Does Molecular Mimicry Explain Epidemiology Linking EBV & MS?
Oligoclonal Antibody in MS Cerebrospinal Fluid Binds EBNA-1 & GlialCAM
EBNA-1--> Molecular Mimicry “Hot Zone”

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What Causes MS?
(What I taught Med Students for 42 years)

- Before January 2022:
- Don’t know – multifactorial----→My teaching point
- Risk factors

  Latitude of birth

  Gender

  Tobacco smoke (RR~2)

  Genes and Family history (MZ twin concordance 25-30%)

  Vitamin D deficiency

  ? viral etiology (EBV, HHV6)

After January 2022-EBV Causes MS!!

Science

REPORTS

Cite as: K. Bjornevik *et al.*, *Science*
10.1126/science.abj8222 (2022).

Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis

Kjetil Bjornevik[1]†, Marianna Cortese[1]†, Brian C. Healy[2,3,4], Jens Kuhle[5], Michael J. Mina[6,7,8], Yumei Leng[6], Stephen J. Elledge[6], David W. Niebuhr[9], Ann I. Scher[9], Kassandra L. Munger[1]‡, Alberto Ascherio[1,10,11]‡*

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system of unknown etiology. We tested the hypothesis that MS is caused by Epstein-Barr virus (EBV) in a cohort comprising more than 10 million young adults on active duty in the US military, 955 of whom were diagnosed with MS during their period of service. Risk of MS increased 32-fold after infection with EBV but was not increased after infection with other viruses, including the similarly transmitted cytomegalovirus. Serum levels of neurofilament light chain, a biomarker of neuroaxonal degeneration, increased only after EBV seroconversion. These findings cannot be explained by any known risk factor for MS and suggest EBV as the leading cause of MS.

Science

PERSPECTIVES

Cite as: W. H. Robinson, L. Steinman, *Science* 10.1126/science.abm7930 (2022).

Epstein-Barr virus and multiple sclerosis

William H. Robinson[1,2] and Lawrence Steinman[3]

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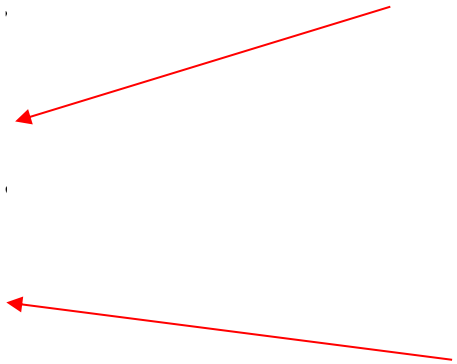
Infection with Epstein-Barr virus is the trigger for the development of multiple sclerosis

Infection with the Epstein-Barr virus (EBV) has long been postulated to trigger multiple sclerosis (MS) (*1*). Prior analyses demonstrated increased serum antibodies to EBV in ~99.5% of MS patients compared with ~94% of healthy individuals (*2*). On page 296 of this issue, Bjornevik *et al.* (*3*) analyzed EBV antibodies in serum from 801 individuals who developed MS among a cohort of >10 million people active in the US military over a 20-year period (1993–2013). Thirty-five of the 801 MS cases were initially EBV seronegative, and 34 became infected with EBV before the onset of MS. EBV seropositivity was nearly ubiquitous at the time of MS development, with only one of 801 MS cases being EBV seronegative at the time of MS onset. These findings provide compelling data that implicate EBV as the trigger for the development of MS.

How does a virus with tropism for B cells develop into a disease of the central nervous system (CNS)? In MS, there is an inflamma-



Embedded in the Epidemiology Paper in Science
A Stunning Clue

Table S1.

Table S1. Viral peptides in the *pre-onset* samples with significantly different antibody binding between cases and controls[1]

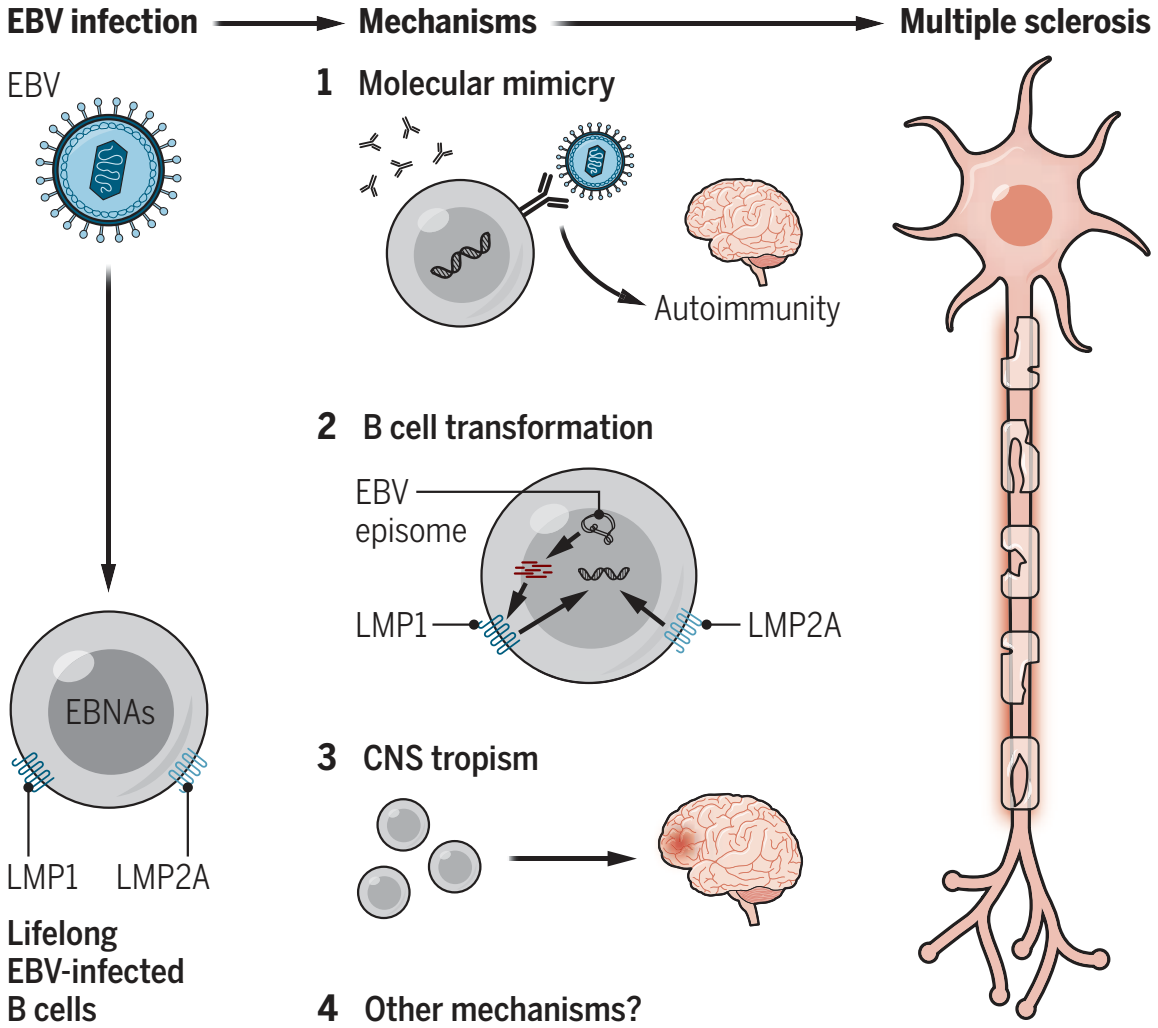
Viral species	Strain	Protein	UniProt ID	Protein length	Peptide start	Peptide end	Proportion in Cases	Proportion in Controls	P-value
Epstein-Barr virus	B95-8	EBNA-1	P03211	641	365	420	0.73	0.2	0.000073
Epstein-Barr virus	B95-8	Capsid protein VP26	P14348	176	113	168	0.87	0.47	0.0022
Epstein-Barr virus	B95-8	Envelope glycoprotein M	P03215	405	365	405	0.37	0.03	0.0025
Epstein-Barr virus	GD1	EBNA-1 (Fragment)	Q5MJ03	237	29	84	0.47	0.1	0.0034
Epstein-Barr virus	B95-8	EBNA-3	P12977	944	701	756	0.63	0.23	0.0038
Epstein-Barr virus	B95-8	EBNA-1	P03211	641	421	476	0.83	0.47	0.0061

Between 365 and 420 in EBNA-1is a Molecular Mimicry Hotspot

Another study showed serum antibodies from MS patients are cross-reactive between **amino acids 411–440 of the viral protein EBV nuclear antigen 1 (EBNA-1) and the human chloride-channel protein, anoctamin 2** (ANO2), which is associated with electrical conduction in axons (*11*). **MS serum antibodies targeting EBNA-1 residues 411–426 that cross-react with myelin basic protein have** also been identified (*12*). Clonally expanded antibodies in the CSF of MS patients targeting EBNA-1 residues **386–405 that cross- react with the CNS cell adhesion molecule, glialCAM, have also been described (*4*).** It is intriguing that three contiguous regions of mimicry have been reported in a small region of the EBNA-1 protein; this may arise through immune surveillance in a process called epitope spreading.

Model for multiple sclerosis development

In at-risk individuals, Epstein-Barr virus (EBV) infection of B cells promotes the development of multiple sclerosis through several possible mechanisms. These include molecular mimicry (**1**) by EBV nuclear antigen 1 (EBNA-1), B cell transformation (**2**) through latent membrane protein 1 (LMP1) and LMP2A, induction of B cell trafficking (**3**) to the central nervous system (CNS), and/or other unknown mechanisms (**4**).



News & views

Medical research

Multiple sclerosis sparked by virus-led autoimmunity

Hartmut Wekerle

Understanding factors that lead to the development of multiple sclerosis might aid efforts to develop new therapies. Clinical data now implicate a viral culprit and immune-system dysfunction as underlying factors in this condition.

Most people who study multiple sclerosis (MS) propose that the factors underlying initiation of the disease enter the central nervous system (CNS) from outside the brain. The debate about the nature of these factors has split researchers into two main camps. Most see autoimmunity as the driving factor for the illness, but a minority invoke viral culprits. Writing in *Nature*, Lanz *et al.*[1] report evidence that might settle this debate through a compromise solution.

Article

Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM

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Check for updates

Tobias V. Lanz[1,2,3,4], R. Camille Brewer[1,4], Peggy P. Ho[5], Jae-Seung Moon[1,4], Kevin M. Jude[6], Daniel Fernandez[7], Ricardo A. Fernandes[6], Alejandro M. Gomez[1,4], Gabriel-Stefan Nadj[1,4], Christopher M. Bartley[8,9], Ryan D. Schubert[10], Isobel A. Hawes[10], Sara E. Vazquez[11], Manasi Iyer[12], J. Bradley Zuchero[12], Bianca Teegen[13], Jeffrey E. Dunn[14], Christopher B. Lock[14], Lucas B. Kipp[14], Victoria C. Cotham[15,16], Beatrix M. Ueberheide[15,16], Blake T. Aftab[17], Mark S. Anderson[18], Joseph L. DeRisi[11,19], Michael R. Wilson[10], Rachael J. M. Bashford-Rogers[20], Michael Platten[2,3,21], K. Christopher Garcia[6], Lawrence Steinman[5] & William H. Robinson[1,4]✉

Multiple sclerosis (MS) is a heterogenous autoimmune disease in which autoreactive lymphocytes attack the myelin sheath of the central nervous system. B lymphocytes in the cerebrospinal fluid (CSF) of patients with MS contribute to inflammation and secrete oligoclonal immunoglobulins[1,2]. Epstein–Barr virus (EBV) infection has been epidemiologically linked to MS, but its pathological role remains unclear[3]. Here we demonstrate high-affinity molecular mimicry between the EBV transcription factor EBV nuclear antigen 1 (EBNA1) and the central nervous system protein glial cell adhesion molecule (GlialCAM) and provide structural and in vivo functional evidence for its relevance. A cross-reactive CSF-derived antibody was initially identified by single-cell sequencing of the paired-chain B-cell repertoire of MS blood and CSF, followed by protein microarray-based testing of recombinantly expressed CSF-derived antibodies against MS-associated viruses. Sequence analysis, affinity measurements and the crystal structure of the EBNA1–peptide epitope in complex with the autoreactive Fab fragment enabled tracking of the development of the naive EBNA1-restricted antibody to a mature EBNA1–GlialCAM cross-reactive antibody. Molecular mimicry is facilitated by a post-translational modification of GlialCAM. EBNA1 immunization exacerbates disease in a mouse model of MS, and anti-EBNA1 and anti-GlialCAM antibodies are prevalent in patients with MS. Our results provide a mechanistic link for the association between MS and EBV and could guide the development of new MS therapies.

Anti-Virals?
EBV Vaccine?
Immune Tolerance?
Other approaches?

Challenges in autoantigen identification

How do we separate what part of our immune history is trapped in the brain in MS patients from what may be driving disease?

Standing on the Shoulders of Giants-Santiago Ramon Cajal

Cajal was fascinated by the medium of photography, and in addition to his self-portraits, famil portraits, still lifes, and micro-photographs, he enjoyed taking a stereoscopic camera on excursion and trips. Here are his photograph of young women on the beach at Biarritz, France (above), and street entertainers in Madrid (opposite).

It Takes a Village, Standing on the Shoulders of Our Colleagues
Kabat JCI 1942-→Some History of Technology Breakthroughs

AN ELECTROPHORETIC STUDY OF THE PROTEIN COMPONENTS IN CEREBROSPINAL FLUID AND THEIR RELATIONSHIP TO THE SERUM PROTEINS[1]

By ELVIN A. KABAT, DAN H. MOORE AND HAROLD LANDOW[2]

(From the Departments of Neurology and Anatomy and the Electrophoresis Laboratory, College of Physicians and Surgeons, Columbia University, and the Neurological Institute, New York)

(Received for publication May 2, 1942)

SUMMARY

1. The electrophoretic pattern of cerebrospinal fluid resembles that of serum.
2. Alterations in the composition of the protein components of serum are reflected in the cerebrospinal fluid, but the changes are not as marked.

In neurosyphilis, however, an increased gamma globulin occurs in cerebrospinal fluid, without similar changes in the blood stream.

3. Colloidal gold activity is associated with the gamma globulin fraction, and albumin has an inhibiting effect on the colloidal gold reaction.

European Neurology

Historical Note

Eur Neurol 2009;62:311–315
DOI: 10.1159/000235944

The Discovery of Oligoclonal Bands: A 50-Year Anniversary

Trygve Holmøy

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The Discovery of Oligoclonal Bands: A 50-Year Anniversary

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Before Electrophoresis

Modern biochemical analysis of human CSF proteins is usually dated back to the discovery of intrathecal synthesis of IgG in 1942 by Elvin Kabat [4]. Before that, the colloidal gold activity method was prevailing for analysis of CSF proteins [5]. The basis for the colloidal gold activity method was that proteins, due to their physicochemical properties, can serve as either flocculants or stabilizers for gold colloids. It did not allow quantification of the globulin fraction, but depended on the relative proportion of albumin to γ-globulin. The colloidal gold method was invented in 1912 by Carl Lange, an assistant physician at the Rudolf Virchow Hospital in Berlin, as a diagnostic test for neurosyphilis [6]. It was indeed most sensitive in patients with neurosyphilis, but some MS patients also displayed an abnormal protein precipitation pattern of CSF proteins, commonly denoted a paretic Lange curve [7]. Although improved by controlling the pH and the size of the gold particles [8], the sensitivity and specificity of the colloidal gold activity method in MS were low and varied greatly between laboratories [9].

