RESEARCH ARTICLE OPEN ACCESS

High-Affinity Human Germline and Mutated Autoantibodies Commonly Cross-React to Self-Antigens in 5 Subgroups of Autoimmune Encephalitis

Laura Stöffler,[1,2] Jakob Kreye,[1,2,3] Friederike A. Arlt,[1,2] Elisa Sanchez-Sendin,[1,2] Julius Hoffmann,[1,2] Hans-Christian Kornau,[1,4] Marieluise Kirchner,[5,6] Philipp Mertins,[5,6] Scott van Hoof,[1,2] Stephanie Wernick,[1,2] Marie Homeyer,[1,2] Sebastian Momsen Reincke,[1,2] and Harald Prüss[1,2]

Correspondence
Prof. Prüss
harald.pruess@charite.de

Neurol Neuroimmunol Neuroinflamm 2026;13:e200608. doi:10.1212/NXI.0000000000200608

Abstract

Background and Objectives

Antineuronal autoantibodies targeting surface membrane proteins are the hallmark of an increasing number of autoimmune encephalitides. The autoantibodies can be directly pathogenic and cause various symptoms ranging from epilepsy and psychosis to amnesia and autonomic dysfunction. It is largely unclear how the humoral autoimmune response is triggered and propagated.

Methods

We analyzed whether affinity maturation leads to increasing affinity of encephalitis-related autoantibodies to their receptors by determining the binding strength of patient-derived monoclonal autoantibodies and their germline ancestors from 11 patients with NMDAR, LGI1, mGluR5, CASPR2, and $GABA_AR$ encephalitis. In addition, binding to foreign and self-antigens was assessed.

Results

For most autoantibodies, affinity maturation generated or increased binding to neuronal surface antigens. However, high numbers of somatic hypermutations were not necessarily needed, as half of germline-encoded variants already recognized the respective antigen with strong binders in all groups. 10%–15% of reverted and mutated monoclonal autoantibodies recognized nuclear antigens, and one-third were reactive to mammalian tissues other than brain. Immunoprecipitation combined with mass spectrometry identified the nuclear antigen proteins major vault protein and translocated promotor region as targets of selected germline ancestors.

Discussion

The findings suggest analogous immunologic mechanisms across 5 different encephalitides, with high-affinity autoantibodies already in the germline pool and frequent reactivity to nuclear antigens. Future studies should determine the contribution of immunologic checkpoint deficiencies, as seen in one patient with a pathogenic immune checkpoint mutation.

MORE ONLINE

Supplementary Material

[1]German Center for Neurodegenerative Diseases (DZNE) Berlin, Berlin, Germany; [2]Department of Neurology and Experimental Neurology, Charité Universitätsmedizin Berlin, Berlin, Germany; [3]Department of Pediatric Neurology, Charité Universitätsmedizin Berlin, Berlin, Germany; [4]Neuroscience Research Center (NWFZ), Charité Universitätsmedizin Berlin, Germany; [5]Core Unit Proteomics, Berlin Institute of Health (BIH), Charité Universitätsmedizin Berlin, Berlin Germany; and [6]Proteomics Platform, Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany.

The Article Processing Charge was funded by the authors.

Glossary

ANA = antinuclear antibodies; **APS-1** = autoimmune polyglandular syndrome type 1; **ASC** = antibody-secreting cells; **AUC** = area under curve; **CASPR2** = contactin associated protein receptor 2; **EMEM** = Eagle's Minimum Essential Medium; **FCS** = fetal calf serum; **$GABA_AR$** = gamma aminobutyric acid A receptor; **HSV** = herpes simplex virus; **HSV-1** = herpes simplex virus type 1; **LGI1** = leucine-rich glioma-inactivated protein 1; **mAbs** = monoclonal autoantibodies; **MBC** = memory B cells; **mGluR5** = metabotropic glutamate receptor type 5; **MVP** = major vault protein; **NMDAR** = N-Methyl-d-aspartate receptor; **NMOSD** = neuromyelitis optica spectrum disorder; **SHM** = somatic hypermutations; **SLE** = systemic lupus erythematosus; **TPR** = translocated promotor region.

Introduction

Antineuronal autoantibodies can lead to autoimmune encephalitis with a variety of neurologic and psychiatric manifestations including psychosis, seizures, pain, memory loss, movement disorders, and vegetative dysfunction.[1] The number of underlying neuronal or glial surface autoantigens is growing continuously and has not reached a plateau yet.[2,3] For many of these encephalitides, experimental evidence from patient-derived monoclonal autoantibodies (mAbs) and their use in animal models demonstrated direct pathogenicity and the development of clinical phenotypes mimicking the human diseases.[4] Mechanisms include the downregulation of target receptors,[5,6] reduction of synaptic currents by receptor inhibition,[7] neutralization of protein-protein interactions,[8] and the activation of cellular cytotoxicity.[9]

To date, the mechanisms by which these autoreactive antibodies arise and persist in the periphery have not been elucidated. In humoral immune defenses against foreign pathogens, such as bacteria and viruses, different mechanisms contribute to antibody diversity and reactivity. These include the random reassortment of V(D)J genes and the insertion of nontemplate n-nucleotides within the CDR3 region.[10] In addition, after antibody-antigen interaction in the germinal centers during B cell affinity maturation, somatic hypermutations (SHM) are inserted into the B cell receptor genes.[11] To reduce the number of autoreactive B cells generated as part of these physiologic mechanisms, high-affine autoreactive B cells are usually negatively selected by distinct central and peripheral immune checkpoints.[12]

Previous work suggested that affinity maturation likewise shapes the autoantibody repertoire in certain human autoimmune diseases such as neuromyelitis optica spectrum disorder (NMOSD) and systemic lupus erythematosus (SLE). Patient-derived mAbs targeting the underlying autoantigens aquaporin-4 and extractable nuclear antigen Ro52, respectively, showed no or low reactivity towards their respective antigens after reversion to the germline configuration.[13,14]

Therefore, it was a surprise when mAbs generated from the CSF of patients with N-Methyl-D-aspartate receptor (NMDAR) encephalitis, the most common form of autoimmune encephalitis, could be cloned from unmutated B cells.[6] Both, unmutated and germline-reverted (previously hypermutated) mAbs showed high binding strength to the NMDAR and resulted in functional effects by reducing synaptic currents in hippocampal neurons.[15] These findings suggested that highly autoreactive naïve B cells targeting the NMDAR escaped B cell counter-selection and are part of the human B cell repertoire.

We therefore hypothesized that antineuronal autoreactivity may be present in the germline configuration of autoantibodies in additional autoimmune encephalitides. We compared the CSF mAb profile of patients with NMDAR, leucine-rich glioma inactivated protein 1 (LGI1), metabotropic glutamate receptor type 5 (mGluR5), contactin associated protein receptor 2 (CASPR2), and gamma aminobutyric acid A receptor ($GABA_AR$) encephalitis. We thus generated germline versions of autoreactive patient-derived mAbs, explored autoimmunity-associated CDR3 sequence irregularities and differences in affinity and specificity, and assessed polyreactivity and cross-reactivity to find distinctive features that associate with autoantibody formation.

Methods

Antibody Generation and Production

Patient-derived monoclonal human anti-NMDAR, -$GABA_AR$, -CASPR2, -LGI1, and -mGluR5 antibodies were previously generated as described.[6,16] An IgG isotype matched nonreactive antibody mGO53 was used as negative control.[10] We generated antibody germline (gl) revertants by returning V gene mutations to the best fit germline V(D)J gene configuration as identified using NCBI IgBlast and cloning into an IgG1 backbone. The reversion strategy was performed, as described.[17] At least 2 mAbs with previously confirmed binding in flow cytometry and immunohistochemistry of murine brain slices from each encephalitis group were selected for reversion.

