTVSSASTK DVWGQGTTVTVSSASTK
	×	4-61/a	QVQLQESGPGLVKPSETLSL	TCTVSGDSVSSG Y YYW	TWIRQPPGKGLEWIGKIR	.YSGRTKYKSSLQSR	VTMSVDTSKNHFSLSLSSVTAADTAVYFCAREVDWGG	AYVSNDAL	DMWGQGTVVTVSSASTK
	× ×	5-51/a	EVQLVQSGAEMKKPGDSLKI	SOQGSGYRFSSYWI	AWVRQKPGKGLEWMGTVHP.	.GDSESRYSPSFEGQ	VTISADMSTNTAYLQWGSLFASDTGIYYCARGLVAHI	(NVWGQGTLVTVSSASTK
	x	5-51/b	EVQLVQSGAEVKKPGESLKI:	SCKSSGYTFTSNRI	GWVRQMPGKGLEWMGIIYP.	.VDSDTTYSPSFQGH	VTISADKSTSTAYLHWSSLKASDTAIYYCAR. LSYCIG	CSGWWSF	DLWGRGTLVTVSSASTK
	x	5-51/d	EVQLVQSEAEVKKPGESLKI	SCKASGYDFTNYWI	GWVRQVPGKGLDWMGIIYP.	.GDSDTRYSPSFEGQ	VTMSADKSTDTAYLQWTSLKASDTAFYYCARHRVRGA	AASLYNWF	DPWGQGTLVTVSSASTK
	x	5-51/e	EVQLVQSGAEVKKPGESLKI: EVOLVOSGAEVKKPGESLRI	SCKASGYNFNTYWI	GWVRQMPGKGLEWMGIIYP. SWVROVPGKGLEWMGRLDP.	.GDSDTRYSPSFQGQ	VTISADKSFSTAYLQWSSLKASDTAMYYCTTYCAADC	GFYFNGM	DVWGQGTRVTVSSASTK
	x	5-a/b	EVQLVQSGAEVKKPGESLRI	SCKASGYSFTSYWI	SWVRQVPGK <mark>GLEWMGR</mark> LDP.	.LDS YTN YSPS FQGH	VSISGDNSISTAYLQWGSLQASDTATYYCARHDYDGS	SGDSLTN	WGQGTLVIVSSASTK
	×	5-a/c 3-53/a	EVQLVQSGAEVKKPGESLRI: EVQLVESGGGLVQPGGSLRL:	SCEGSGYSFINYWI SCVASDFSVGDNYM	SWVRQMPDKDLEWMGRIDP. TWVRQAPGKGLDWVSMIY	.SGGSTHYADSVRGR	VTISVDKSISTAYLQWSSLKASDTAIYYGAR.SL FTISRDKSKNTLYLQMNSLRVEDTALYYCAR.VPGHA.		WGQGTLVTVSSASTK
	×	4-39/a	QLQLQESGPGLVKPSETLSL	CIVSGGSIGSSSYFW	GWFRQPPGKRLEWIGTIY	. YSGDT YYNPSLKSR	LSLSLDTSKNLVSLKLHSVTAADTAVYYCAR. PPLYYD	SE	DYWGQGTFVTVSSASTK
	×	1-46/a	QVQLVQSGAEVKKPGASVKV:	SCKASGFTFSA YYI	HWLRQAPGQGLEWMGRIDP.	.SGVSTGYAQKFQAR	VTMTKDTSTSTGYMELSSLRSDDTAI YYCAR GRLAVG	CAPFYF	DSWGQGTLVTVSSASTK
	×	2-05/a 3-07/a	QITLKESGPTLVKPTQTLTL EVQLVESGGGLVOPGGSLTV	CTVSGFSLNSYRVGV SCAASGFTFVSYWM	AWIRQPPGKALEWLALIH SWVROAPGKGLEWVANIKO.	. WODDKRYSPSLTRR . DGSEKYYVDSLEGR	LTITKDTSKNHVVLTLTNMEAVDTATYYCAHR.TD FTISRDNAKKSLYLHMNSLRAEDTAVYYCVR.GLALTR	PFGRM	DVWGQGTTVTVSSASTK DVWGQGTTVTVSSASTK
	×	3-11/a	QVQLVESGGGLVRPGGSLRL	SCAASGFTFSDYYM	SWIRQAPGKGLEWVSYISN.	. REGYTKDADSVTGR	FIISRDNAKSSLYLQMNNLRAEDTAVYYCARGGGGSK	IKWF	DPWGQGTLVTVSSASTK
	×	3-11/b 3-30/a	QVQLVESGGGLVRPGGSLRL QVQLVESGGGVVQPGRSLRL	SCAASGFTFSDYM SCAASGFTFSSYAM	SWIRQAPGKGLEWVSYISN. HWVRQAPGKGLEWVAVISY.	 DGSNKYYADSVKGR 	FIISRDNAKSSLYLQMNNLRAEDTAVYYCARGGGGSK FTISRDNSKNTLYLQMNSLRAEDTAVYYCARSPMVPL	1K	DPWGPGTLVTVSQASTK DYWGQGTLVTVSSASTK
	X	3-33/a	QVQLVESGGGVVQPGRSLRL:	SCSATGFTFRSYGM	HWVRQAPGKGLEWVAVIWY.	.DGSKEYYPDSVKGR	FTISRDNSKNTLYLQMNTLRDEDTAVYYCAR. GSGRWS	PYFYAM	DFWGQGTTVTVSSASTK