Detection of Intrathecal Synthesis of IgG

In 1930 the Noble Prize laureate Arne Tiselius published his thesis on moving boundary electrophoresis [10]. By this method, sharp electrophoretic boundaries of ionized molecules could be obtained, and this allowed Tiselius to describe moving boundaries corresponding to albumin, α-, β- and γ-globulin in serum [11]. The biochemist Elvin Kabat learned moving boundary electrophoresis from Tiselius in Uppsala [11]. Kabat returned to Columbia University in New York in 1941, and in 1942 he used the Tiselius method to demonstrate that colloidal gold activity was associated with the γ-globulin fraction in CSF [1]. The most impressive increase in γ-globulin in CSF was noted in patients with neurosyphilis. One of the five MS patients studied displayed a high concentration of γ-globulin in CSF, but this patient also had a high concentration of γ-globulin in serum. The conclusion that 'some formation of gamma globulin could take place within the tissues of the central nervous system and be poured into the cerebrospinal fluid' [1] was therefore based on observations in neurosyphilis and not in MS.

Denise Karcher 1959

separate individual IgG subfractions. In 1959, Denise Karcher reported that agar electrophoresis was superior to paper elecrophoresis for separation of γ-globulins in CSF [21]. In this report, individual IgG subfractions were not directly visible by inspection of the agar gel. However, densitometry revealed that the IgG fraction in the CSF from a patient with subacute sclerosing panencephalitis

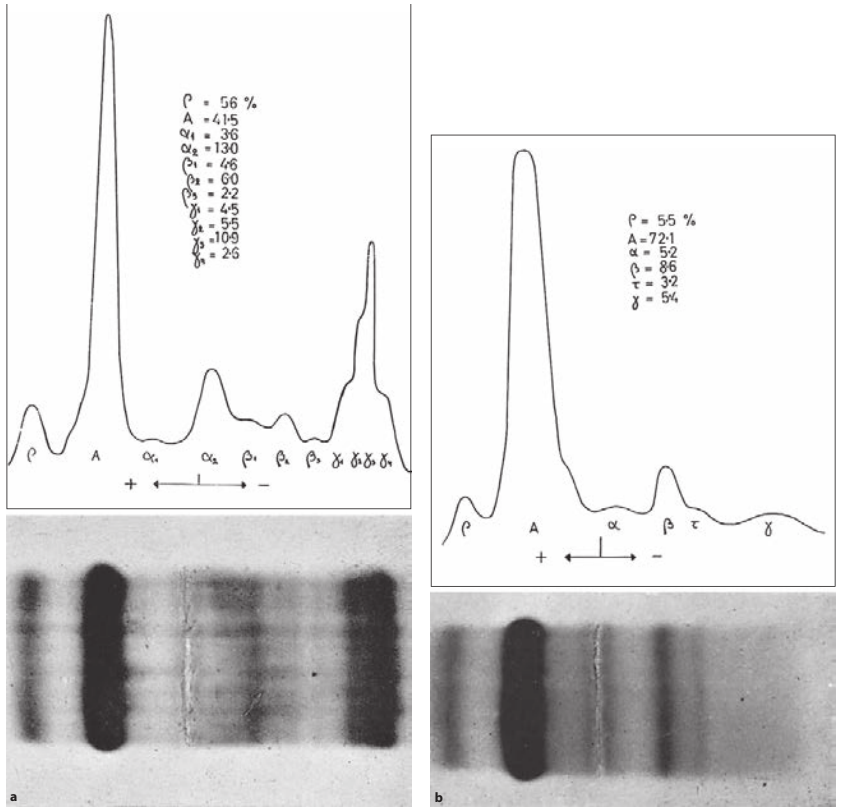


Fig. 1. The first demonstration of different mobilities of γ-globulin subfractions in CSF. **a** Densitometry (upper panel) of agar electrophoresis (lower panel) of concentrated CSF from a patient with subacute sclerosing panencephalitis displayes four subfractions of γ-globulin (γ_{1-4}). **b** Densitometry of agar electrophoresis of normal CSF. Reproduced from Karcher et al. [21] with permission from Wiley.

(which was known as subacute sclerosing leukoencephalitis at that time) was subdivided into 4 subfractions denoted γ_{1-4} (fig. 1). This picture is probably as close to the birth of the OCB as we can get, and prompted the conclusion that: 'Electrophoresis in agar gel reveals in the CSF of subacute sclerosing leukoencephalitis patients an increase in the γ-globulins, which can be subdivided into several individual fractions. These subfractions which differ in mobility may probably also differ in chemical composition' [21].

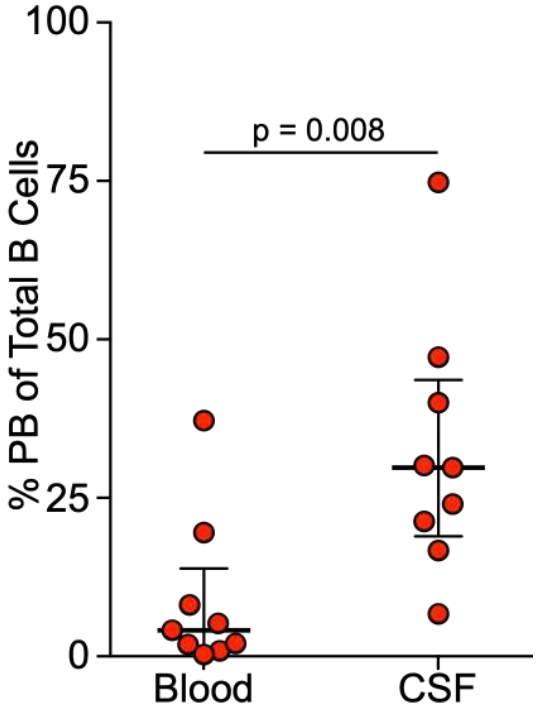
A 2005;1079:24–40.
18 Van Sande M, Karcher D, Lowenthal A: Electrophoretic examinations of proteins in blood and cerebrospinal fluid in multiple sclerosis patients. Acta Neurol Psychiatr Belg 1957;57:407–415.
19 Lowenthal A, Karcher D, Van Sande M: Electrophophoretic studies of central nervous system proteins. Exp Neurol 1959;1:233–247.

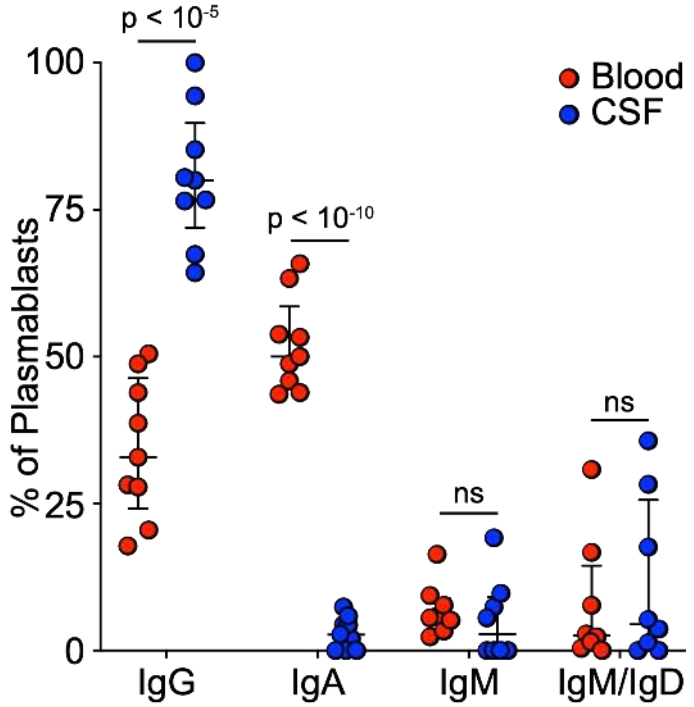
Intrathecal Synthesis of OCB

Lowenthal, van Sande and Karcher did not include serum samples in their initial reports [21, 22], and it was therefore not possible to tell whether the IgG fractions appearing as OCB were produced intrathecally or elsewhere. The importance of comparing the patterns of OCB in CSF and serum in order to obtain qualitative evidence of intrathecal synthesis of IgG was particularly underscored by Laterre, who also showed that the γ-globulin fraction of normal CSF was composed of both IgG and of tissue-like proteins [28]. Hans Link demonstrated that OCB could be detected in CSF from patients who did not display quantitative evidence for intrathecal synthesis of IgG [29, 30]. In a study of 2,043 patients, Laterre indeed demonstrated that intrathecally synthesized OCB were detectable with agar electrophoresis in 86.9% of patients with definite MS, and that this assay was more sensitive than quantitative assays in detecting intrathecal IgG synthesis [31].

289–304.
29 Link H: Qualitative changes in immunoglobulin G in multiple sclerosis-cerebrospinal fluid. Acta Neurol Scand 1967;43:180.
30 Link H: Immunoglobulin G and low molecular weight proteins in human cerebrospinal fluid. Chemical and immunological characterisation with special reference to multiple sclerosis. Acta Neurol Scand 1967;43:1–136.
31 Laterre EC, Callewaert A, Heremans JF, Sfaello Z: Electrophoretic morphology of gamma globulins in cerebrospinal fluid of multiple sclerosis and other diseases of the nervous system. Neurology 1970;20:982–990.

Elevated Levels of Activated Plasmablasts in the CSF of MS patients





Tobias Lanz

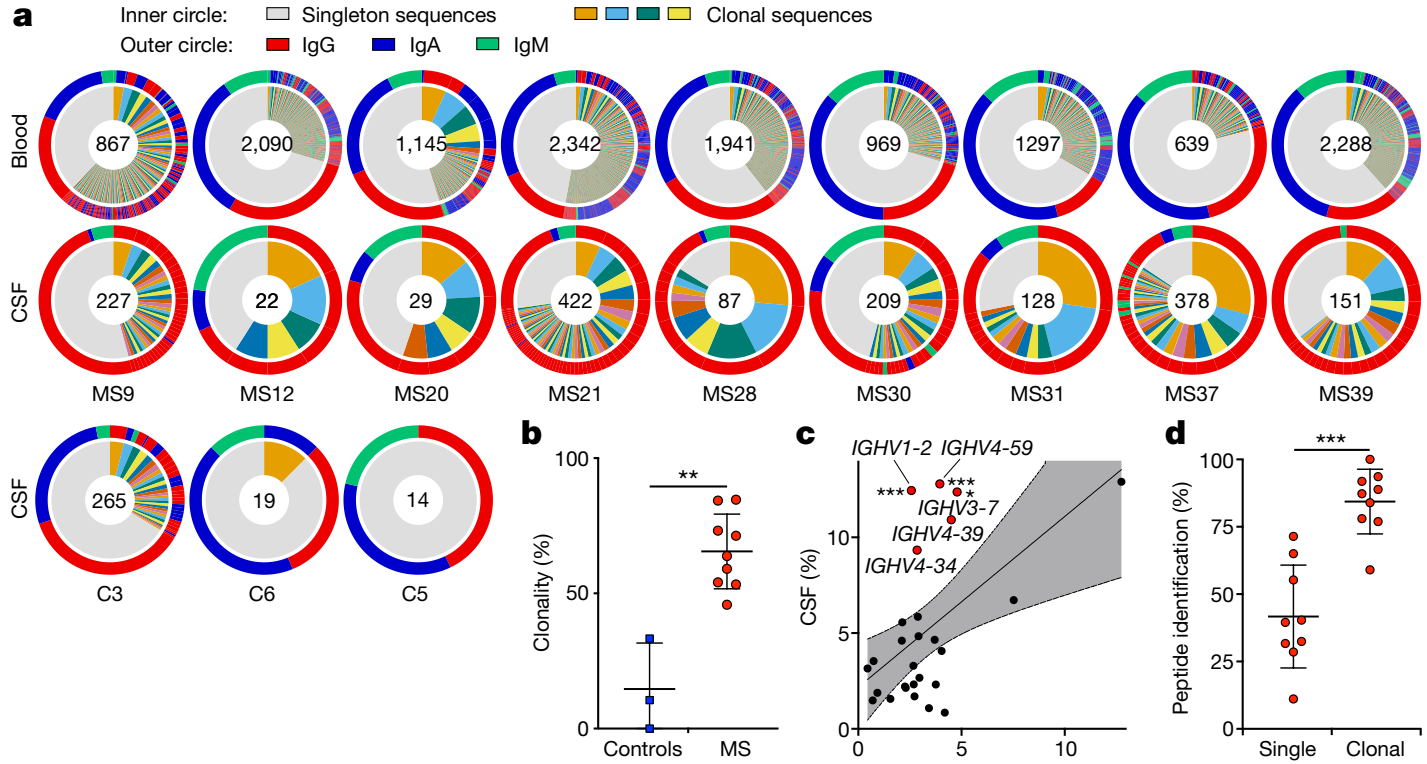
Bill Robinson

Peggy Ho

The B cell repertoire in MS CSF PB is highly clonal

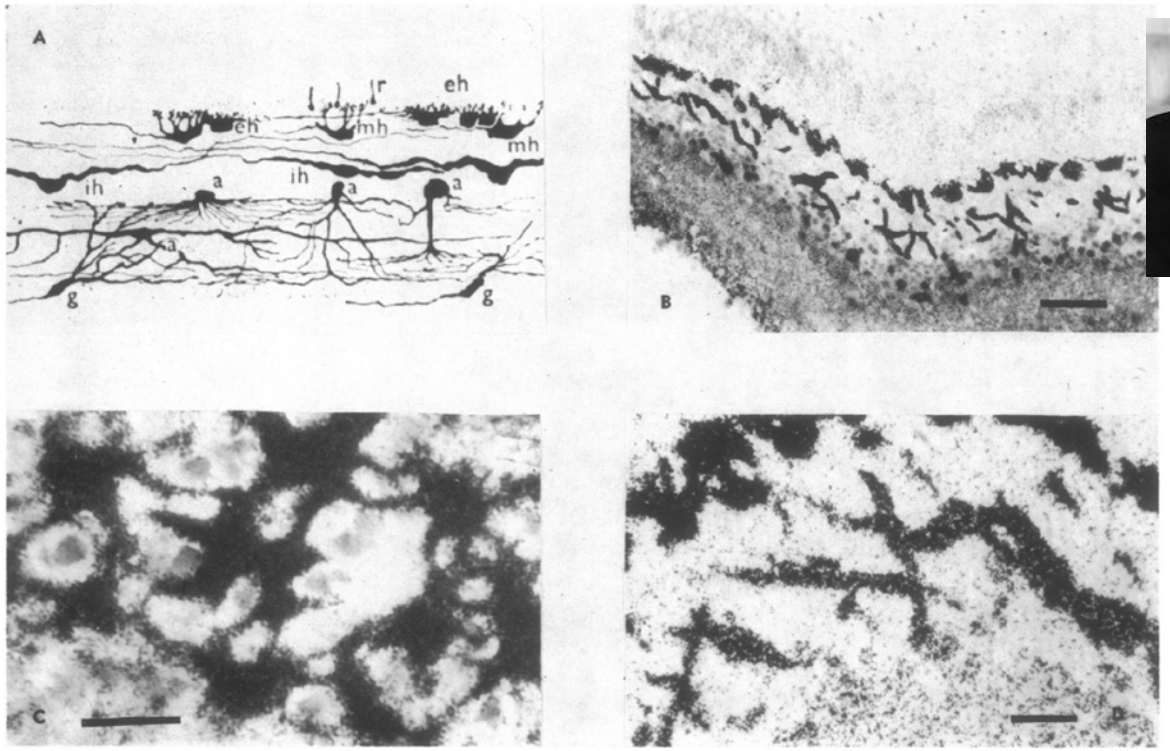
Clonal PB predominant source of oligoclonal Ig in MS CSF