Sequence Analysis

Clonotypes, mAb sequences, CDR3-charge, CDR3-length, and CDR3-hydrophobicity as well as the number of SHM were previously analyzed using our established method

(BASE).[18] Sequences from antibodies previously published were reanalyzed, and new antibodies were added.[6,19-21]

Flow Cytometric Quantification of Binding Strength

Human Embryonic Kidney 293T cells (HEK293T) were cotransfected with enhanced green fluorescent protein (EGFP) and the antigens (GluN1/GluN2B, $GABA_AR$ A1/B3/G2, CASPR2, LGI1, mGLuR5). Polyethyleneimine was used as transfection agent. For GluN1/GluN2B transfected cells, MK-801 was added for NMDAR antagonism. Cells were harvested 2 days after transfection with 1 minute incubation of trypsin and then incubated with primary antibodies (diluted in FACS buffer: 2% heat-inactivated fetal calf serum (FCS) in 1x phosphate buffered saline (PBS)) for 45 minutes on ice, washed with FACS buffer, and stained with the secondary antibody (Alexa Flour-647 goat-anti-human, Life Technologies, 1:400 in FACS buffer) for 20 minutes. DAPI (4',6-Diamidin-2-phenylindol, 0.1 ng/mL)1:100) was added to the cells, and samples were analyzed using FACSCanto™ II (BD Biosciences). Quantification of antibody binding strength was performed as described.[22] The mean fluorescence intensity (MFI) was calculated from the EGFP-positive live single cells. To reduce interexperimental variability, MFI values were normalized by dividing the MFI of each sample by the MFI of the 2 strongest binding mutated mAbs of each target at a concentration of 20 μg/mL. One-site specific binding nonlinear regression models were calculated using GraphPad Prism (9.2.0) software. As a relative value for mAb binding strength, the parameters c_{50} (mAb concentration when reaching 50% of the maximal MFI) and the area under curve (AUC) were calculated. We set an AUC value of 86.2 as a cutoff for specific binding, based on the mean AUC of the negative control mAb mGO53 and its 3 times SD.

Immunohistochemistry of Brain and Organ Tissues

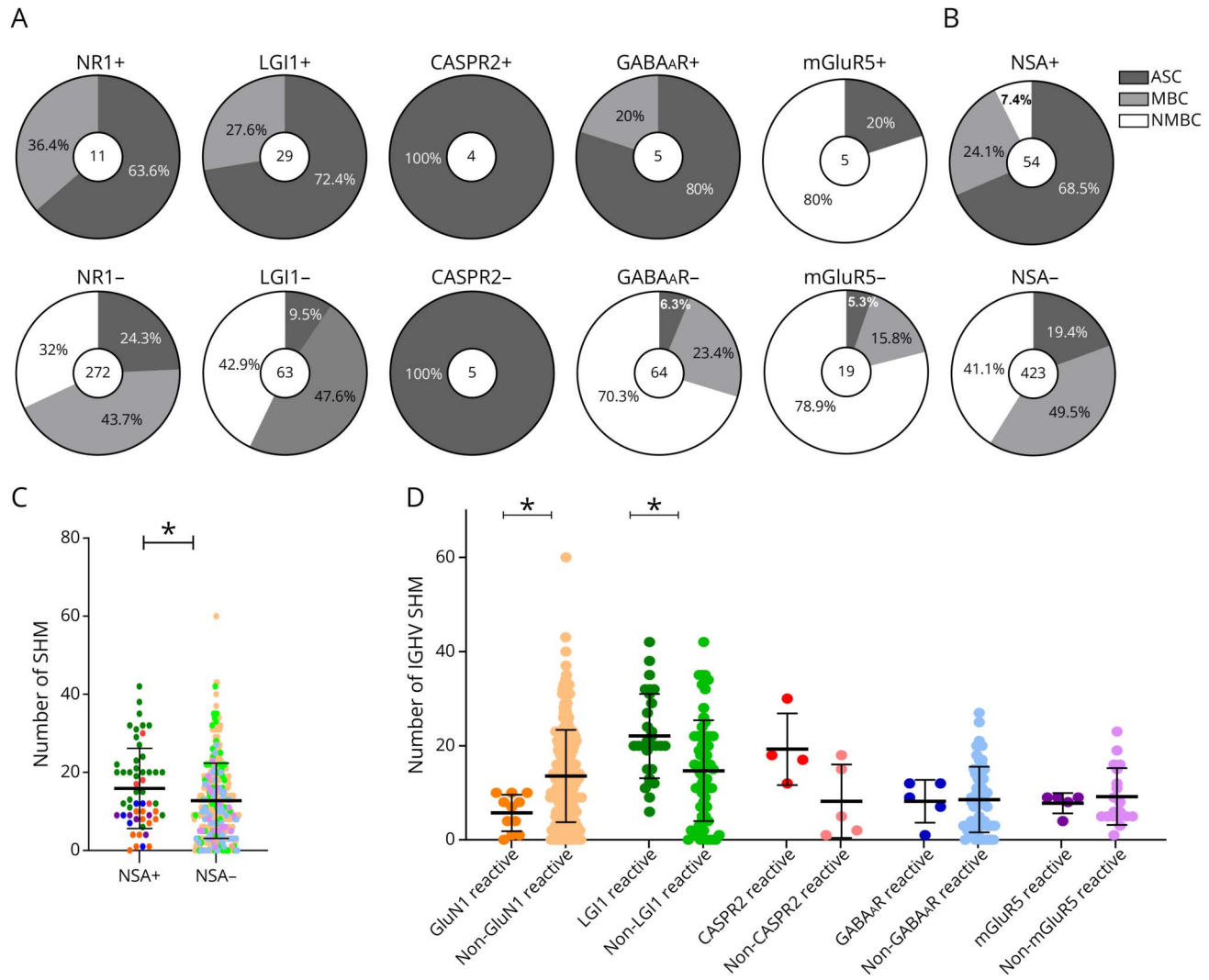
Mice were sacrificed by cervical dislocation, brains and tissues were shock frozen, stored at −20°C, and cut in 20 μm slices. Slices were fixed for 5 minutes with paraformaldehyde (PFA), washed with PBS, and preincubated with blocking solution

Table 1 Summary of Patient Characteristics Regarding Age Group at Diagnosis (<25, 25–50, 50–75, >75), Symptoms, Disease Progression, and Comorbidities

ID	CSF mAbs	Age	Main symptoms	ICU	Immunotherapy	Residual symptoms	Tumor association	Autoimmune comorbidities
003	Anti-NMDAR	<25	Psychosis, cognitive impairment	Yes	Plasmapheresis, rituximab	Slight memory impairment	Teratoma	Hypothyroidism
007	Anti-NMDAR	<25	Paresthesia, delirium, paresis, seizures	Yes	Steroids, plasmapheresis, rituximab	Slight impairment of information processing speed	No	Multiple infectious diseases
008	Anti-NMDAR	<25	Epileptic seizures, psychosis	No	Steroids, plasmapheresis, rituximab	No	No	Autoimmune thyroiditis with hypothyroidism
080	Anti-NMDAR	<25	Psychosis, loss of consciousness and memory, paresthesia, seizures	Yes	Steroids, IVIGS, IA, plasmapheresis, rituximab, bortezomib, MTX	Motor impairment, prolonged ICU treatment	Teratoma	No
054	Anti-LGI1	50–75	Faciobrachial dystonic seizures, absences	No	Steroids, IA, rituximab, MTX	Subjectively impaired memory	NET	No
060	Anti-LGI1	50–75	Cognitive impairment, myoclonus and seizures	No	Steroids, IA, plasmapheresis, rituximab	Impairment of memory and orientation	No	No
137	Anti-LGI1	50–75	Faciobrachial dystonic seizures, falls	No	Steroids, plasmapheresis, rituximab	Gait disorder, mild cognitive impairment	Pancreatic head carcinoma	Lichen sclerosus et atrophicus
187	Anti-CASPR2	50–75	Morvan syndrome	No	Steroids, plasmapheresis, rituximab	Small fiber neuropathy	No	No
219	Anti-CASPR2	50–75	Cognitive impairment, character change, myoclonus, dysarthrophonia	Yes	Steroids, IVIGs, IA, plasmapheresis, rituximab, bortezomib, daratumumab	Remission after extensive treatment	Base of tongue SCC	Hypothyroidism
113	Anti-$GABA_AR$	<25	Fever, catatonia, encephalopathy	Yes	Steroids, IVIGS, plasmapheresis	No	No	APS-1
136	Anti-mGluR5	<25	Headache, photophobia, dysarthria, hallucinations	Yes	Steroids, IVIGS, rituximab, bortezomib, MMF	Remission	No	No

Abbreviations: IA = immunoabsorption; IVIG = IV Immunoglobulin; MTX = methotrexate.
Five of 11 patients had comorbidities related to autoimmunity.

Figure 1 Features of Antibodies Reactive to Neuronal Surface Antigens (NSA)



Phenotype distribution of B cells was different for NSA+ and NSA- antibodies in autoimmune encephalitis subgroups (A), with ASCs being the predominant source for NSA+ mAbs, while MBCs and NMBCs produced most NSA- mAbs (B). NSA+ mAbs had a higher number of SHM across all encephalitides (C, each dot indicates one mAb). In NMDARE, SHM numbers were lower in NSA+ mAbs, while they were higher in NSA+ mAbs in LGI1E (Mann-Whitney *U* test), and not statistically significantly different in the other subtypes (D). Bars indicate mean and SD. ASC = antibody-secreting cells; mAbs = monoclonal autoantibodies; MBC = memory B cells; NMBC = non-memory B cell; SHM = somatic hypermutation.