	× ×	3-33/c	QVQLVESGGGVVQPGRSLRL	SCSATGFTFRSYGM	HWVRQAPGKGLEWVAVIWY.	.DGSKEYYPDSVKGR	FTISRDNSKNTLYLQMNTLRDEDTAVYYCARGSGRWS	PYFYAM	DFWGQGTTVTVS.ASTK
	X	4-31/a 4-59/a	QVQLQESGPGLVKPSQPLSL OVOLOESGPGLVKPSETLSL	DCTVSGGSISGGSYYW	SWIRQQPGKGLEWIGYIY SWIROSPEKGLEWLGYIF	.YSGTNNYNPSLKSR .DIGITNYNPSLKSR	VTISIDTSKNOFSLEMTSVTAADTAVYFCVRDKVVAG LTLSLDTSKNOFSLELSSVTAADTAVYFCARTPLRCA	NHYYYYGM AGTCVKYNYGL	DVWGQGTTVTVSSASTK DAWGHGTTVIVSSASTK
	X	5-51/a	EVQLVQSGAEVKKPGESLKI	SCKTSGDNFKNYWM	GWVRQMPGKGLEWMGII FS .	.GDSETRYSPSFQGQ	VTISFDKSTKTAYVHWSSLKASDTAMYFCASLKPVFP	SDSLDYHYDDSGHYSVLPG	R. WGQGTLVTVSSASTK
	х	6-01/a	QVQLQQSGPGLVKPSQTLSL	ngvisgdsvasnifaw	NWI RQSPSRGLEWLGRAYRR	.SIYYNDDMVSVKSR	ITITSDTSRNQLSLHLNSVTPEDTAVYYGARGNLN		DIWAQGTVVTVSSASTK
	×	1-08/a	QVQLVQSGAEVKKPGASVKV	CKASGYT LAS YDI	NWVRQTTGQGLEWMGWVNP.	.NSGDTGYAQKFQGR	VTMTRDTSISTAYMELSNLRSEDTALYYCARVKPGPY	QF	DPWGQGTLVTVSSASTK
	×	1-24/a 1-46/a	QVQLVQSGAEVKKPGASVKV:	SCKASGYSFTNYYL	HWVRQAPGKGFEWMGIINT.	.GGGSTGYAQKFQGR	VINTEDISTDIAIMENSSLASEDIAVIICAN GROEDW VINTEDISTSTVHMELSSLRYEDTAVYYCAR DLRPTA	iKGAF	DIWGQGTMVTVSSASTK
	×	1-69/a	QVQLVQSGTEVKKPGSSVKV	SCKGSGGNFNNYAI	NWVRQAPGQGLEWMGQIIP.	.FFDTTNDAQKFQGR	VTITADKSTSTAYMELSSLTYEDTAVYYCARDGGPTG	DYYGM	DVWGQGTTVTVSSASTK
	X X	3-07/a	EVQLVESGGGLVQPGGSLRL	SCAASGFTFGTYWM	SWVRQAPGKGLEWVANIKQ.	DGNEKYYVDSVK	FTISEDNAKNSLYLQMNSLRAEDTAVYYCAR. GHCYGG	CYAGHF	DYWGQGTLVTVSSASTK
	×	3-09/a 3-11/a	EVQLVESGGGLVQPGRSLRL: OVOLVESGGGLVKPGGSLRL:	SCAASGFTFVDYAM SCAASGFRFSDYYM	HWVRQTPGKGLEWVSGISW. AWLROAPGKGPEWLSYVST.	.NSGSIGYADSVRGR .TSTNIYYADSVKGR	FTISEDNAKNSLFLOMNSLETEDTAFYYCIKGGLLEF FTISEDNAKNSLFLOMNSLEAEDTANYYCARVELDLE	RELIAPF	DYWGQGILVTVSSASTK DVWGOGTTVTVSSASTK
	х	3+23/a	EVQLLESGGGLVQPGGSLR	SCAASGFTFSNYAM	TWVRQAPGKGLEWVSAVIS.	.NGLNTYYADSVKGR	FTISRDNSKNTLYLHMNSLRPEDTAVYYCAKVGVVVK	/FLIGLASPDWYF	DLWGRGTLVTVSSASTK
	×	3-23/b 3-23/c	EVQLLESGGGLVQPGGSLRL: EVQLLESGGTFVQSGGSLRL:	SCTASGFTFSNYAV SCAASGFTFSTYSM	NWVRQTPMKGLEWVSVITA. SWVRQAPGKGLEWVSAFRA.	SGRTDYPDSVKGR .SDGATFYADSVKGR	FTISRDNSKNMLYLQMSSLRAEDTAIYYCAK. BRTTEP FTVSRDKSRNTLYLQMNSLRADDTAVYYCAK. PHPPAR	YCGADCP.	DSWGQGTLVTVSSASTK DYWGQGTQVTVSSASTK
	x	3-23/d	EVQLLESGGGLVHPGGSLRL	CAGSGFAFSS FAM	SWVRQAPGKGLEWVSVVSV.	. SGS STFYADSVKGR	FTMSRDNSKNTLYLOMNSLRAEDTAVYYCAK GLNHCS	ASCRPGA	WGQGTLVTVSSASTK