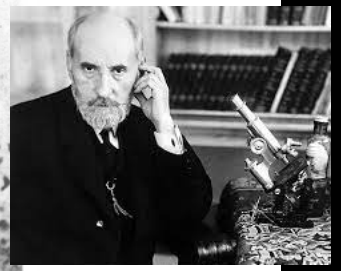
IGHV4-59, IGHV4-39, IGHV4-34, IGHV1-2, and IGHV3-7, preferred



(Technology Breakthrough based on Robinson, Sokolove, YC Tan Patent from Stanford OTL)

Close Encounters of the Cajal Kind-1971





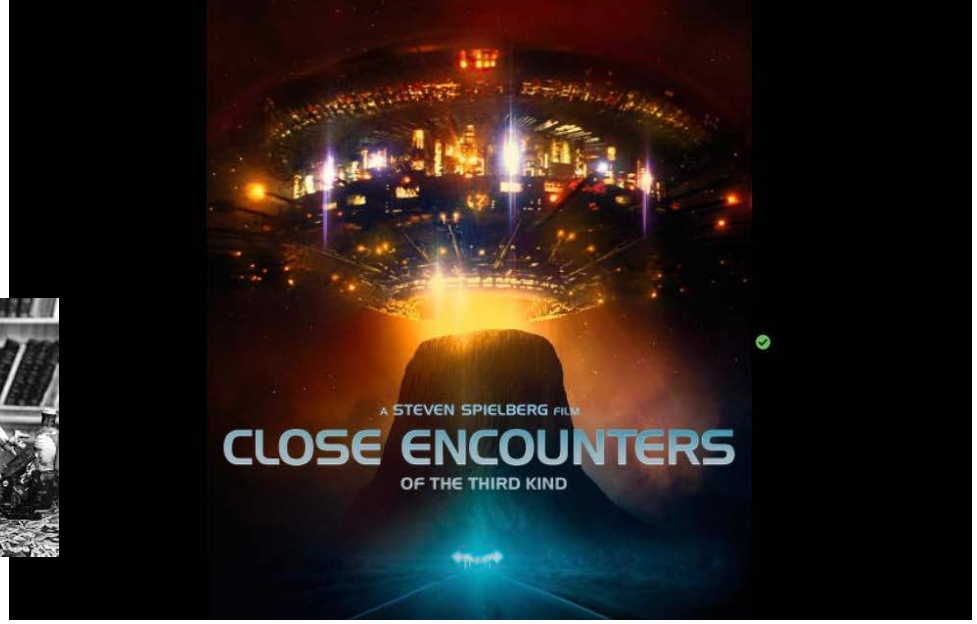


FIG. 4. (*A*) A vertical section of the teleost retina, stained by the Golgi method from Cajal (19). The section shows the horizontal connections in the retina. *r*, rods; *eh*, external horizontal cells; *mh*, intermediate horizontal cells; *ih*, internal horizontal cells; *a*, amacrine cells; *g*, ganglion cells.

B, *C*, and *D* are sections through retinas that have been incubated in [^{3}H]GABA under light stimulation for 1 hr. Scale 40 μm. (*B*) Oblique section showing external horizontal cells and their processes, which extend vertically toward receptors and laterally toward neighboring horizontal cells. The stellate geometry of the internal horizontal cells is also revealed. On the vitreous side of the inner nuclear layer, there is heavy labeling in what may be amacrine cells. Scale: 40 μm. (*C*) *En face* section through external horizontal cells, revealing extensive coupling between neighboring cells. Scale: 20 μm. (*D*) Oblique section through the internal horizontal cells, showing their stellate shape. Scale: 20 μm.

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 11, pp. 2777–2781, November 1971

The Uptake of [γ-^{3}H] Aminobutyric Acid in the Goldfish Retina

(*Carissius auratus*/horizontal cells/amacrine cells/light-stimulated retinas)

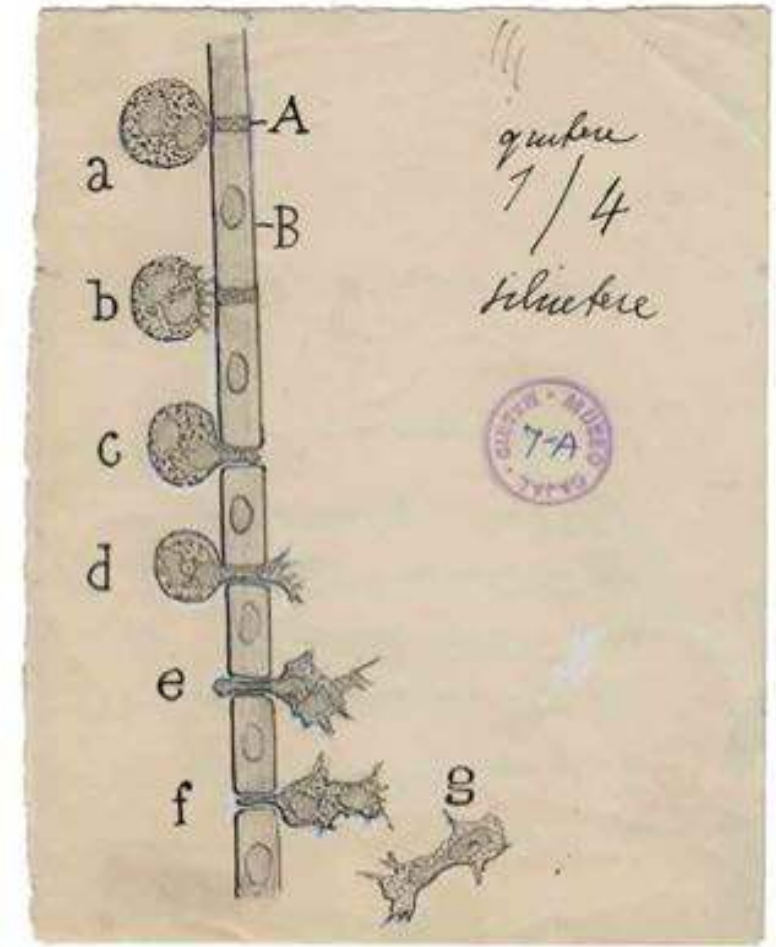
DOMINIC M. K. LAM AND LAWRENCE STEINMAN

Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115

Communicated by David H. Hubel, August 30, 1971

Having Grown Up in Los Angeles (my home was 3 blocks from MGM)
I have been interested in traffic
(as well as making movies)

Close Encounter of the Cajal Kind-2012, work published in Nature 1992



White blood cell migration across a blood vessel, 1918. [Cajal Institute / CSIC / Madrid]

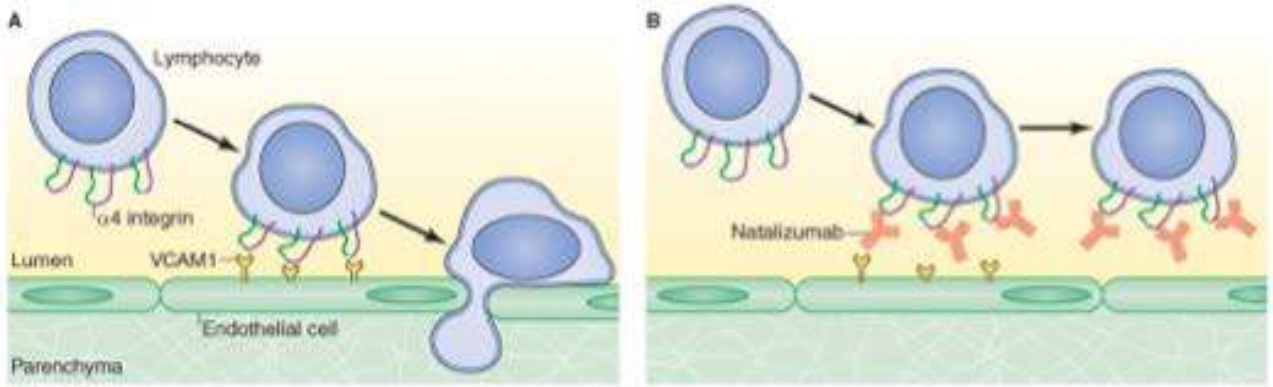
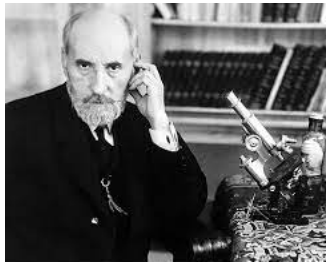


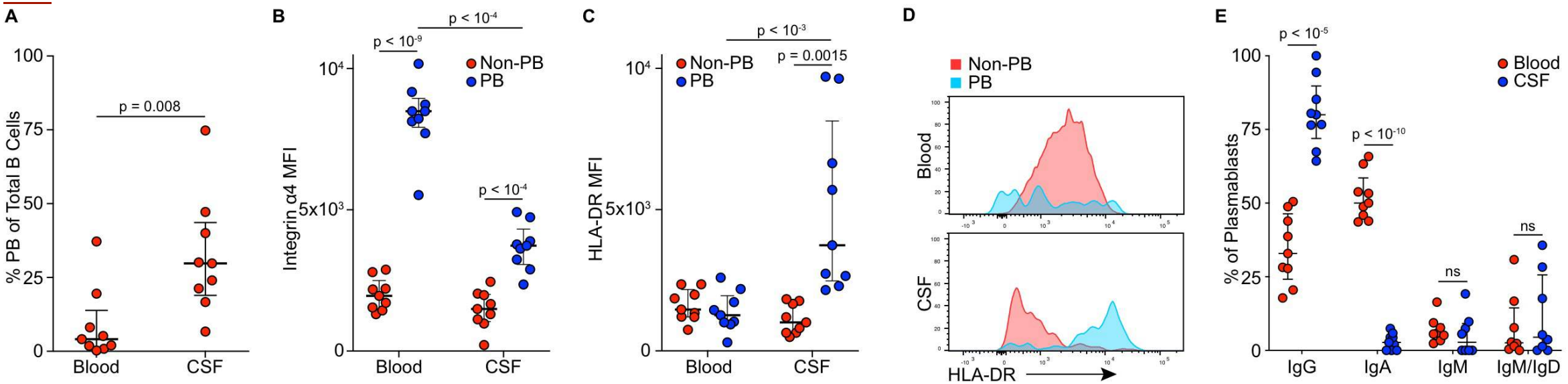
Figure 1. **Natalizumab blocks lymphocyte homing in MS.** (A) α4 integrin binds to vascular cell adhesion molecule 1 (VCAM1) and to osteopontin (not depicted) on inflamed brain endothelium. This interaction gives lymphocytes access to the central nervous system (CNS). The presence of immune cells in the brain is a prominent feature of MS. (B) Natalizumab, a humanized antibody to α4 integrin, blocks binding of lymphocytes to VCAM and osteopontin on inflamed brain endothelium, thereby preventing lymphocyte entry into the CNS.

The discovery of natalizumab, a potent therapeutic for multiple sclerosis



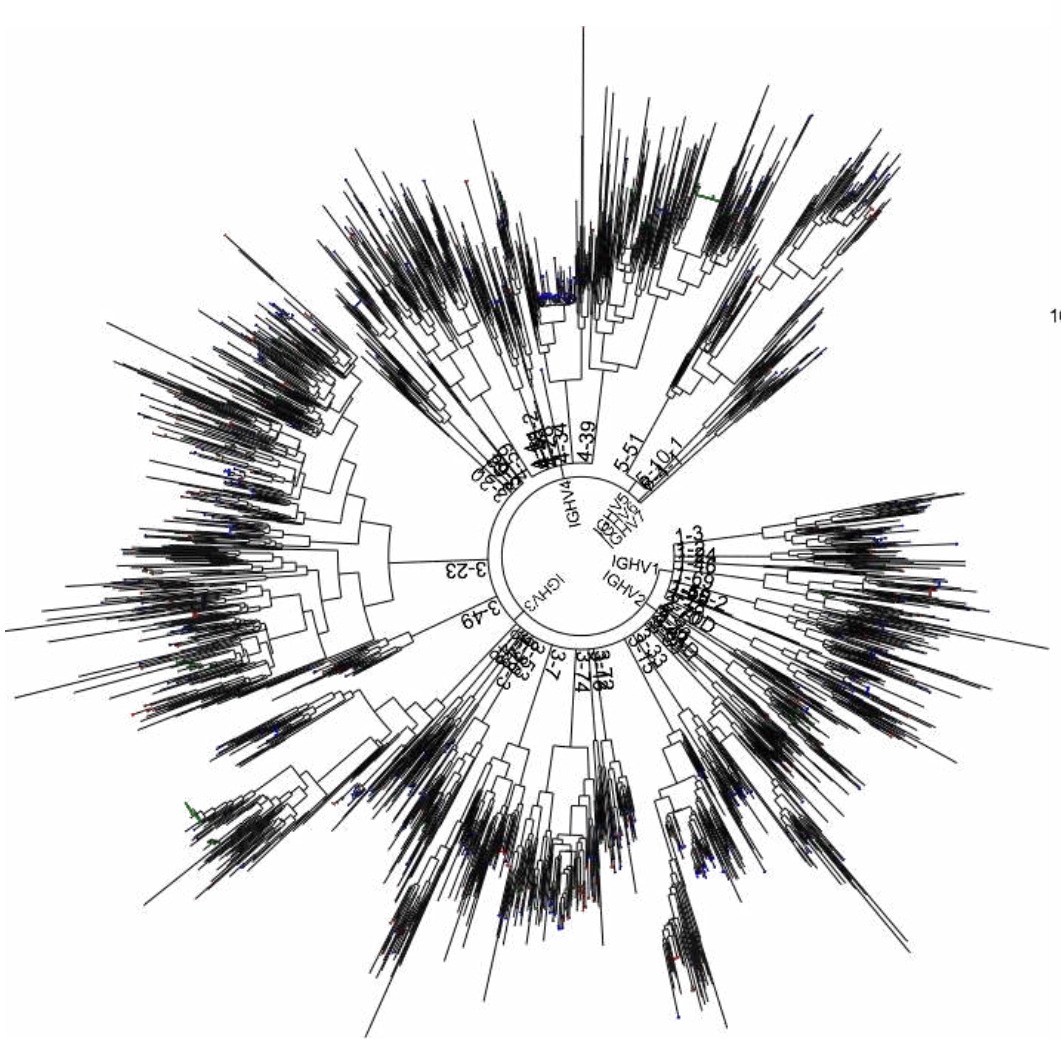
Multiple sclerosis (MS) is the major inflammatory demyelinating disease of the central nervous system. There is strong evidence that an immune response in the brain is a critical component of the disease. In 1992, in a collaboration between academia and biotechnology, my colleagues and I showed that α4 integrin was the critical molecule involved in the homing of immune cells into the inflamed brain. Was it sheer luck that these results led to the development of a drug for MS?