(5% normal goat serum, 2% bovine serum albumin and 0.05% sodium acid in 1x PBS). Primary antibodies were incubated at 4°C overnight, washed, and incubated for 2 hours at room temperature with the secondary (Alexa Flour-488 goat anti-human-IgG, Invitrogen, 1:1,000) and 5 minutes with DRAQ 5 (Thermo Fisher Scientific, 1:1,000). Tissue was mounted with mounting medium (Immu-mount, Thermo Fisher Scientific). For image acquisition, fluorescence microscopy (Leica DMI3000, LEICA SPE) was used. Staining was repeated 3 times.

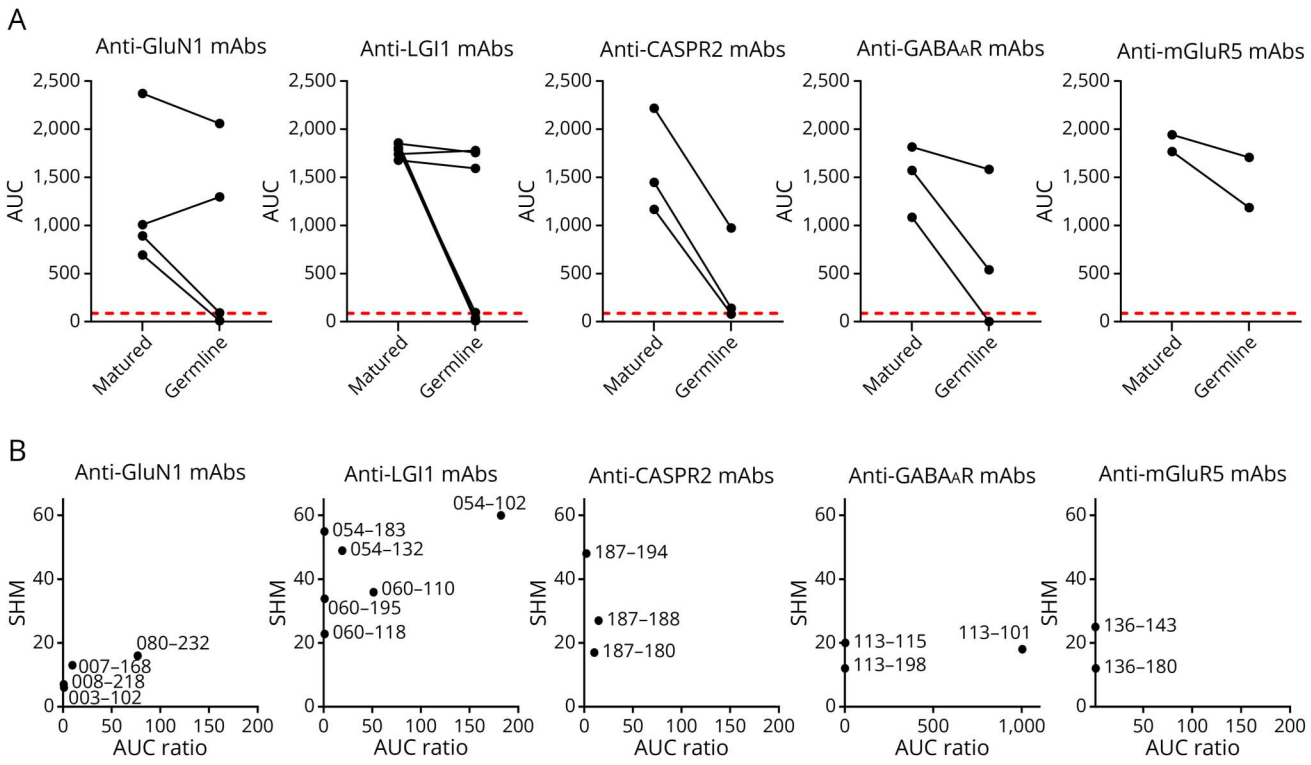
Immunohistochemistry on HEp-2-Cells

The HEp-2 assay was performed using NOVA Lite HEp-2 ANA Kit (Inova Diagnostics) following the manufacturer's instructions. Alexa Fluor-488 goat anti-human was used for secondary staining, and images were obtained with fluorescence microscopy (LEICA DMI3000).

Polyreactivity ELISA

A polyreactivity ELISA was performed as described.[10,16] In brief, protein high-binding plates were incubated over night with 6 different antigens (10 µg/mL calf thymus double-stranded DNA (ds-DNA) (Sigma; #D8515), 10 µg/mL single-stranded DNA (ss-DNA) (from double-stranded DNA prepared by heating at 95°C for 30 minutes, immediate aliquotation and freezing at −20°C), 10 µg/mL lipopolysaccharide (LPS) from Escherichia coli (Sigma; #L2637), 10 µg/mL keyhole limpet hemocyanin (KLH) (Sigma; #H8283), 10 µg/mL Cardiolipin (Sigma; #C0563), and 5 µg/mL human insulin (Sigma; #I9278). Plates were

Figure 2 Quantification of Binding Strength of Germline and Mutated mAbs



Binding strength was quantified using flow cytometry. Binding was determined by the area under curve (AUC) of serial dilution binding as well as by HalfMax concentrations (50% of MFI_{max}) (Table 2). The AUC was calculated for each mutated mAb and its germline reverted counterpart, revealing that at least one germline mAb in every encephalitis subgroup maintained strong binding (A). The dotted line marks the threshold for positive binding. Comparison of the number of SHM to AUC ratios (AUC mutated mAb/germline mAb); small AUC ratio = gl binding strength close to mutated mAb binding)] showed no negative correlation of SHM numbers and gl mAb binding (B). mAbs = monoclonal autoantibodies; SHM = somatic hypermutation.

washed, blocked (0.05% Tween and 1 mM EDTA in 1xPBS), and incubated with reverted and maturated mAbs (1 μg/mL in blocking solution). As positive controls, polyreactive mAbs #eiJB40 and #ED38 were used.[10,23] After washing, the secondary (HRP-labeled goat anti-human IgG Fc antibody (1:5,000; Dianova)) was added. Plates were incubated with 1-Step Ultra TMB ELISA Substrate Solution, and the reaction stopped with 2M sulfuric acid. Polyreactivity was detected at OD450, and mAbs were considered as polyreactive when OD450 was above the OD450 of #eiJB40 minus 2 SDs of #eiJB40.

Herpes Simplex Virus ELISA

Herpes simplex virus (HSV) ELISA was performed with the ANTI-HSV-1/2-Pool ELISA (Euroimmun, #EI 2531-9601-1 G) displaying whole HSV1 and HSV2 antigen. mAbs were diluted to 4 μg/mL, and the assay was performed following the manufacturer's instructions.

HSV1 CBA

Vero E6 cells were cultivated in Eagle's Minimum Essential Medium (EMEM) supplemented with 5% FCS, Gentamycin sulphate, Nonessential Amino Acid solution, Pyruvate, and Glutamax. For HSV1 infection, cells were seeded and cultivated until reach of 80% confluence. Cells were washed twice with PBS and incubated with HSV1 containing supernatant at multiplicity of infection of 3 diluted in EMEM media. One hour after inoculation, supernatant was removed from cells and media supplemented with 2% FCS was applied, and then cells were cultivated at 37°C for an additional 21 hours. Afterward, plates were fixated with 1:1 methanol:acetone solution for 20 minutes at −20°C, blocked in BS for 1 hour at RT, and stained with monoclonal antibodies at 5 μg/mL following our standard staining protocol.