	×	3-23/é 3-23/f	EVQLLESGGGLVQPGGSLRL	SCAASGFTFRN YAM	RWVRQAPGKGLEWVSGISD.	.TGGSTYYADSVKGR	FTISRDNSKNTVYLQMNSLRAEDTAVYYCAK. DQIAAA	ARYYNYGL	DVWGQGTTVTVSSASTK
	×	3-33/a	QVQLVESGGGVVQPGRSLRL OVOLVESGGGVVQPGRSLRL	SCVASGFVFGTYGM	HWVROAPGKGLEWVASIFD.	. DSSNRHYADSVKGR	FTISRONSKATVFLOMNSLRVEDTAVYYCAR WIGRON	(DYWGQGTLVTVSSASTK DVWGOGTTVTVSSASTK
	x X	3-49/a	EVOLVESGGGLVKPGRSLRL	SCTASGFTFDD YTM	TWFROAPGKGLEWVAFIKSK	AFGATIEYAASVKGR	FTISEDDSKSIAYLOMNSLKTEDTAVYYCTRDPGYSG	DWDLGTYVPRF	DYWGOGTLVTVSSASTK
	×	3-53/a 3-74/a	EVQLVESGGGLIQPGGSLRL: EVQLVESGGDLVOPGGSLRL:	SCAASGENVSTTYN SCAASGITLSGYWM	TWVRQAPGKGLEWVSVIY HWVRQAPGKGLVWVALVKS.	.MGGGRYYADSVKGR .DGTSTVYADSVRGR	FTVSKDNSKNTIYLQANSLRAEDTAIYFCAR., VHYFYG FTISRDDAKNTLYLQMNSLRAEDTAVYYCAT, MDA	GSSDMGFFGAF	JIWGQGTMLTVSSASTK .YWGQGTLVTVSSASTK
	x	4-34/a	QVQLQQWGAGLLKPSETLSL	ICAVYGGSFSGYYW	TWIRQSPEKGLEWIGESY	.HTGKTKYNPSLRSR	VTISVDTSKNHFSLSLRSVTAADTAVYYCAR AGSSSN	YPTPRHYF	DSWSQGTRVTVSSASTK
	×	4-59/a 4-59/b	QVQLQESGPGLVKPSETLSL/ QVQLQESGPGLVRPSETLSL/	PCAVSGDAISSSYW PCSISGGSINSYYW	SWIRQSPGKGLEWIAFIH SWIRQPPGKGLEWLGYIS	. YNGTSNFNPSLKGR .FSGSPDYNPSVKSR	VTTSVDTSKSRFSLRLTSVSAPDTAVYYCAKVSAPS. LTISLDKPKNOVFLRLTSVAAADTAVYYCARGG	YLYF AF	DLWGRGTLVTVSSASTK DFWGHGTMVVVSAASTK
	x	4-59/c	QVQLQESGPGLVKPSETLSL	PCTVSDGSISNYYW	SWIRQPPGKGLEWIGYIY	.YTGNTNYNPSLKSR	VTISVDTSKNOFSLKLTSVTAADTAVYYCAR EGPGYS	(AL	DYWGQGTLVTVSSASTK
	×	4-59/d 5-51/a	EVQLVQESGPGLVKPSETLSL EVQLVQTGAEVKKPGESLRI	SCKASGYTFSR., YVI	SWINQSPGKGLESIGYIS GWVRQVPGKGLEWLGIVYP.	.GDSDTRYGPSLOGO	TIRSUUTSKNUTSLKLNSVTAADTAVYYCARWAAAGN VTISADESIATAYLQWSSLKASDTAMYFCATGTLRSA	EAF	NIWGQGTMVTVSSASTK
	×	5-51/b	EVQLVQSGAEVKKPGESLKI	SCKGSGYSFTN FWI	GWVRQMPGKGLEWMGIIYP.	. GDS DI RYSPSFOGO	VTISADKSISIAYLQWTSLKASDTAMYYCAR. RRKGYC	GGICYDF	DYWGQGTLVTVSSASTK
	X X	5-51/d	EVQLVQSGAEVKKPGESLQI	SOQGSGYNFIDYWI	AWVRQMPGKGLEWMGVIYP.	.GDSDTRYSPSFQGQ	<pre>viisAdASdSTTILER#SSLKASDTAMTFGARPTCTNN VTISVDKSISTAYLQWSSLKASDTAMYYCATTAGFT.</pre>	YF	DFWGQGTLVAVSSASTK
	×	5-51/e	EVOLVOSGPEVKKPGESLKI	SOQGSTYSFTSDWI	AWVRQMPGKGLEWMGIIYP.	.GDSDTRYSPSFQGQ	VTFSADKSISTAYLQWSSLKASDTALYFCAR RGSWGT	SFHYGL	DVWGQGTTVTVSSASTK
	x	6-61/a	QVQLQQSGPGLVKPSQTLSL	TCAI SGDSVGTTFAAW	NWI RQSPSRGLEWLGRTYYR	.SKWYSDYALSVRGR	ITIKSDTSKNHFSLQLNSVTPEDTAVYYCAR DSVRGE	FGLLHDSFYYGM	DVWGQGTTVTVSSASTK