Plasmablasts Enriched in CSF of MS Patients Express α4 integrin
Remember: Natalizumab Targets α4β1 integrin

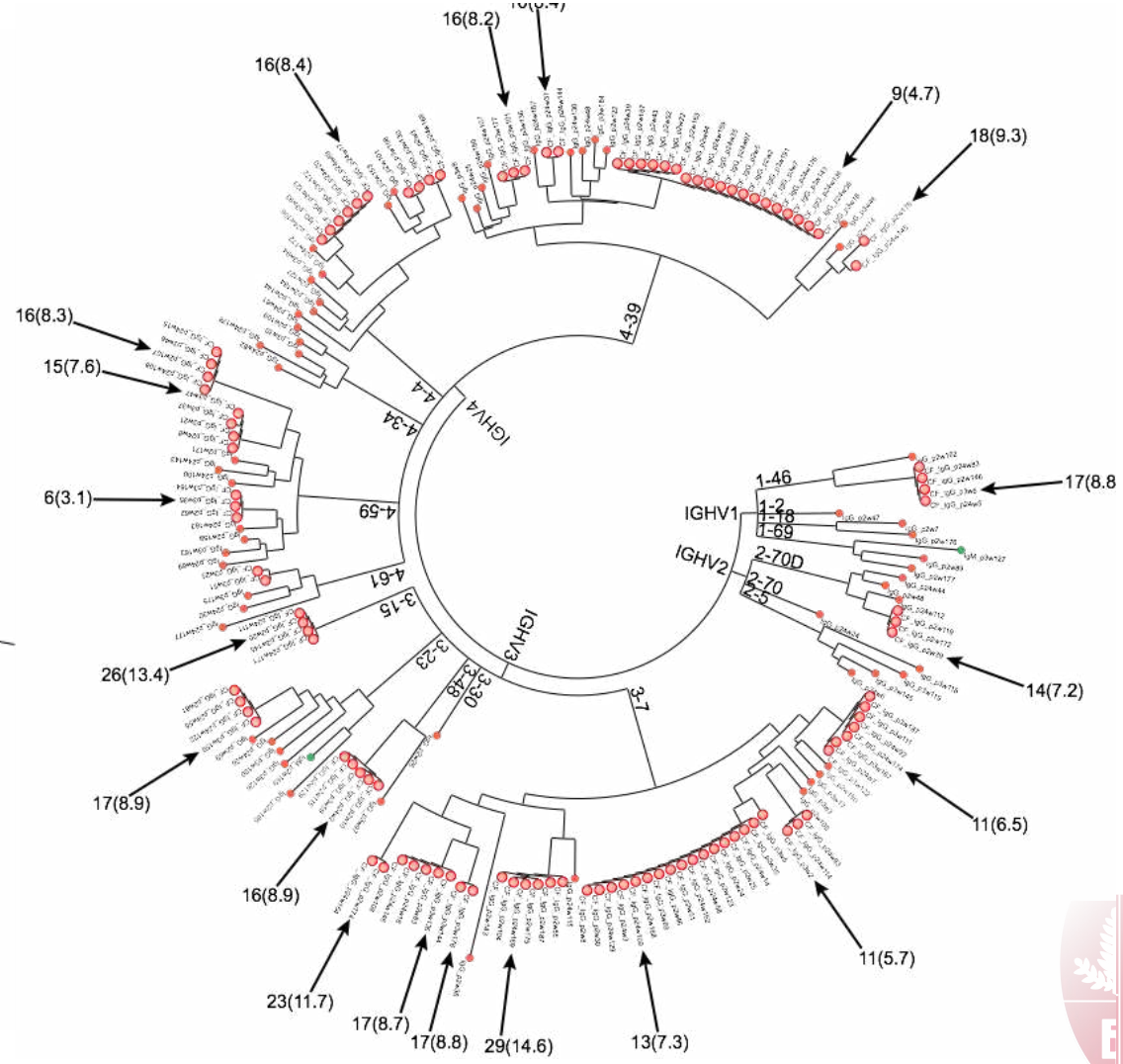


Plasmablasts get into the CSF, but they cannot escape

The CSF Repertoire is Highly Clonal, Consistent with Perpetual Antigen Stimulation and Accounting for Oligoclonal Bands, A Cornerstone of Diagnostic Testing

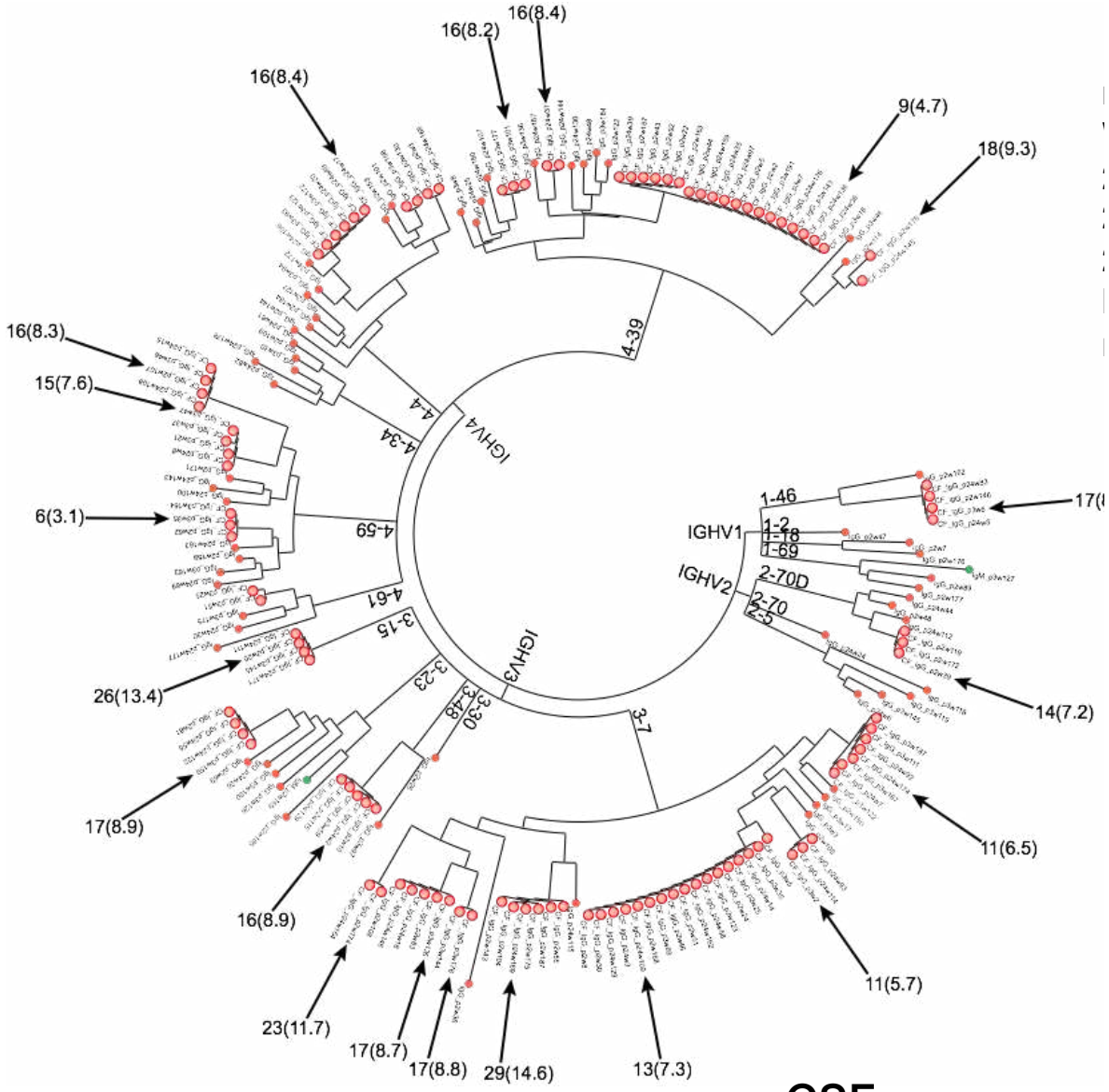


Blood



CSF

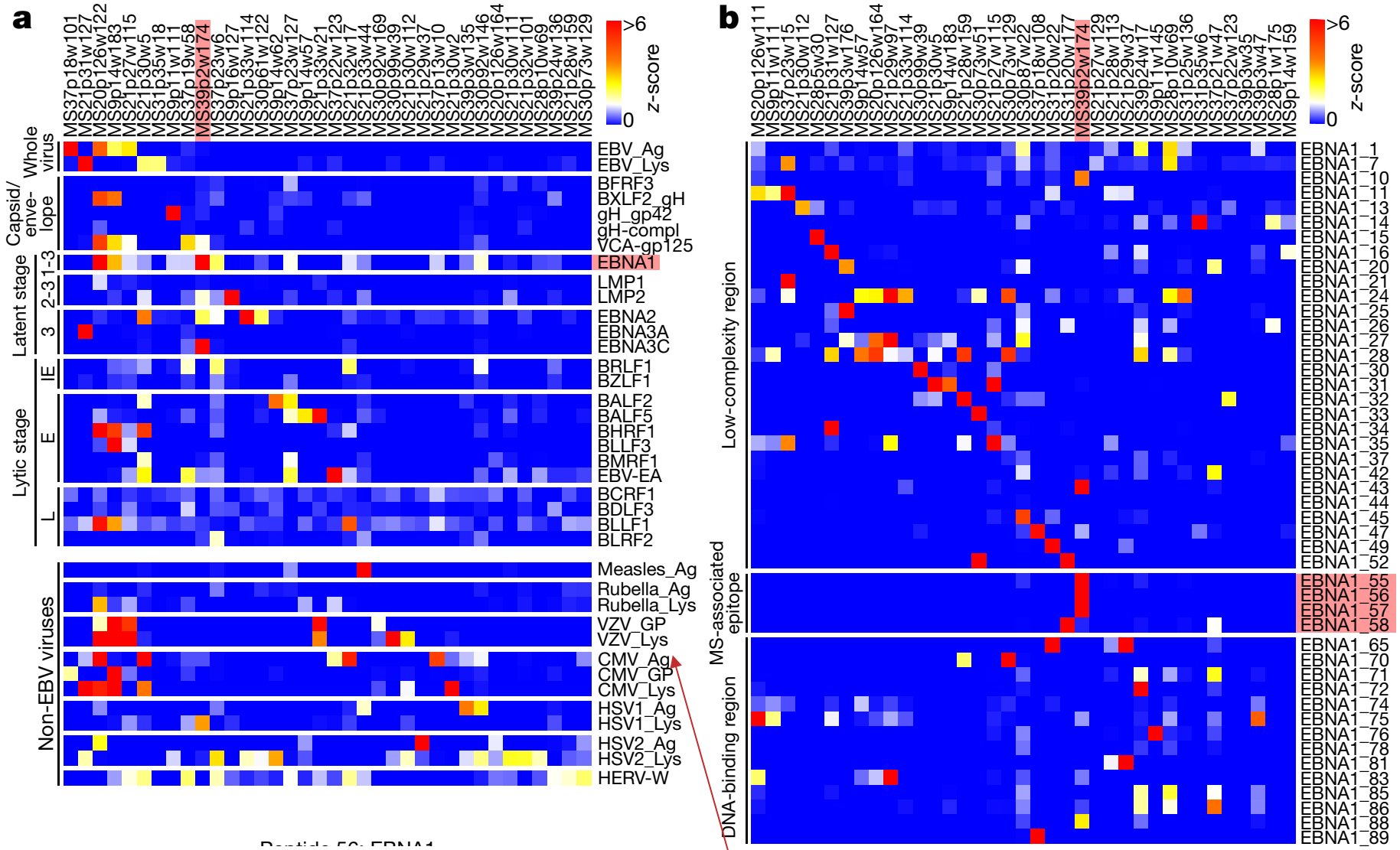
Representative Clonal Antibodies were Selected for further Evaluation



CSF

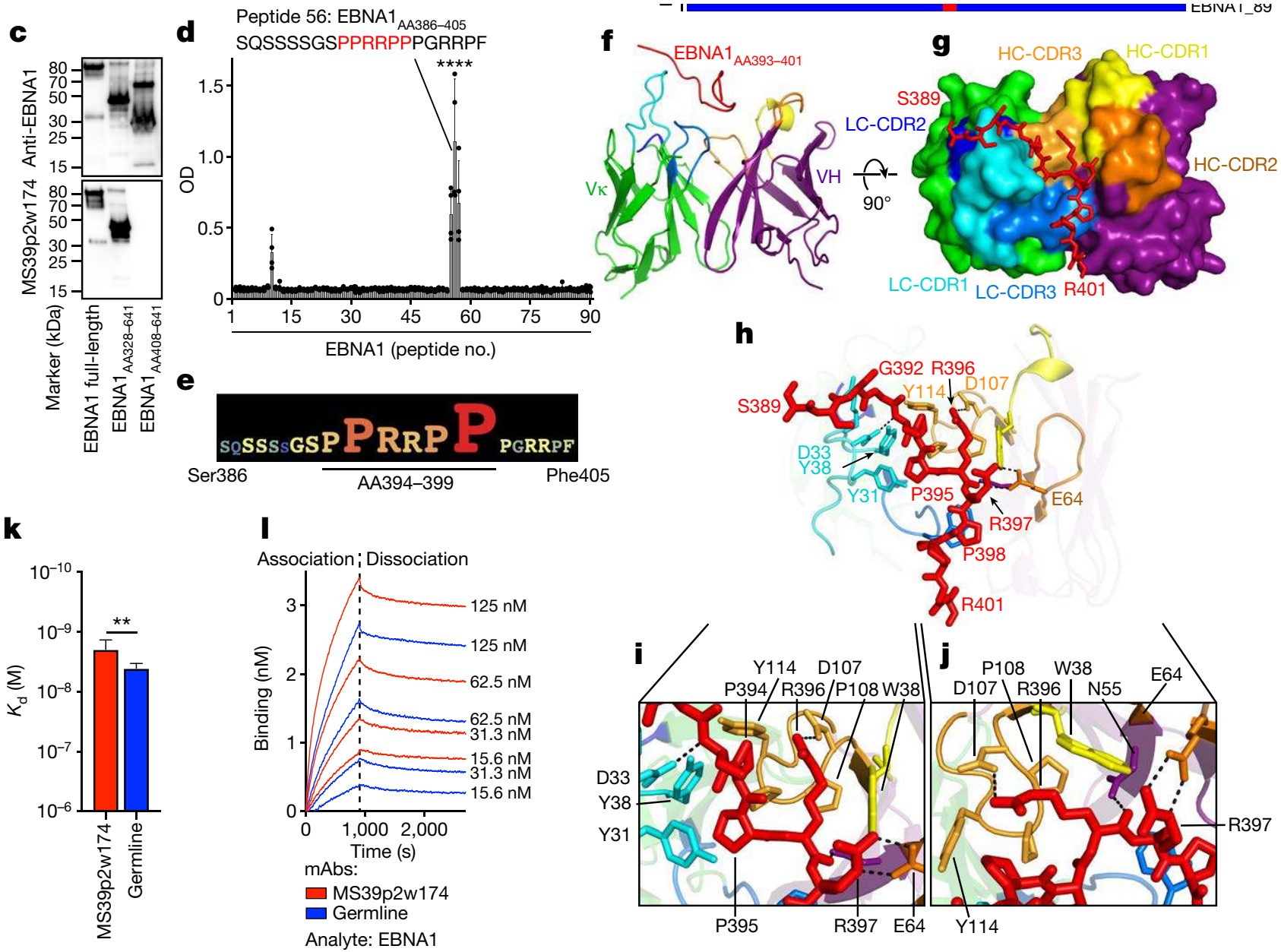
mAbs were used to probe protein microarrays
What was on the microarrays>
2 EBV viral lysates,
23 recombinant latent and lytic EBV proteins,
240 peptides spanning four prominent EBV proteins,
lysates of 7 other MS-associated viruses, including measles virus, rubella virus, and varicella-zoster virus