Immunoprecipitation Coupled With Mass Spectrometry

Immunoprecipitation was performed using murine colon and kidney tissue.[24] After cryo-grinding and solubilization in lysis buffer the tissue was centrifuged at 18.000 rpm for 30 minutes at 4°C. MAbs were coupled to Dynabeads Protein G for 30 minutes, washed, and incubated with the tissue lysates for 1 hour at 4°C. After washing, antibody-dynabead complexes were digested with LysC and trypsin and measured using LC-MS (liquid chromatography tandem with mass spectrometry)–based proteomic analysis. Peptides were separated using a 98 minutes gradient on a reversed-phase C18 column using a high-performance liquid chromatography (HPLC) system (Thermo Fisher Scientific) and analyzed on an Q Exactive Plus instrument

Table 2 Quantification of the Binding Strengths of Mutated and Reverted mAb

Antigen	Antibody	C50 (µg/mL)	AUC
NR1/2B	003-102	0.29	2,372
	003-102 gl I	0.25	2058
	007-168	2.43	891
	007-168 gl	43.22	91
	008-218	1.31	1,007
	008-218 gl	0.66	1,297
	080-232	17.39	693
	080-232 gl	16.70	9.0
LGI1	054-102	0.33	1805
	054-102 gl	69.25	9.91
	054-132	0.31	1788
	054-132 gl	42.48	92.8
	054-183	0.16	1740
	054-183 gl	0.22	1779
	060-110	0.26	1859
	060-110 gl	n.d.	36.2
	060-118	0.10	1851
	060-118 gl	0.09	1757
	060-195	0.17	1,679
	060-195 gl	0.31	1,594
CASPR2	187-180	1.83	1,448
	187-180 gl	27.93	139.9
	187-188	2.52	1,166
	187-188 gl	0.16	78.4
	187-194	0.14	2,218
	187-194 gl	0.34	973.2
GABRA	113-101	1.03	1,084
	113-101 gl	n.d.	1.1
	113-115	0.63	1,571
	113-115 gl	18.28	538.9
	113-198	0.49	1815
	113-198 gl	0.62	1,583
mGluR5	136-143	0.03	1943
	136-143 gl	0,09	1708
	136-180	0.04	1787
	136-180 gl	3.46	1,185

Half max binding concentrations (c50, 50% of MFI_{max}) were derived from nonlinear regression models created from flow cytometry results. N.d. was reached when c50 was not determinable within the concatenation range.

(Thermo Fisher Scientific) in data dependent mode. Raw data were processed with MaxQuant software package using human and mouse UniProt database (HUMAN.2020-06; MOUSE.2019-07) with a false discovery rate of 1% for peptide and protein identifications. For statistical analysis, label-free quantification intensities were used. By group comparison against all (Student *t* test and significance cutoff false discovery rate at 1%), antibody targets were identified.

Cell-Based Assays

HEK293T cells were seeded on cover slips coated with PLL on 24 well plates and 1 day later transfected with the respective antigens (major vault protein [MVP], translocated promotor region [TPR]) using PEI. 48 hours later, TPR plates were fixed with PFA and methanol. Primaries were diluted in blocking solution to a concentration of 5 µg/mL and incubated at 4°C overnight. After washing, secondary was added and images were obtained with Leica SPE.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (9.2.0).

Standard Protocol Approvals, Registrations, and Patient Consents

Experiments and human sample collection were approved by the Charité University hospital Review Board (No EA1/258/18). Written informed consent was obtained from each subject in this study.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

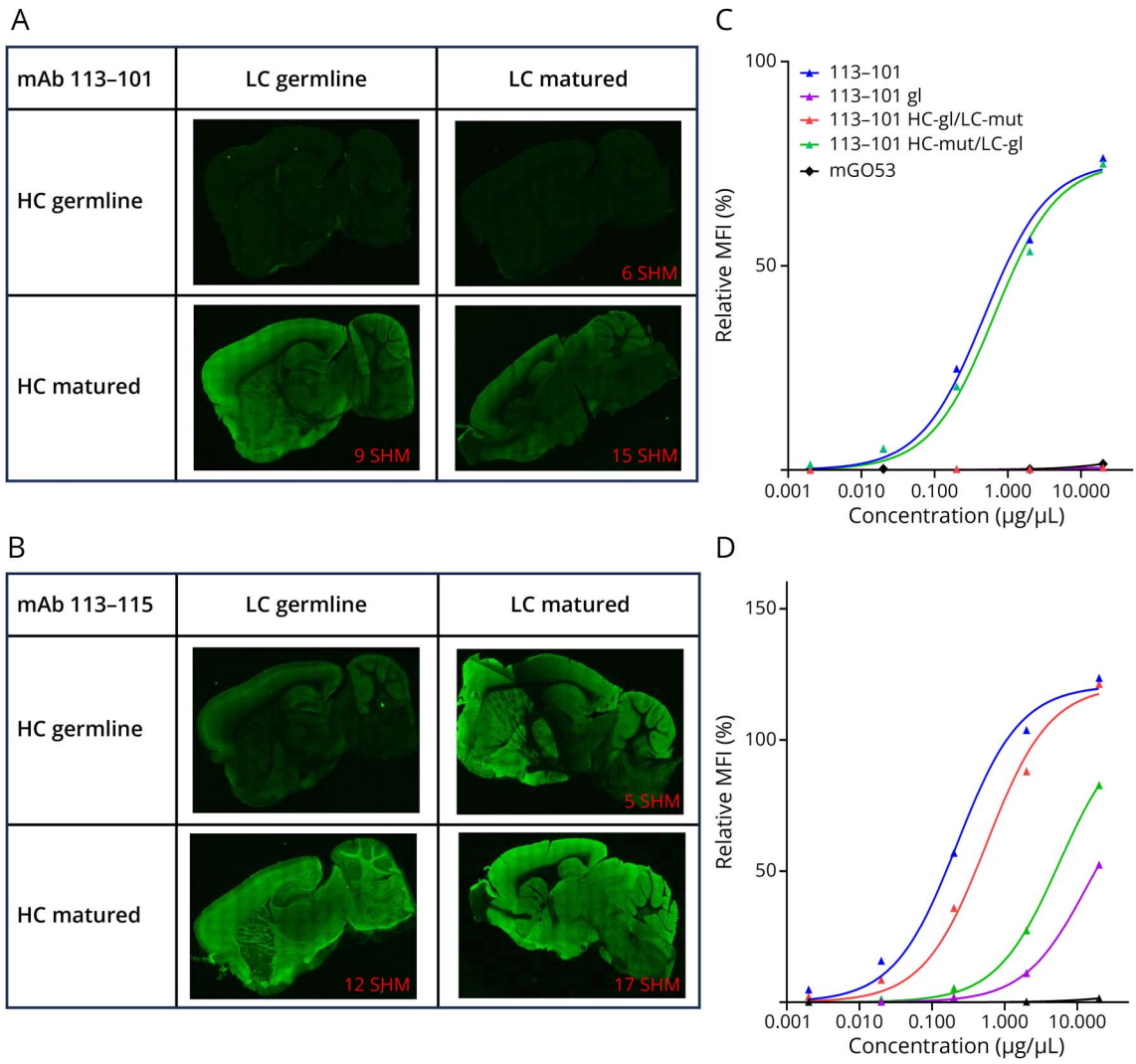
Results

We compared the CSF antibody repertoire of 11 patients from 5 different autoimmune encephalitides, belonging to NMDAR (n = 4), LGI1 (n = 3), CASPR2 (n = 2), $GABA_AR$ (n = 1), and mGluR5 (n = 1) encephalitis (Table 1). In total, 54 mAbs bound to 1 of the 5 underlying antigens, here collectively termed neuronal surface antigens (NSA). In addition, 423 mAbs from the same patients were analyzed, which did not react to these NSA (non-NSA).

NSA-Reactive Autoantibodies Derived Predominantly From Antibody-Secreting Cells and Memory B Cells

To explore distinctive patterns, we compared the phenotypes of the cells from which NSA-reactive mAbs were derived. Except for mGluR5, between 64 and 100% of mAbs were derived from antibody-secreting cells (ASCs) and the remaining from memory B cells (MBC) (Figure 1A). Only 1 of 5 mGluR5 mAbs derived from an ASC and the other 4 from non-MBCs (NMBCs). Combining all 5 autoimmune encephalitis groups, 92.6% of NSA mAbs were cloned from ASCs and MBCs that have supposedly undergone affinity

Figure 3 Binding Characterization After Single Chain Germline Reversion



Staining of $GABA_AR$ reactive 113-101, its germline counterpart (113-101gl) and the 2 hybrid versions with either heavy or light chain in its germline version (113-101 HCgl/LCmut and 113-101 HCmut/LCgl) on murine brain sections (×10) (A, B). Quantification of binding strength using flow cytometry demonstrated that binding of mAb 113-101 depended solely on HC SHM (C), while reactivity of mAb 113-115 markedly increased with affinity-maturated HC and/or LC (D). $GABA_AR$ = gamma aminobutyric acid A receptor; mAbs = monoclonal autoantibodies; SHM = somatic hypermutation.