DIGHTGSESSASVORDET TERASGESS....LANYQQKFOKVPKLLTYAATIGSVFSF933535TEFTIISISJOEDFATTG.SKTOBA.. DIGHTGSESSV5ASVORDET TERASGVISS...LANYQQKFOKAPKLLTYAASIGSVFSF933535TEFTIISISJOEDFATTG.SKTOBA.. DIGHTGSESVSASVORDET TERASGVISS...LANYQQKFOKAPKLLTYAASIGSVFSF933535TEFTIISISJOEDFATTG.SKONSFP. DIGHTGSESVSASVORDET TERASGVISS...LANYQQKFOKAPKLLTYAASIGSVFSF933535TEFTIISISJOEDFATTG.CONNFFP. DIGHTGSESVSASVORDETTERASGVISS...LANYQQKFOKAPKLLTYAASIGSVFSF933535TEFTIISISJOEDFATTG.CONNFFP. DIGHTGSESVSASVORDETTERASGPRSS...LANYQQKFOKAPKLLTYAASIGSVFSF933535TEFTIISISJOEDFATTG.CONNFFP. DIGHTGSESVSASVORDETTERASGPRSS...LANYQQKFOKAPKLLTYAASIGSVFSF933535TEFTIISISJOEDFATTG.CONNFFP. DIGHTSSFSV3ASVORDETTERASGPRSS...LANYQQKFOKAPKLLTYAASIGSVFSF933535TEFTIISISJOEDFATTG.CONNFFP. DIGHTSSFSV3ASVORDETTERASGPRSS...LANYQQKFOKAPKLLTYAASIGSVFSF933535TEFTIISISJOEDFATTG.CONNFFP.

×

L5/a L5/b

FGQGTKLEI

.ITFGQGTRLEIK .ITFGQGTRLEIK

WED

Supplementary Fig. 3



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



Σ=65

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
VH4-59 CDR2 rev-out
VH4-59 CDR2 rev-in

5'-AGGGAAGGGACTGGAGTGGC 5'-TGATATGGTGACTCGACTCTC 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 VK1-O2 CDR1 rev-out VK1-O2 CDR1 rev-in 5'-TCACTTGCCGGGCAAGTCG 5'-ATGCACCATAGATCAGGAGC 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 μ l 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 PepMapTM, 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 PepMapTM, 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 PepMapTM, 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