Antibody MS39p2w174 Binds an MS-Associated Epitope of EBNA1



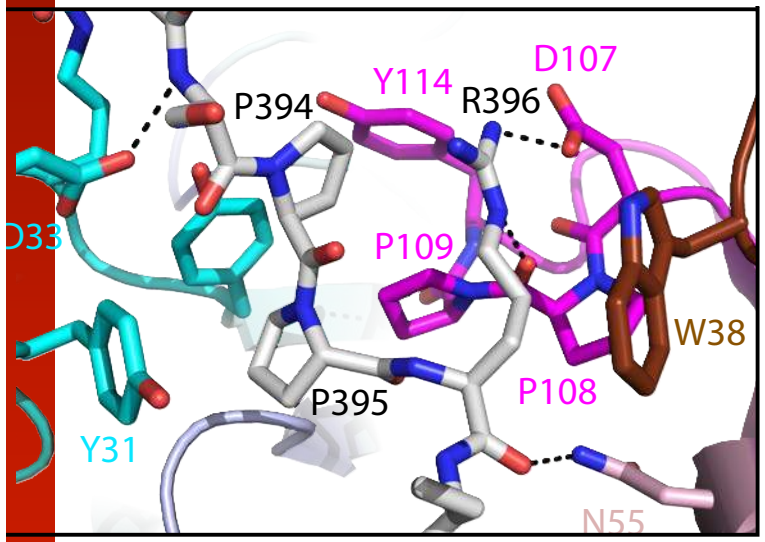
Note that other monoclonals bind Measles, Rubella, Varicella-Zoster, CMV

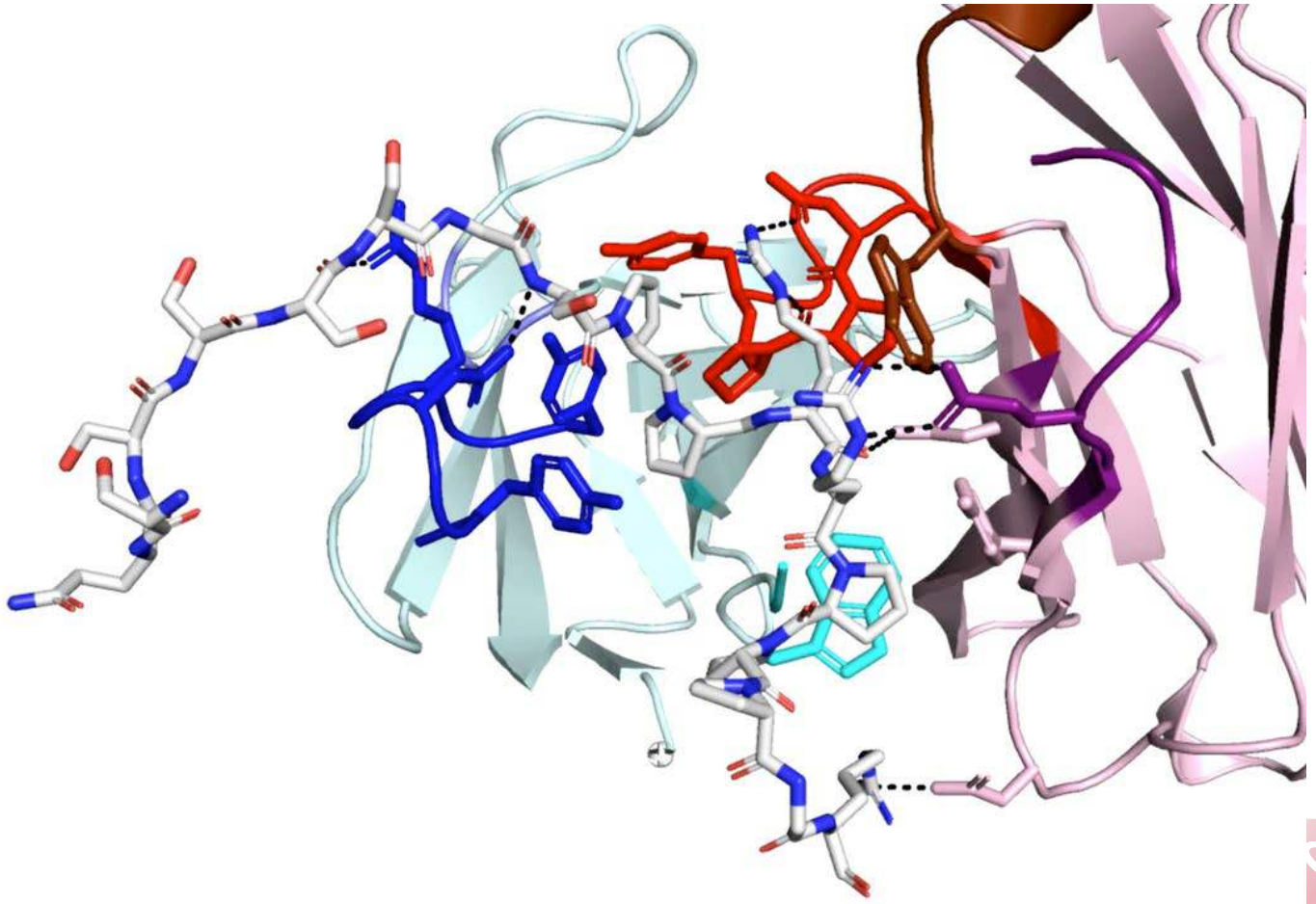
MS Clonal CSF Antibody Binds to EBNA1 AA386-405



Structural Analysis of the Antigen-Antibody Complex

A Hydrophobic Cage around Prolines and H-Bonds to Arginines Generate Tight Interactions between EBNA1 and Antibody





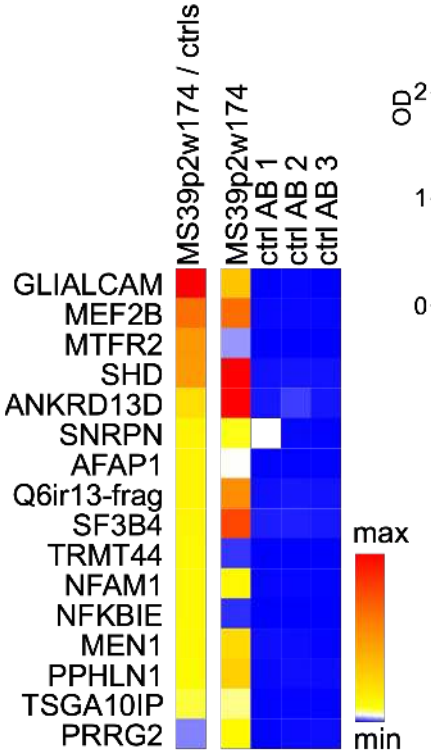
Proline Cage for EBNA

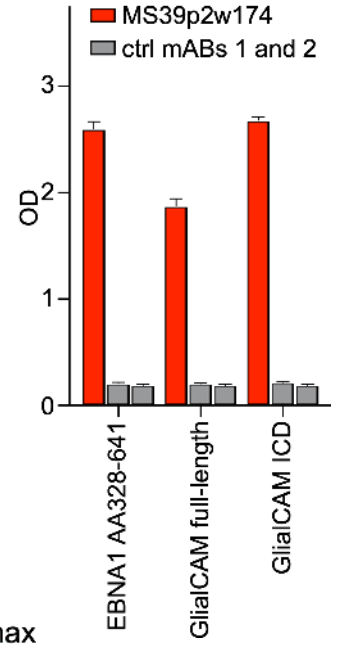
Antibody MS39p2w174 binds GlialCAM

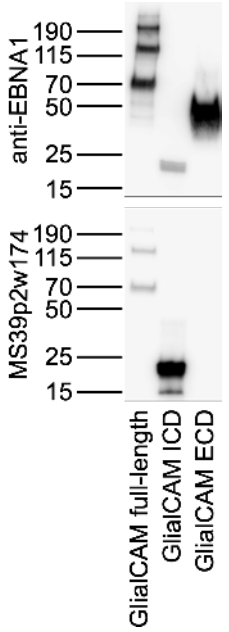
We probed mAb MS39p2w174 on a HuProt protein microarray, which represents >16,000 proteins spanning >80% of the human proteome

Antibody MS39p2w174 binds GlialCAM

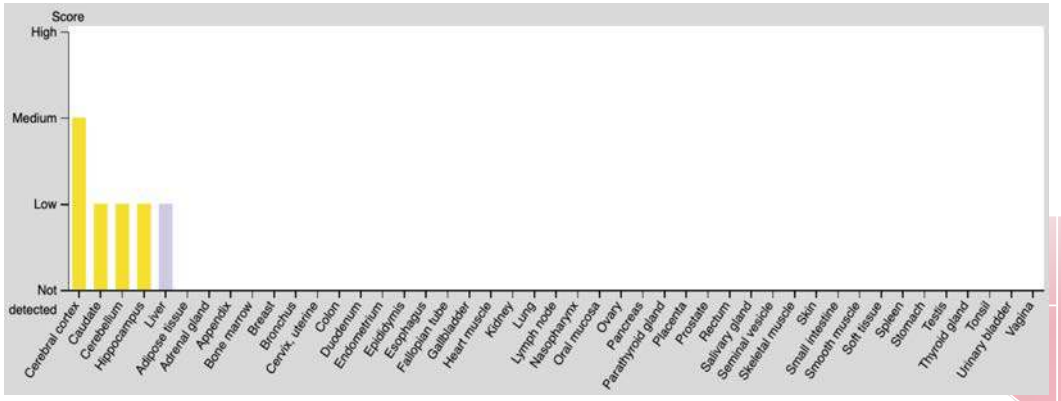
GlialCAM







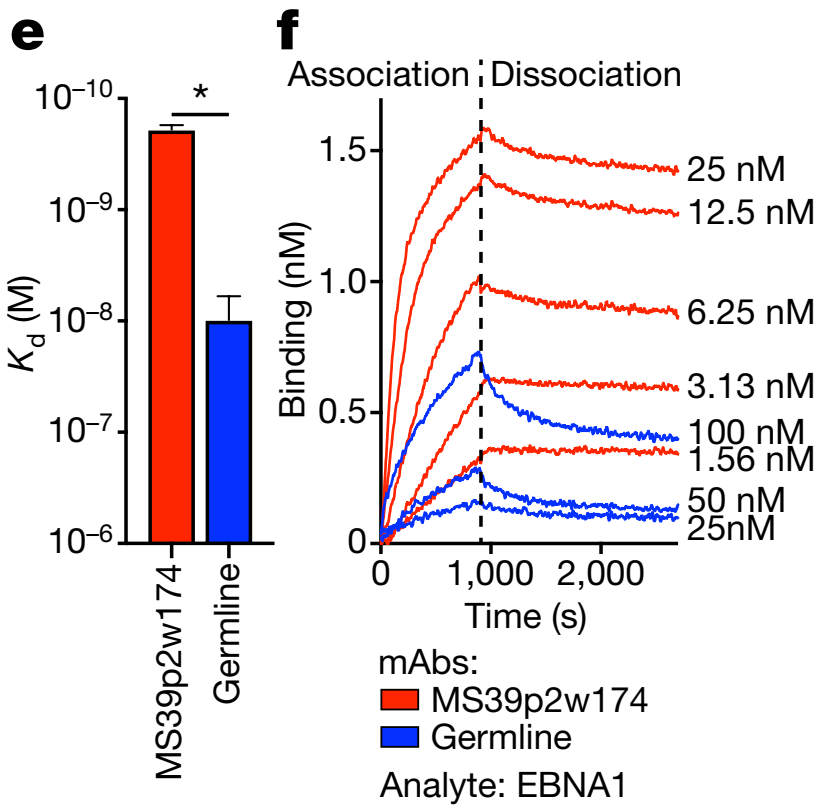
- Glial cellular adhesion molecule
- Expressed in oligodendrocytes and astrocytes
- Involved in chloride and water homeostasis
- Mutation causes megalencephalic leukoencephalopathy with subcortical cysts (MLC)
- Chaperone of Aquaporin-4



We probed mAb MS39p2w174 on a HuProt protein microarray, which represents >16,000 proteins spanning >80% of the human proteome