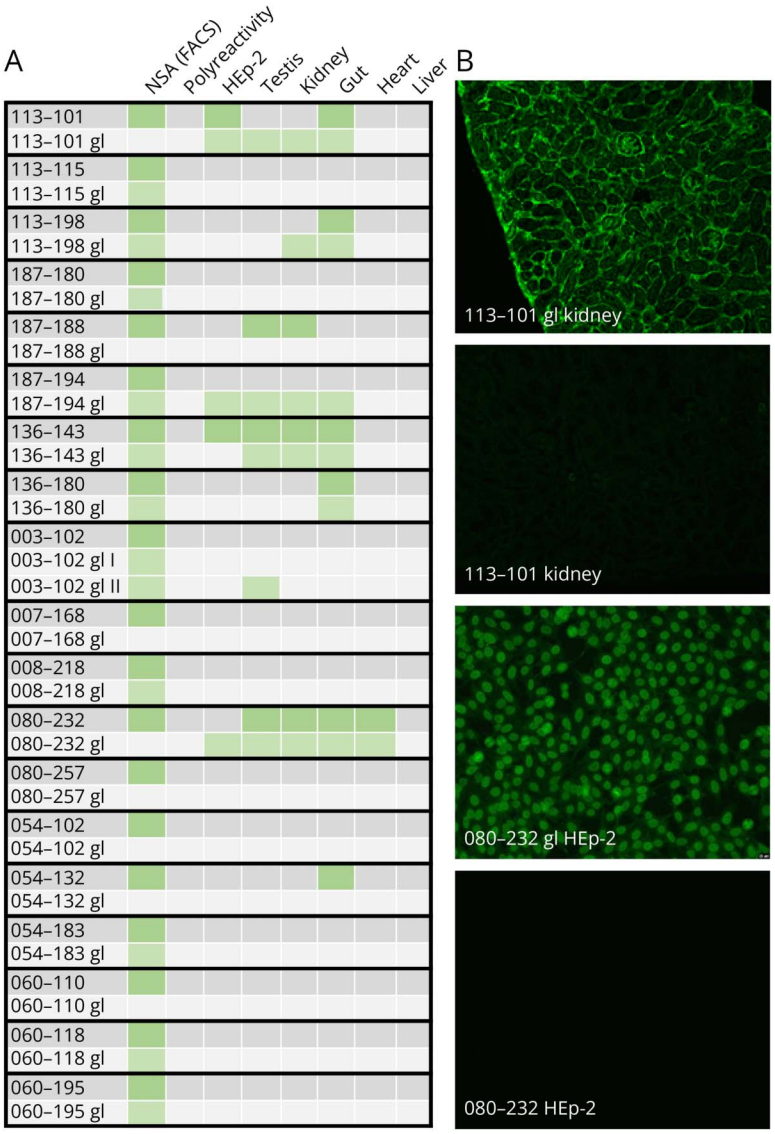
maturation, whereas non-NSA mAbs mainly derived from NMBCs or MBCs (Figure 1B).

NSA mAbs derived from 19 gene families used for immunoglobulin heavy chain variable region (IGHV) gene formation, with frequencies ranging from 1 to 9 antibodies per gene family. The most common gene family was IGHV3-11 with 6 LGI1, 1 CASPR2, and 2 mGluR5 mAbs (eFigure 1A). For none of the encephalitis subgroups, mAbs were restricted to one or few VH genes, indicating diverse polyclonal responses.

LGI1 mAbs Displayed the Highest and NMDAR mAbs the Lowest Numbers of SHM

We next analyzed the CDR3 regions to detect characteristic sequence features using BASE.[18] NSA-reactive mAbs did not differ in terms of length, isoelectric point (pI), or charge of the IGHV-CDR3 region when compared with non-NSA-reactive mAbs of the same patients. These data are available in the eSAP (eFigure 1B-D). NSA-reactive mAbs displayed the elevated mean numbers of SHMs compared to non-NSA-reactive mAbs (mean of 17.7 vs 15.9; Mann-Whitney *U* test; $p = 0.03$) (Figure 1C). However, mAb-SHM were not equally distributed between encephalitis groups. As previously described, mAbs reactive to the GluN1 subunit of the NMDAR had significantly less SHM than non–GluN1-reactive mAbs from the same patients (Mann-Whitney *U* test; $p = 0.003$), and LGI1 mAbs had a significantly higher number of SHMs than non–LGI1-reactive mAbs (Mann-Whitney *U* test; $p = 0.002$).[6,19] SHM numbers of CASPR2 mAbs were higher, and $GABA_AR$ and mGluR5 mAbs slightly lower compared with their nonreactive counterparts of the

Figure 4 Cross-Reactivity of Germline and Mutated mABs



Autoreactivity is summarized in a reactivity map displaying NSA binding, polyreactivity, HEp-2, and tissue reactivity (green bars show reactivity) (A). Exemplary immunohistochemistry stainings include kidney-reactive germline mAb 113-101gl (derived from $GABA_AR$-reactive 113-101) and its non–kidney-reactive maturated counterpart (×10) (B) and positive HEp-2 staining of 080-232gl (derived from NMDAR reactive 080-232) and its nonreactive maturated version (×20) (C). mAbs = monoclonal autoantibodies.

CSF of the same patients, however, not reaching statistical significance, potentially related to the low mAb number (Figure 1D).

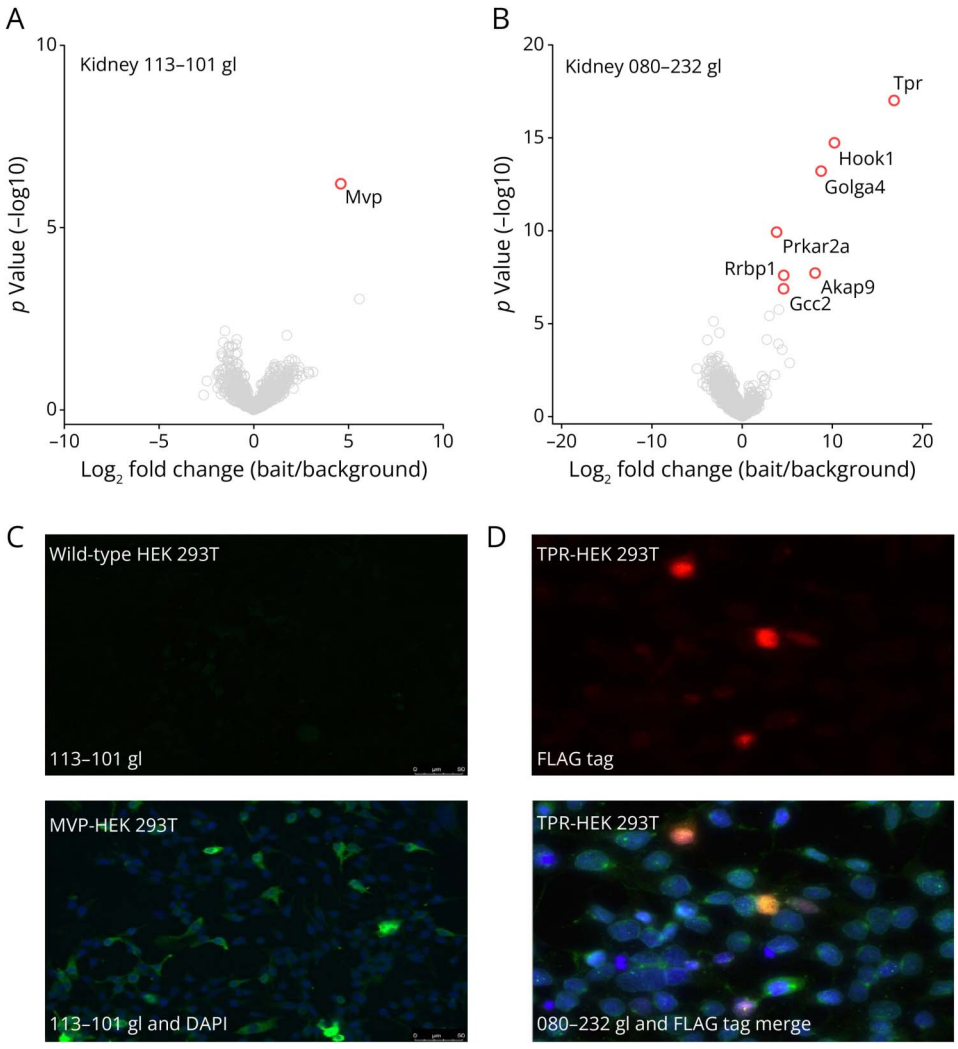
Antibodies Reverted to Germline Bound NSA With High Binding Strength

As mAbs from the encephalitis subgroups revealed different SHM numbers, we next explored to which extent affinity maturation contributed to antigen binding. For this, we generated germline-configured mAbs by removing the V and J gene SHMs based on sequence comparison with IgBlast from 19 representative antibodies (≥2 per group), thus resulting in the unmutated ancestors of the mAbs.

Relative binding strength was quantified using flow cytometry with HEK cells overexpressing the respective NSA together with EGFP (eFigure 2A). EGFP expression correlated with the expression of the respective NSA, as detected by mAb binding (exemplarily shown for $GABA_AR$ in eFigure 2B). Mean fluorescence intensity (MFI) of mAb binding correlated with increasing mAb concentrations (eFigure 2C), thus being suitable for creating serial dilution binding curves of mutated and reverted mAbs (eFigure 2D). Visualization of binding strength quantification is available in the eSAP.

Across all groups, 11/19 (57.9%) of the germline-reverted mAbs were reactive to their respective NSA (Figure 2, E–I; NMDAR 50% [2/4],], LGI1 50% [3/6], CASPR2 66.6% [2/3], $GABA_AR$ 66.6% [2/3], and mGluR5 100% [2/2]). Half-maximal antibody binding concentrations (c50) ranged from 0.03 to 69.25 μg/mL (Table 2). A small AUC ratio (AUC

Figure 5 Target Identification of 2 Germline mAbs



Immunoprecipitation and mass spectrometry were performed with murine kidney homogenate incubated with mAbs 113-101gl (A) and 080-232gl (B) (maturated versions and mGo53 were used as controls). Volcano plots show the target enrichment of MVP (113-101gl) and TPR (080-232gl). Shown are *p* value (-log10) (y-axis) and log2 fold change (bait/background) (x –axis) of target mAbs against all other mAbs. Antigens were confirmed in cell-based assays overexpressing MVP (×20) (C) and TPR (×40) (D), respectively. Staining of untransfected HEK293T cells was used as negative control (shown for 113-101gl). TPR was cotransfected with a Flag-Tag, showing overlapping staining patterns of 080-232gl and Flag-Tag expression. mAbs = monoclonal autoantibodies; MVP = major vault protein; TPR = translocated promotor region.

maturated mAb/germline mAb) represents a germline mAb binding strength close to its maturated counterpart. There was no correlation between binding strength/AUC ratios and numbers of SHM (Figure 2B). Interestingly, although most hypermutated mAbs had increased binding strengths, at least one germline mAb of each encephalitis group bound its respective antigen with similar (060-118gl) or even elevated (008-218gl) affinity compared to the hypermutated mAb (Figure 2A; Table 2).

Heavy or Light Chain SHMs Can Determine Neuronal Binding

We next asked whether mutations on heavy chains (HC) or light chains (LC) contribute to enhanced binding to neuronal surface antigens. We selected 2 anti-$GABA_AR$ mAbs that lost most of their binding when reverted to the germline configuration (mAb 113-101 and mAb 113–115). For both mAbs, we created hybrid versions in which either only the HC or the LC carried the SHMs (Figure 3) and tested them for binding strength using immunohistochemistry on murine brain sections (Figure 3, A and B) and flow cytometry (Figure 3, C and D). For mAb 113-101, brain reactivity and binding to $GABA_AR$-transfected HEK cells depended solely on HC SHM. Neither the germline version nor the hybrid version with only light chain mutations showed $GABA_AR$ binding on mouse brain (Figure 3A) or against human $GABA_AR$ (Figure 3C). By contrast, mAb 113–115 showed some $GABA_AR$ reactivity already in its germline configuration, but it markedly increased with affinity-maturated HC and/or LC (Figure 3, B and D). This finding is consistent with structural data that revealed SHM-dependent binding to the receptor with a greater surface area binding by the 113–115 heavy than light chain.[25]

Several Patient-Derived mAbs and Their Unmutated Germline Ancestors Were Reactive to Additional Self-Antigens

As a large fraction of encephalitis patient-derived germline-reverted mAbs reacted to NSA, it seems possible that they

fulfill beneficial functions such as molecular mimicry with pathogens. Given the well-established association of NMDAR autoantibody development following herpes simplex virus type 1 (HSV-1) encephalitis,[26,27] we screened the NMDAR mAbs and their germline ancestors for binding to HSV-1 using ELISA (eFigure 3A) and immunofluorescence on HSV-1-expressing cells (data not shown). All mAbs were negative for HSV-1 in the ELISA and cell-based assay.

We next investigated potential (poly-) reactivity to self-antigens using ELISA, HEp-2 cells and murine tissues. First, germline and mutated mAbs were screened for polyreactivity using an ELISA displaying 6 common autoantigens (double-stranded DNA [ds-DNA], single-stranded DNA [ss-DNA], lipopolysaccharide [LPS], keyhole limpet haemocyanin [KLH], cardiolipin, and human insulin). None of the patient-derived or reverted mAbs displayed polyreactivity (eFigure 3B). Second, we examined whether some mAbs represented antinuclear antibodies (ANA), a frequent finding in patients with systemic autoimmunity. Both, germline and the respective maturated mAbs were screened on HEp-2 cells (eFigure 3C). Two of 19 maturated mAbs (mAbs: 113-101, 136–143) and 3 of 20 germline mAbs (mAbs: 113-101gl, 187-194gl, 080-232gl) bound to HEp-2 cell-expressed self-antigens.

Third, we assessed binding to different murine tissues (kidney, gut, heart, liver, and testis), which showed comparable frequencies among maturated and germline mAbs (eFigure 3D). Seven mutated mAbs (113-101, 113–198, 187–188, 136–143, 136–180, 080–232, 054–132) and 7 reverted mAbs (113-101gl, 113-198gl, 187-194gl, 136-143gl, 136-180gl, 003-102gl II, 080-232gl) displayed strong binding to different murine tissues (Figure 4A). NSAs, especially the NMDAR, can be expressed outside the brain. However, only one maturated anti-NMDAR mAb showed reactivity to peripheral tissue, suggesting that tissue binding is not directed against the NMDAR, but specific to this individual mAb. Interestingly, in some instances, tissue autoreactivity was seen only with the germline mAb but was lost after affinity maturation. For example, the germline mAb 113-101gl showed strong binding to murine kidney glomeruli and around renal tubules, while its $GABA_AR$-reactive maturated counterpart 113-101 did not bind to renal tissue (Figure 4B). Similarly, mAb 080-232gl reacted with nuclear autoantigens on HEp-2 cells in its germline configuration, but not in the mutated version (Figure 4C). However, this mAb showed strong NMDAR binding already in the germline version.

Identification of Nuclear Antigens as Targets of Selected Germline Antibodies

Given that these germline mAbs bound to antigens on murine tissue or HEp-2 cells that were different to the encephalitis-defining neuronal surface antigens, we next aimed to identify the underlying target proteins using the 2 mAbs 113-101gl and 080-232gl. Immunoprecipitation and mass spectrometry of mAbs incubated with mouse tissue homogenate, revealed MVP as a potential autoantibody target of 113-101gl (Figure 5A) and TPR as a potential target of 080-232gl, in line with the nuclear staining patterns on HEp-2-cells (Figure 5B). Target specificity of the mAbs was confirmed in HEK cell-based assays overexpressing MVP and TPR, respectively (Figure 5, C and D). Both identified antigens are often associated with systemic autoimmune diseases. Interestingly, the respective mAbs 113-101 and 080–232 have lost binding to these antigens during affinity maturation.

Discussion

In this study, we investigated (auto)antigen reactivity of monoclonal patient-derived autoantibodies and their germline reverted counterparts from patients with 5 different autoimmune encephalitides. Across diseases, considerable heterogeneity regarding IgG gene families or the composition of heavy chain CDR3 regions including length, charge, or hydrophobicity was observed. In 4 of 5 groups, mAbs were predominantly produced by ASCs or MBCs and had undergone affinity maturation, with low SHM numbers in NMDAR encephalitis, and, contrarily, high numbers in LGI1 encephalitis. However, high numbers of SHM were not required for strong antigen binding, as all 5 encephalitides contained clones with strong target binding in their germline-reverted autoantibody pool. Several unmutated and mutated mAbs showed strong binding to self-antigens beyond the encephalitis-defining neuronal surface proteins. These included established ANA antigens which have previously been associated with autoimmune diseases, such as MVP and TPR.