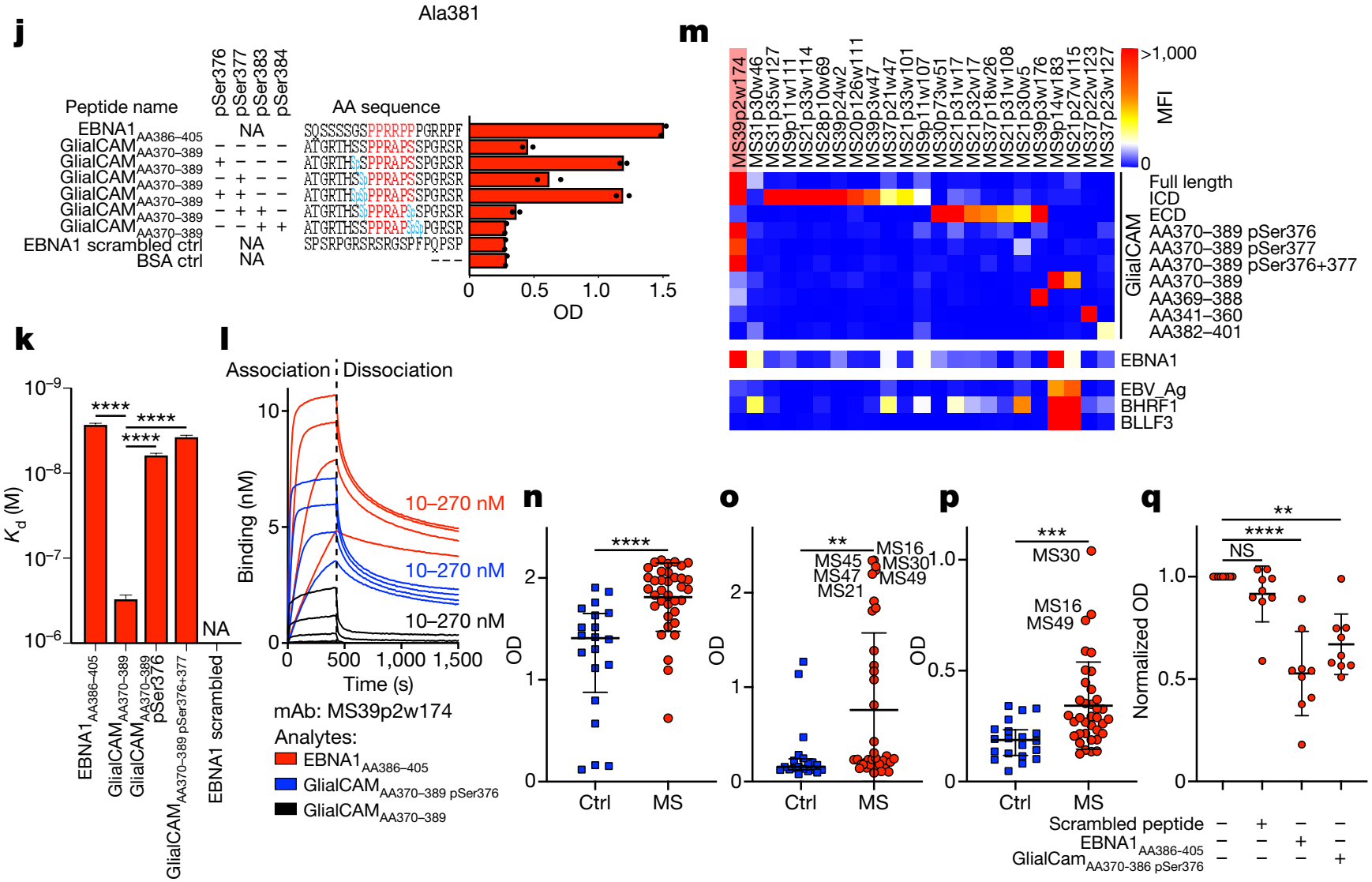
proteinat
a

Affinity Maturation from Germline is Necessary for High Affinity Binding to GlialCAM



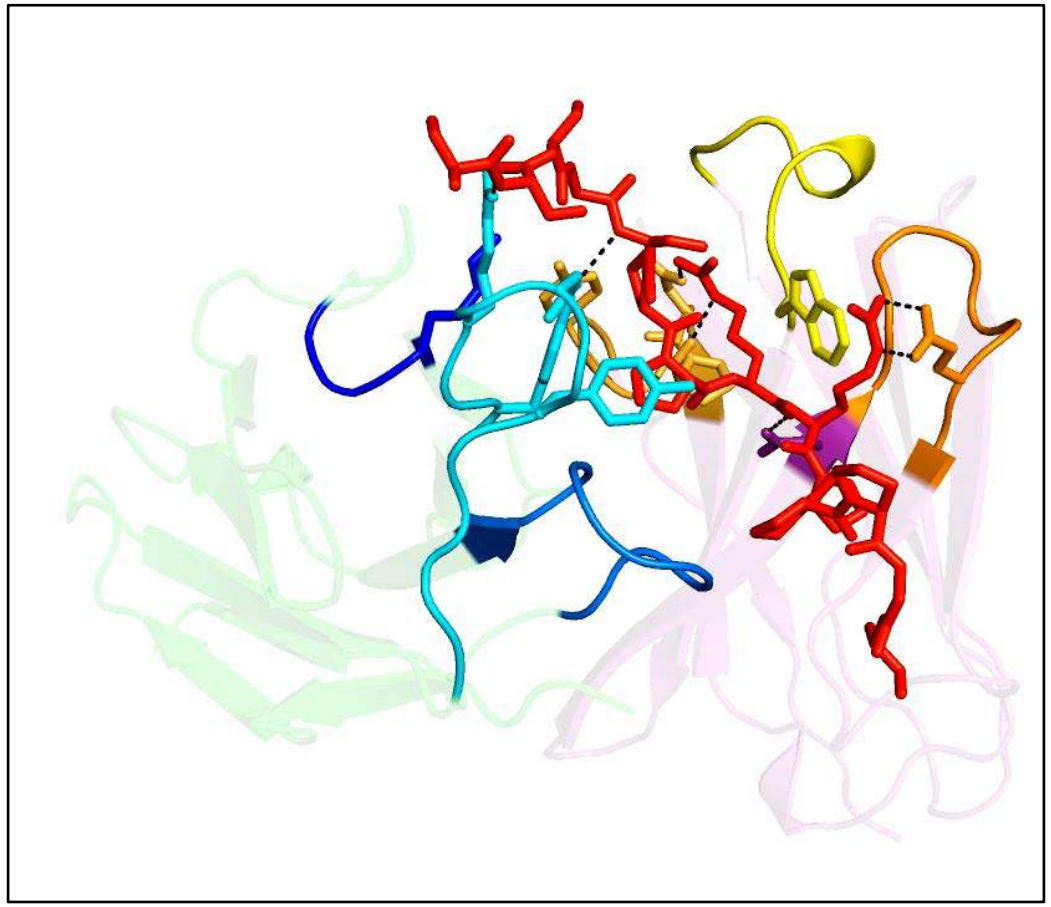
Nearly everyone is infected with EBV, but only a small fraction develop MS. Thus, other factors, such as genetic susceptibility, are important in MS pathogenesis. Certain genes, such as those encoding the antigen-presenting human leukocyte antigen (HLA) proteins, determine the portion of a pro- tein that is presented to the immune system. **Other genes control modifications in EBV-associated proteins, including phosphorylation. Such genes are critical for modulating molecular mimicry (*4*, *11*).** Thus, given these additional gating factors in MS pathogenesis, **infection with EBV is likely to be necessary, but not sufficient**, to trigger development of MS. Infection with EBV is the initial pathogenic step in MS, but additional fuses must be ignited for the full pathophysiology

Key Roles of Phosphorylation in Adjacent Serine Residues This Might be One of the Gating Steps that Modulates Risk

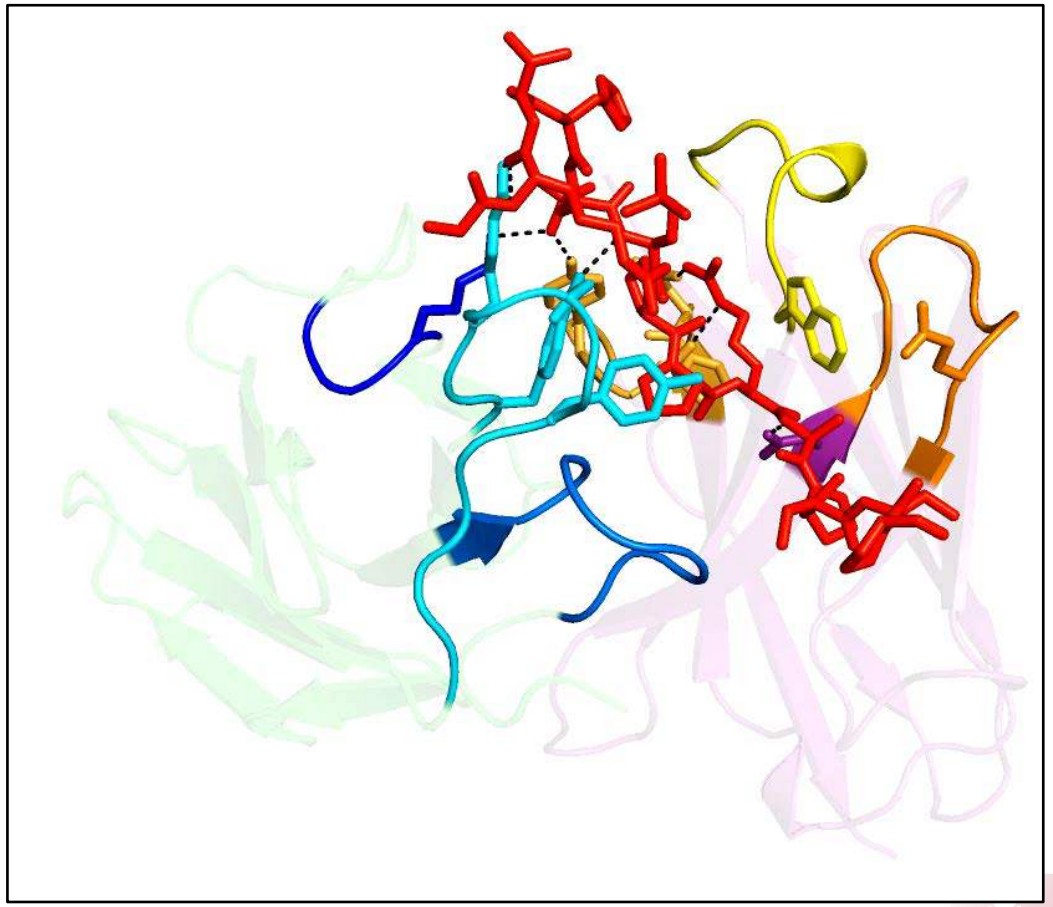


Serine Phosphorylation Enables Fab / GlialCAM Interaction

Fab / $EBNA1_{AA386-405}$



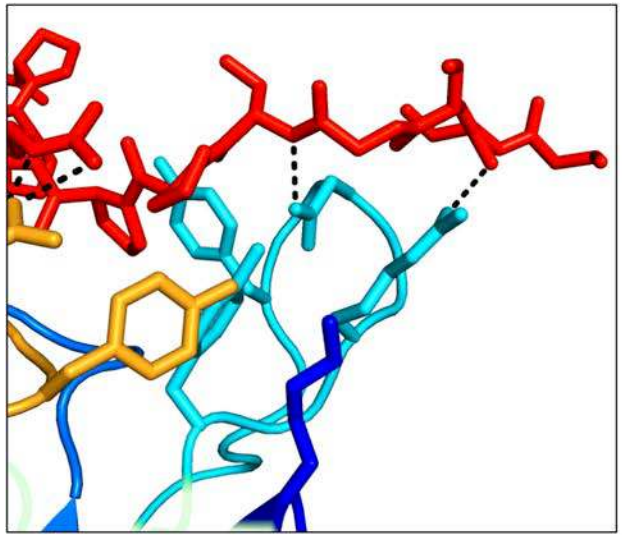
Fab / $GlialCAM_{370-389}$



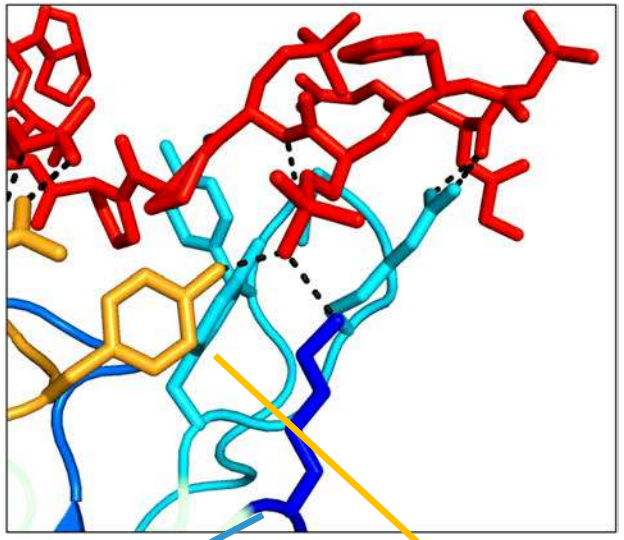
Several kinases have been described as risk genes for MS, including *MERTK*, *MAPK1*, *MAPK3* and *TYK2*, which potentially contribute to alternative phosphorylation patterns in the CNS