We have previously shown in patients with NMDAR encephalitis that germline reverted ancestors can have strong binding to the NMDAR and that unmutated NMDAR-binding mAbs can reduce synaptic currents in electrophysiologic studies.[15] By back-mutating patient-derived mAbs, we demonstrate in this study that, likewise, patients with LGI1, CASPR2, $GABA_AR$, and mGluR5 encephalitis can have strongly binding autoantibodies already in the germline antibody pool. In most individuals, immunologic checkpoints limit the expansion of APCs and B cells producing these autoantibodies. Therefore, the rare autoimmune encephalitides might be a consequence of dysfunctional immune checkpoints. In susceptible individuals, ANAs may be a starting point for developing encephalitis-causing autoantibodies.

Indeed, there are several findings supporting the hypothesis of immunologic alterations in autoimmune encephalitis patients, including genetic contributors. In our cohort, 5 of 11 patients had ≥1 autoimmune disease such as hypothyroidism or lichen sclerosus, indicating an elevated susceptibility to the development of autoimmunity. In line with this hypothesis,

several autoimmune encephalitides are associated with certain HLA haplotypes, such as HLA-DRB*07:01 in LGI1 encephalitis or HLA-DRB1*11:01 in CASPR2 encephalitis hinting toward a possible genetic susceptibility.[28-30] Strikingly, in one patient, the hypothesis of immunologic checkpoint deficiency was confirmed years later by detection of a pathogenic *AIRE* mutation, leading to autoimmune polyglandular syndrome type 1 (APS-1).[31] This genetic immune checkpoint defect leads to severely dysfunctional humoral autoimmunity conveyed through autoantibodies targeting cytokines and interferons[32] and in our patient to the development of $GABA_AR$ autoantibodies.

Immunologic alterations in autoimmune encephalitides seem to also affect $CD4^+$ T helper cell (T_H) function. Contrary to the expectations, we previously detected a reduced number of NMDAR-specific T_H cells in patients with NMDAR encephalitis.[33] Similarly, in the related disease NMOSD driven by aquaporin-4 (AQP4) autoantibodies, T_H cells against AQP4 had an exhaustion phenotype expressing FOXP3, likely resulting from adaptation to chronic (self-) stimulation in autoimmune diseases.[34] Interestingly, in NMOSD, but also in SLE and APS-1, SHMs were required for autoantibody binding and specificity as germline-reverted mAbs showed little or no binding capacity to their respective antigens.[13,14,35]

These findings raise the question of why several germline antibodies show strong binding to autoimmune encephalitis-related neuronal proteins. One explanation for NSA reactive mAbs could be a yet undetermined positive function, e.g., for viral and bacterial defense, cancer suppression, signaling pathway modification, or neuromodulation. Generally, high antibody diversity of antibody specificities is necessary to cover all potential 3-dimensional epitopes including self-antigens, otherwise viruses would use these "holes in the repertoire" and rapidly develop surface structures closely resembling autoantigens, hindering efficient antiviral immune responses.

An additional mechanism leading to strong binding of (neuronal) self-antigens may be unrelated to a protective function but be a mere consequence of imperfect tolerance. An incomplete counter-selection of autoreactive B cells at central or peripheral checkpoints will then lead to the persistence of autoantibodies in the circulation.[36] For example in NMOSD, compromised central tolerance led to higher frequencies of immature autoreactive naïve B cells compared with healthy controls.[13] Similar findings have been described in the neuromuscular disease myasthenia gravis and in Sjögren syndrome.[37,38]

In this study, we did not anticipate the high frequency of self-reactive germline-reverted mAbs including murine tissue binding and HEp2 cell reactivity. In 2 germline mAbs, immunoprecipitation plus mass spectrometry identified targets that were previously associated with systemic autoimmunity. The NMDARE-derived reverted mAb 080-232gl reacted with TPR, a protein that is part of the nuclear pore complex and associated with a variety of autoimmune diseases including SLE.[39] Interestingly, cross-reactivity between a subset of anti-dsDNA antibodies and NMDA receptors has already been demonstrated in patients with SLE.[40]

The target of $GABA_ARE$-derived reverted mAb 113-101gl, MVP, has been previously linked to rheumatoid arthritis.[41] While mAb 113-101gl bound to MVP but not to the $GABA_AR$, its mutated counterpart lost binding to MVP but gained $GABA_AR$ reactivity. MVP and TPR are ubiquitously expressed intracellular targets. It is currently unclear why germline mAbs react to those antigens and whether they can reach them in vivo. It will be intriguing to find out in the future, why and how exactly mAbs against nuclear antigens mature further to become reactive against neuronal surface proteins. Owing to the lack of conformationally stabilized NSA suitable for surface plasmon resonance, we could not directly compare the affinity of mAbs to nuclear antigens and neuronal targets in this study.

Future screening for ANAs and tissue reactivity in a larger cohort of patients is needed to support the hypothesis of defective immune tolerance mechanisms in the pathogenesis of autoimmune encephalitis. In one case, this concept was already visible by the detection of a pathogenic checkpoint mutation in the *AIRE* gene. The observation of permissive tolerance in autoimmune encephalitis is substantiated by a recent study identifying defective tolerance as a key pathogenic mechanism in CASPR2 encephalitis.[42]

The rarity of autoimmune encephalitis implies that several mechanisms may have to come together in susceptible people for development of disease. These may include genetic risk factors and immunostimulatory events such as viral infections or cancer but potentially also the natural escape of a small fraction of self-reactive autoantibody producing B cells during B-cell development. Disentangling these mechanisms will help to develop novel up-stream treatments for autoimmune encephalitis, such as immune checkpoint modulators, beyond current concepts of immunosuppression or removal of autoantibodies.

Author Contributions

L. Stöffler: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. J. Kreye: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. F.A. Arlt: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. E. Sanchez-Sendin: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. J. Hoffmann: drafting/revision of the

manuscript for content, including medical writing for content. H.-C. Kornau: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. M. Kirchner: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. P. Mertins: drafting/revision of the manuscript for content, including medical writing for content. S. van Hoof: drafting/revision of the manuscript for content, including medical writing for content. S. Wernick: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. M. Homeyer: drafting/revision of the manuscript for content, including medical writing for content. S.M. Reincke: drafting/revision of the manuscript for content, including medical writing for content. H. Prüss: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data.

Study Funding

This study was supported by grants from the German Research Foundation (DFG) to H.-C.K. (Research Unit FOR 3004 "SYNABS," project 415914819) and H.P. (grants FOR3004, PR1274/9-1, clinical research unit 5023/1 "BECAUSE-Y" [project number 504745852]), by grants from the Helmholtz Association (HIL-A03 BaoBab) and the German Federal Ministry of Research, Technology and Space (BMFTR) (Connect-Generate 16 GW0279K) to H.P.

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NN for full disclosures.

Publication History

Received by *Neurology® Neuroimmunology & Neuroinflammation* January 28, 2026. Accepted in final form April 7, 2026. Submitted and externally peer reviewed. The handling editor was Associate Editor Romana Höftberger, MD.

References

1. Prüss H. Autoantibodies in neurological disease. *Nat Rev Immunol*. 2021;21(12):798-813. doi:10.1038/s41577-021-00543-w
2. Dalmau J, Graus F. Antibody-mediated encephalitis. *N Engl J Med*. 2018;378(9):840-851. doi:10.1056/NEJMra1708712
3. Varley JA, Strippel C, Handel A, Irani SR. Autoimmune encephalitis: recent clinical and biological advances. *J Neurol*. 2023;270(8):4118-4131. doi:10.1007/s00415-023-11685-3
4. Duong SL, Prüss H. Molecular disease mechanisms of human antineuronal monoclonal autoantibodies. *Trends Mol Med*. 2023;29(1):20-34. doi:10.1016/j.molmed.2022.09.011
5. Hughes EG, Peng X, Gleichman AJ, et al. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J Neurosci*. 2010;30(17):5866-5875. doi:10.1523/JNEUROSCI.0167-10.2010
6. Kreye J, Wenke NK, Chayka M, et al. Human cerebrospinal fluid monoclonal N-methyl-D-aspartate receptor autoantibodies are sufficient for encephalitis pathogenesis. *Brain*. 2016;139(10):2641-2652. doi:10.1093/brain/aww208
7. Crisp SJ, Dixon CL, Jacobson L, et al. Glycine receptor autoantibodies disrupt inhibitory neurotransmission. *Brain*. 2019;142(11):3398-3410. doi:10.1093/brain/awz297
8. Ohkawa T, Fukata Y, Yamasaki M, et al. Autoantibodies to epilepsy-related LGI1 in limbic encephalitis neutralize LGI1-ADAM22 interaction and reduce synaptic AMPA receptors. *J Neurosci*. 2013;33(46):18161-18174. doi:10.1523/JNEUROSCI.3506-13.2013
9. Brilot F, Dale RC, Selter RC, et al. Antibodies to native myelin oligodendrocyte glycoprotein in children with inflammatory demyelinating central nervous system disease. *Ann Neurol*. 2009;66(6):833-842. doi:10.1002/ana.21916
10. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. *Science*. 2003;301(5638):1374-1377. doi:10.1126/science.1086907
11. Victora GD, Nussenzweig MC. Germinal centers. *Annu Rev Immunol*. 2022;40(1):413-442. doi:10.1146/annurev-immunol-120419-022408
12. Meffre E, Wardemann H. B-cell tolerance checkpoints in health and autoimmunity. *Curr Opin Immunol*. 2008;20(6):632-638. doi:10.1016/j.coi.2008.09.001
13. Cotzomi E, Stathopoulos P, Lee CS, et al. Early B cell tolerance defects in neuromyelitis optica favour anti-AQP4 autoantibody production. *Brain*. 2019;142(6):1598-1615. doi:10.1093/brain/awz106
14. Mietzner B, Tsuiji M, Scheid J, et al. Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors. *Proc Natl Acad Sci U S A*. 2008;105(28):9727-9732. doi:10.1073/pnas.0803644105
15. Wenke NK, Kreye J, Andrzejak E, et al. N-methyl-D-aspartate receptor dysfunction by unmutated human antibodies against the NR1 subunit. *Ann Neurol*. 2019;85(5):771-776. doi:10.1002/ana.25460
16. Tiller T, Meffre E, Yurasov S, Tsuiji M, Nussenzweig MC, Wardemann H. Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. *J Immunological Methods*. 2008;329(1-2):112-124. doi:10.1016/j.jim.2007.09.017
17. Murugan R, Buchauer L, Triller G, et al. Clonal selection drives protective memory B cell responses in controlled human malaria infection. *Sci Immunol*. 2018;3(20):eaap8029. doi:10.1126/sciimmunol.aap8029
18. Reincke SM, Prüss H, Kreye J. Brain antibody sequence evaluation (BASE): an easy-to-use software for complete data analysis in single cell immunoglobulin cloning. *BMC Bioinformatics*. 2020;21(1):446. doi:10.1186/s12859-020-03741-w
19. Kornau HC, Kreye J, Stumpf A, et al. Human cerebrospinal fluid monoclonal LGI1 autoantibodies increase neuronal excitability. *Ann Neurol*. 2020;87(3):405-418. doi:10.1002/ana.25666
20. Kreye J, Wright SK, van Casteren A, et al. Encephalitis patient-derived monoclonal GABAA receptor antibodies cause epileptic seizures. *J Exp Med*. 2021;218(11):e20210012. doi:10.1084/jem.20210012
21. van Hoof S, Kreye J, Cordero-Gomez C, et al. Human cerebrospinal fluid monoclonal CASPR2 autoantibodies induce changes in electrophysiology, functional MRI, and behavior in rodent models. *Brain Behav Immun*. 2024;122:266-278. doi:10.1016/j.bbi.2024.08.027
22. Ly LT, Kreye J, Jurek B, et al. Affinities of human NMDA receptor autoantibodies: implications for disease mechanisms and clinical diagnostics. *J Neurol*. 2018;265(11):2625-2632. doi:10.1007/s00415-018-9042-1
23. Meffre E, Schaefer A, Wardemann H, Wilson P, Davis E, Nussenzweig MC. Surrogate light chain expressing human peripheral B cells produce self-reactive antibodies. *J Exp Med*. 2004;199(1):145-150. doi:10.1084/jem.20031550
24. Arlt FA, Breuer A, Trampenau E, et al. High serum prevalence of autoreactive IgG antibodies against peripheral nerve structures in patients with neurological post-COVID-19 vaccination syndrome. *Front Immunol*. 2024;15:1404800. doi:10.3389/fimmu.2024.1404800
25. Noviello CM, Kreye J, Teng J, Prüss H, Hibbs RE. Structural mechanisms of GABA(A) receptor autoimmune encephalitis. *Cell*. 2022;185(14):2469-2477 e13. doi:10.1016/j.cell.2022.06.025
26. Armangue T, Leypoldt F, Malaga I, et al. Herpes simplex virus encephalitis is a trigger of brain autoimmunity. *Ann Neurol*. 2014;75(2):317-323. doi:10.1002/ana.24083
27. Prüss H, Finke C, Holtje M, et al. N-methyl-D-aspartate receptor antibodies in herpes simplex encephalitis. *Ann Neurol*. 2012;72(6):902-911. doi:10.1002/ana.23689
28. Binks S, Varley J, Lee W, et al. Distinct HLA associations of LGI1 and CASPR2-antibody diseases. *Brain*. 2018;141(8):2263-2271. doi:10.1093/brain/awy109
29. Gaig C, Graus F, Compta Y, et al. Clinical manifestations of the anti-IgLON5 disease. *Neurology*. 2017;88(18):1736-1743. doi:10.1212/WNL.0000000000003887
30. Mueller SH, Farber A, Prüss H, et al. Genetic predisposition in anti-LGI1 and anti-NMDA receptor encephalitis. *Ann Neurol*. 2018;83(4):863-869. doi:10.1002/ana.25216
31. Meisel C, Akbil B, Meyer T, et al. Mild COVID-19 despite autoantibodies against type I IFNs in autoimmune polyendocrine syndrome type 1. *J Clin Invest*. 2021;131(14):e150867. doi:10.1172/JCI150867
32. Meager A, Visvalingam K, Peterson P, et al. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. *PLoS Med*. 2006;3(7):e289. doi:10.1371/journal.pmed.0030289
33. Dao LM, Machule ML, Bacher P, et al. Decreased inflammatory cytokine production of antigen-specific CD4(+) T cells in NMDA receptor encephalitis. *J Neurol*. 2021;268(6):2123-2131. doi:10.1007/s00415-020-10371-y
34. Saggau C, Bacher P, Esser D, et al. Autoantigen-specific CD4(+) T cells acquire an exhausted phenotype and persist in human antigen-specific autoimmune diseases. *Immunity*. 2024;57(10):2416-2432 e8. doi:10.1016/j.immuni.2024.08.005
35. Di Zenzo G, Di Lullo G, Corti D, et al. Pemphigus autoantibodies generated through somatic mutations target the desmoglein-3 cis-interface. *J Clin Invest*. 2012;122(10):3781-3790. doi:10.1172/JCI64413
36. Meffre E, O'Connor KC. Impaired B-cell tolerance checkpoints promote the development of autoimmune diseases and pathogenic autoantibodies. *Immunol Rev*. 2019;292(1):90-101. doi:10.1111/imr.12821
37. Lee JY, Stathopoulos P, Gupta S, et al. Compromised fidelity of B-cell tolerance checkpoints in AChR and MuSK myasthenia gravis. *Ann Clin Transl Neurol*. 2016;3(6):443-454. doi:10.1002/acn3.311

38. Glauzy S, Sng J, Bannock JM, et al. Defective early B cell tolerance checkpoints in Sjogren's syndrome patients. *Arthritis Rheumatol*. 2017;69(11):2203-2208. doi:10.1002/art.40215
39. Ou Y, Enarson P, Rattner JB, Barr SG, Fritzler MJ. The nuclear pore complex protein Tpr is a common autoantigen in sera that demonstrate nuclear envelope staining by indirect immunofluorescence. *Clin Exp Immunol*. 2004;136(2):379-387. doi:10.1111/j.1365-2249.2004.02432.x
40. DeGiorgio LA, Konstantinov KN, Lee SC, Hardin JA, Volpe BT, Diamond B. A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. *Nat Med*. 2001;7(11):1189-1193. doi:10.1038/nm1101-1189
41. Marinou D, Katsifis G, Barouta G, et al. Major vault protein/lung resistance related protein: a novel biomarker for rheumatoid arthritis. *Clin Exp Rheumatol*. 2021;39(5):1033-1042. doi:10.55563/clinexprheumatol/pcozc1
42. Sun B, Fernandes D, Soltys J, et al. Permissive central tolerance plus defective peripheral checkpoints license pathogenic memory B cells in CASPR2-antibody encephalitis. *Sci Adv*. 2025;11(16):eadr9986. doi:10.1126/sciadv.adr9986