Serine Phosphorylation Enables Fab / GlialCAM Interaction

Fab / EBNA1$_{AA386-405}$



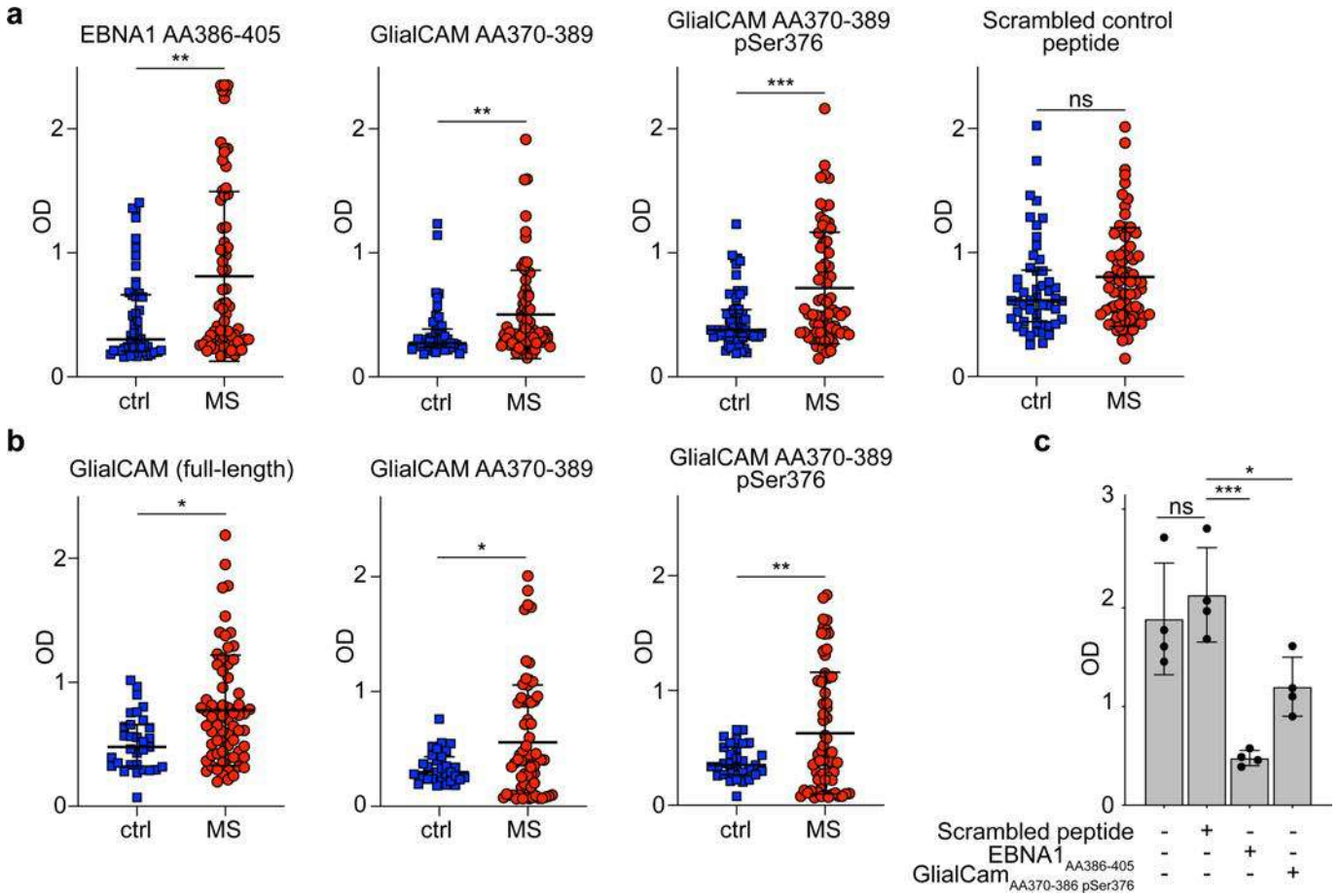
Fab / GlialCAM$_{370-389}$



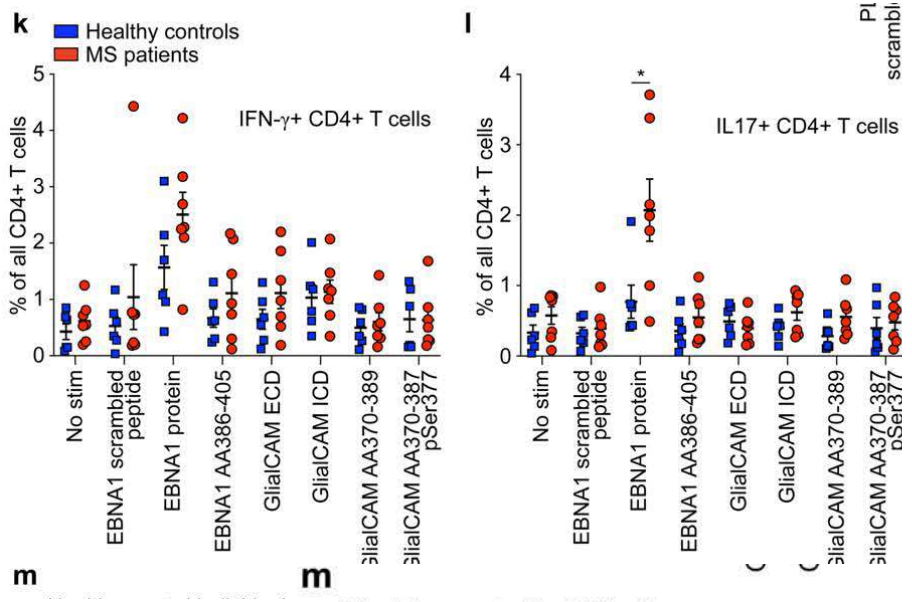
	1	FR1	27	LCDR1	39	FR2	56	LCDR2	66	FR3	105	LCDR3	118	FR4
LC														
MS39p2w174	DVVLTQSPLSLPVILGQPASISCRSSQSLVYS.DGRTYLNWFQQRPGQSPRLIYKI.....SKRDSGVP.ERFSGSG..SGTDFTLEISRVEAEDVGIYYCMQGSH....WPVTFGQGTKVEIK.													
GL IGKV2-30 / IGKJ1	M T N V N D K V T V													

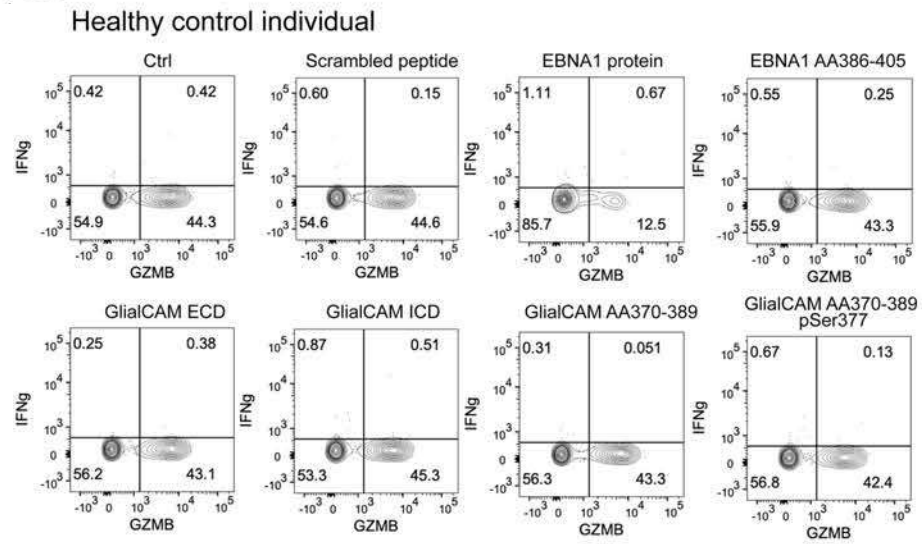
	1	FR1	27	HCDR1	39	FR2	56	HCDR2	66	FR3	105	HCDR3	118	FR4
HC														
MS39p2w174	QVQLVESGG.GLVKPGGSLRLSCVSSGFTF....SNYWMSWVRQAPGGGLEWVANINQD..GSEKYYVDSVK.GRFTSSRDNTKNSLFLQLNSLRAEDTGIYYCTRDPP....YFDNWGQGTLVTVSS													
GL IGHV3-7 / IGHJ4	E Q AA S K K I A Y M AV A Y													

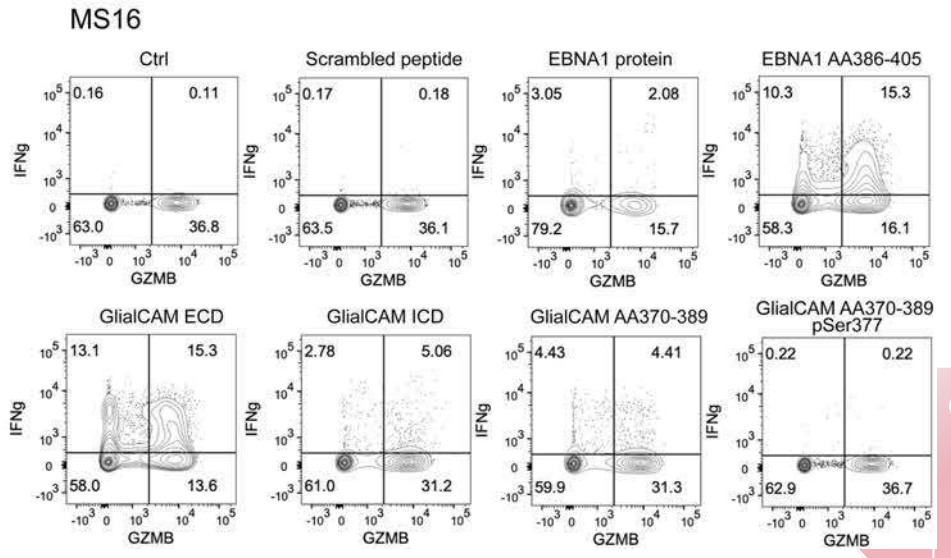
Anti-GlialCAM Reactivity is a Broader Phenomenon in MS Patients ”Snapshot” in Time in Blood Compartment Might Underestimate Earlier Presence in Blood and CSF



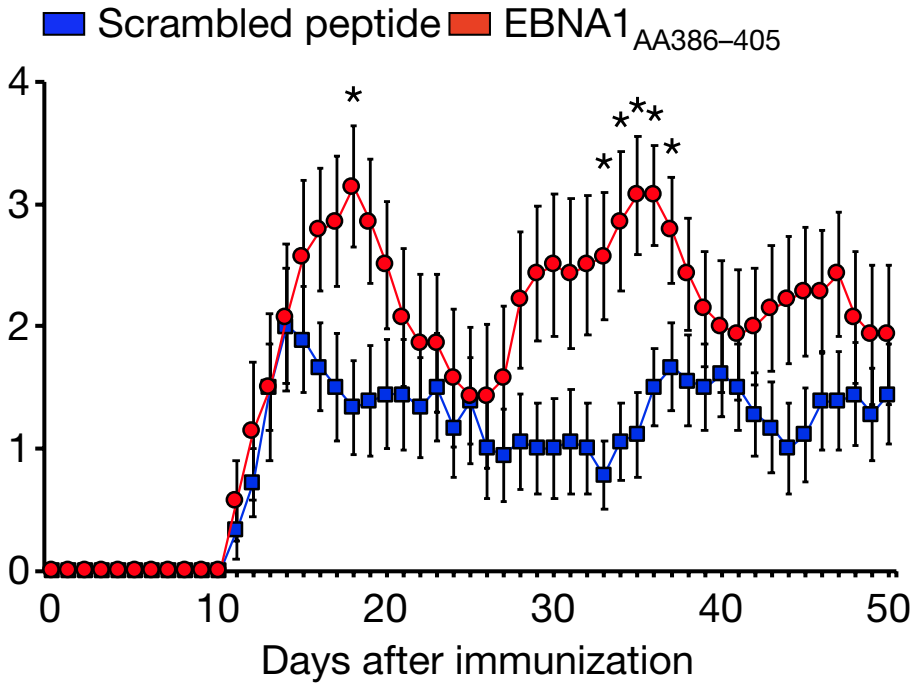
T cell Response to GlialCAM in MS Blood Signals in IFN-γ IL-17 and granzyme T cells

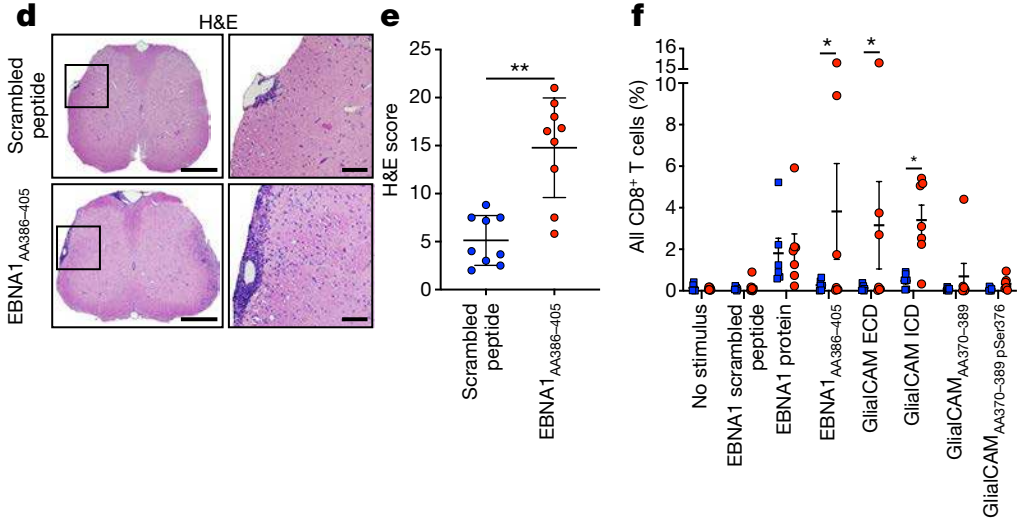






Immunization with EBNA1 Aggravates EAE With Homage to Koch's Postulates





Embedded in the Epidemiology Paper in Science
A Stunning Clue

Table S1.

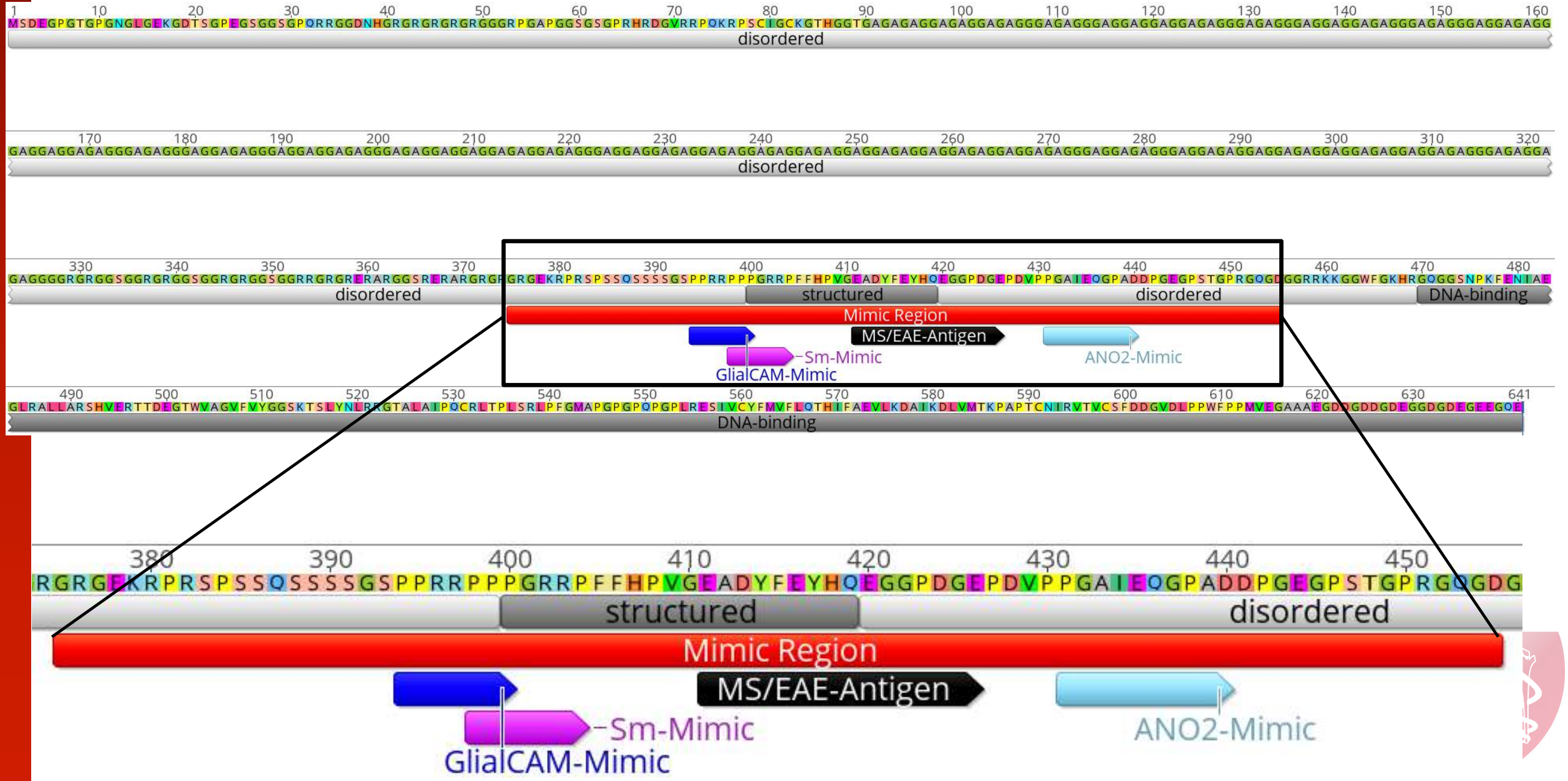
Table S1. Viral peptides in the *pre-onset* samples with significantly different antibody binding between cases and controls[1]

Viral species	Strain	Protein	UniProt ID	Protein length	Peptide start	Peptide end	Proportion in Cases	Proportion in Controls	P-value
Epstein-Barr virus	B95-8	EBNA-1	P03211	641	365	420	0.73	0.2	0.000073
Epstein-Barr virus	B95-8	Capsid protein VP26	P14348	176	113	168	0.87	0.47	0.0022
Epstein-Barr virus	B95-8	Envelope glycoprotein M	P03215	405	365	405	0.37	0.03	0.0025
Epstein-Barr virus	GD1	EBNA-1 (Fragment)	Q5MJ03	237	29	84	0.47	0.1	0.0034
Epstein-Barr virus	B95-8	EBNA-3	P12977	944	701	756	0.63	0.23	0.0038
Epstein-Barr virus	B95-8	EBNA-1	P03211	641	421	476	0.83	0.47	0.0061

Between 365 and 420 in EBNA-1is a Molecular Mimicry Hotspot

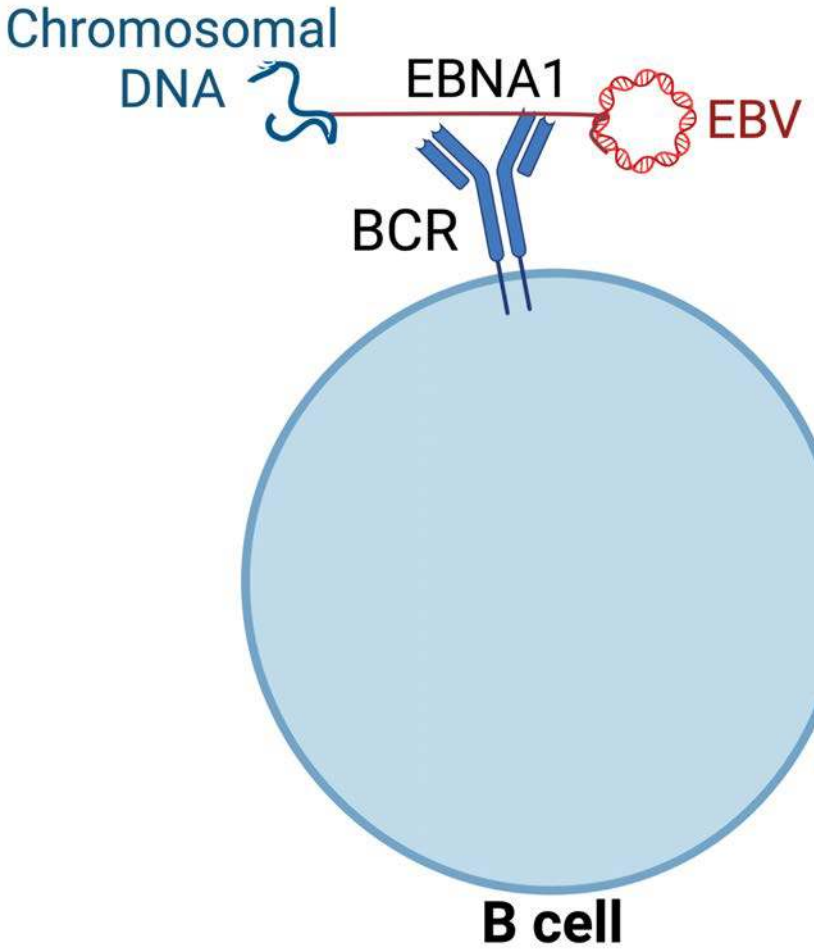
Another study showed serum antibodies from MS patients are cross-reactive between **amino acids 411–440 of the viral protein EBV nuclear antigen 1 (EBNA-1) and the human chloride-channel protein, anoctamin 2** (ANO2), which is associated with electrical conduction in axons (*11*). **MS serum antibodies targeting EBNA-1 residues 411–426 that cross-react with myelin basic protein have** also been identified (*12*). Clonally expanded antibodies in the CSF of MS patients targeting EBNA-1 residues **386–405 that cross- react with the CNS cell adhesion molecule, glialCAM, have also been described (*4*).** It is intriguing that three contiguous regions of mimicry have been reported in a small region of the EBNA-1 protein; this may arise through immune surveillance in a process called epitope spreading.

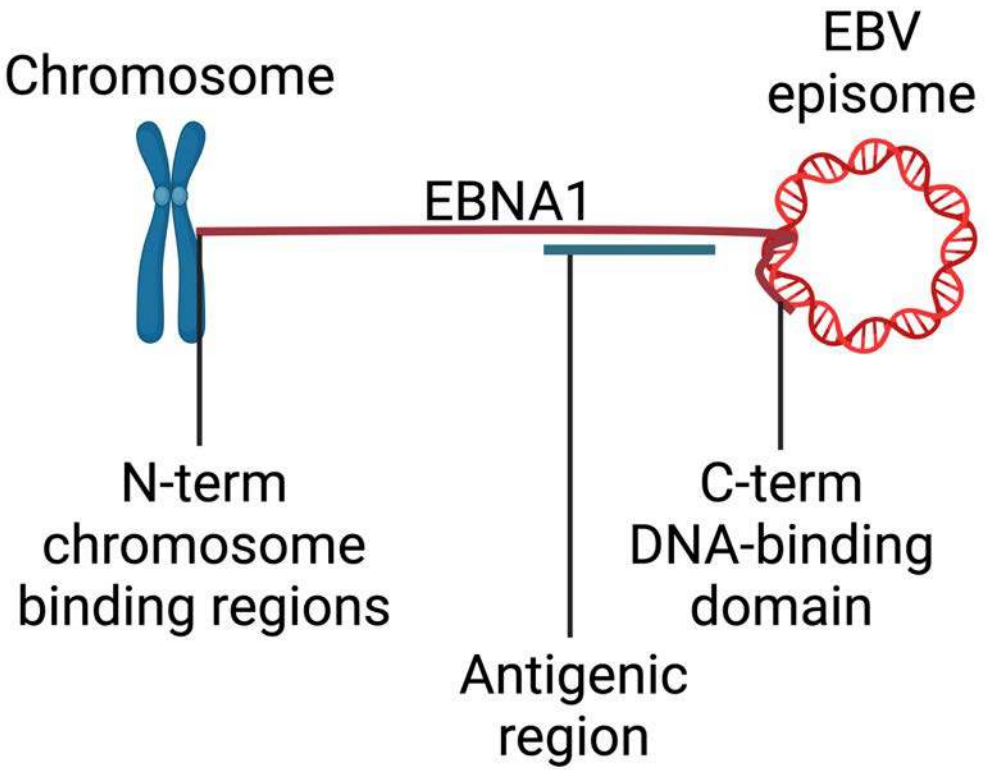
Multiple Autoimmune Mimics in a Small Region of EBNA1



Antigenicity of DNA-Binding Proteins

DNA binding proteins are known antigens in many autoimmune disorders





How do we eliminate/tolerize these T and B cells

- Many ways:
- Nanoparticles with antigen, Engineered Dendritic Cells, Antigen coupled to RBC are all promising
- Plasmid and RNA Inverse Vaccines are promising, Science 2021→Tolerize to GlialCAM?

One of the very most promising approaches is using RNA without adjuvanticity of the liposome:

"We hypothesized that the use of such nucleoside-modified, purified mRNA (m1Y mRNA) for in vivo delivery of autoimmune disease target antigens into CD11c+ APCs in a noninflammatory context would enable systemic tolerogenic antigen presentation in lymphoid tissues."

Tolerizing RNA Vaccine from BioNTech, Science Jan 8 2021

RESEARCH ARTICLE

MULTIPLE SCLEROSIS

A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis

Christina Krienke[1,2], Laura Kolb[1]*, Elif Diken[1]*, Michael Streuber[1], Sarah Kirchhoff[1], Thomas Bukur[1], Özlem Akilli-Öztürk[1], Lena M. Kranz[3], Hendrik Berger[3], Jutta Petschenka[1,4], Mustafa Diken[1,3], Sebastian Kreiter[1,3], Nir Yogev[5,6], Ari Waisman[2,5], Katalin Karikó[3], Özlem Türeci[3,7], Ugur Sahin[1,2,3]†

The ability to control autoreactive T cells without inducing systemic immune suppression is the major goal for treatment of autoimmune diseases. The key challenge is the safe and efficient delivery of pharmaceutically well-defined antigens in a noninflammatory context. Here, we show that systemic delivery of nanoparticle-formulated 1 methylpseudouridine-modified messenger RNA (m1Ψ mRNA) coding for disease-related autoantigens results in antigen presentation on splenic $CD11c^+$ antigen-presenting cells in the absence of costimulatory signals. In several mouse models of multiple sclerosis, the disease is suppressed by treatment with such m1Ψ mRNA. The treatment effect is associated with a reduction of effector T cells and the development of regulatory T cell (T_{reg} cell) populations. Notably, these T_{reg} cells execute strong bystander immunosuppression and thus improve disease induced by cognate and noncognate autoantigens.

Tolerizing DNA Vaccine Nature Biotech 2003, Phase 2 Trial 2008, Now gearing up tolerization to GlialCAM

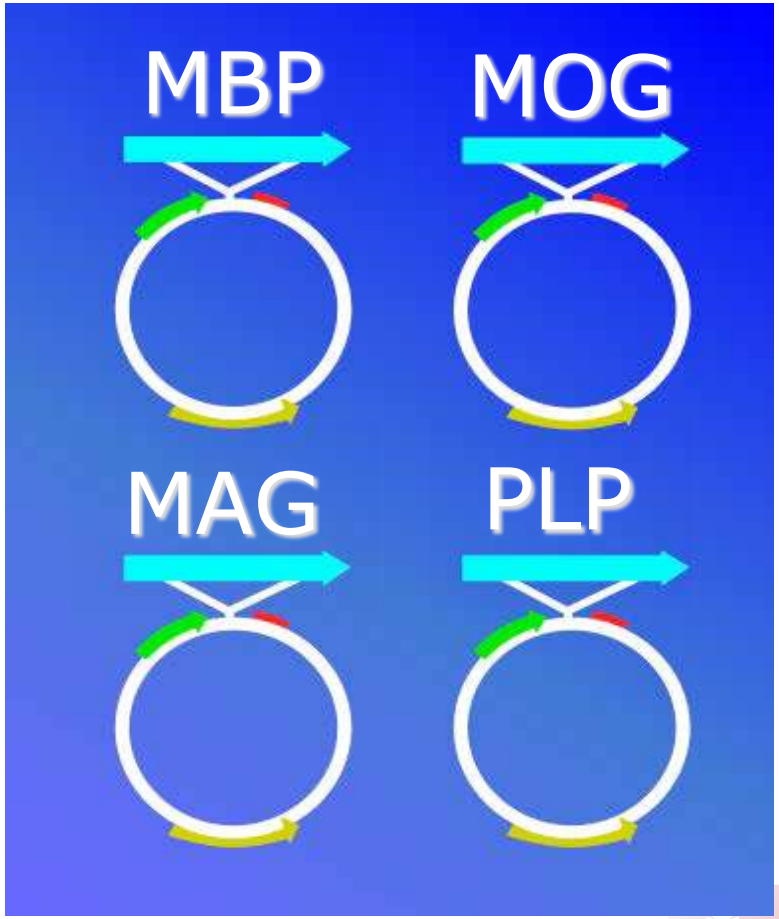
nature biotechnology

ARTICLES

Protein microarrays guide tolerizing DNA vaccine treatment of autoimmune encephalomyelitis

William H Robinson[1–4], Paulo Fontoura[1], Byung J Lee[1,2], Henry E Neuman de Vegvar[1,3], Jennifer Tom[1,2], Rosetta Pedotti[1], Carla D DiGennaro[1,2], Dennis J Mitchell[1], Derek Fong[1,2], Peggy P-K Ho[1], Pedro J Ruiz[1], Emanual Maverakis[5], David B Stevens[6], Claude C A Bernard[7], Roland Martin[8], Vijay K Kuchroo[9], Johannes M van Noort[10], Claude P Genain[11], Sandra Amor[12], Tomas Olsson[13], Paul J Utz[2,4,14], Hideki Garren[4,14] & Lawrence Steinman[1,4,14]

The diversity of autoimmune responses poses a formidable challenge to the development of antigen-specific tolerizing therapy. We developed 'myelin proteome' microarrays to profile the evolution of autoantibody responses in experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis (MS). Increased diversity of autoantibody responses in acute EAE predicted a more severe clinical course. Chronic EAE was associated with previously undescribed extensive intra- and intermolecular epitope spreading of autoreactive B-cell responses. Array analysis of autoantigens targeted in acute EAE was used to guide the choice of autoantigen cDNAs to be incorporated into expression plasmids so as to generate tolerizing vaccines. Tolerizing DNA vaccines encoding a greater number of array-determined myelin targets proved superior in treating established EAE and reduced epitope spreading of autoreactive B-cell responses. Proteomic monitoring of autoantibody responses provides a useful approach to monitor autoimmune disease and to develop and tailor disease- and patient-specific tolerizing DNA vaccines.



Autoantibody Array Technology

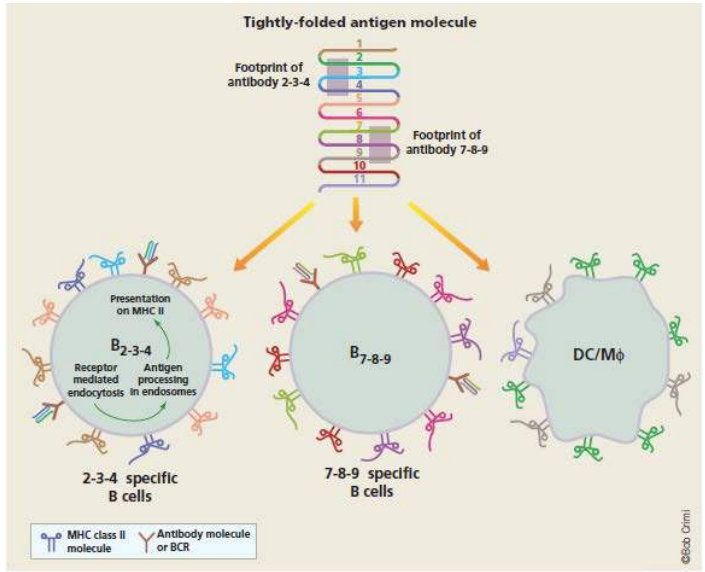
- A Personalized Therapy of Autoimmune Diseases
- Eli Sercarz wrote this News and Views Accompanying the 2003 Nature Biotech

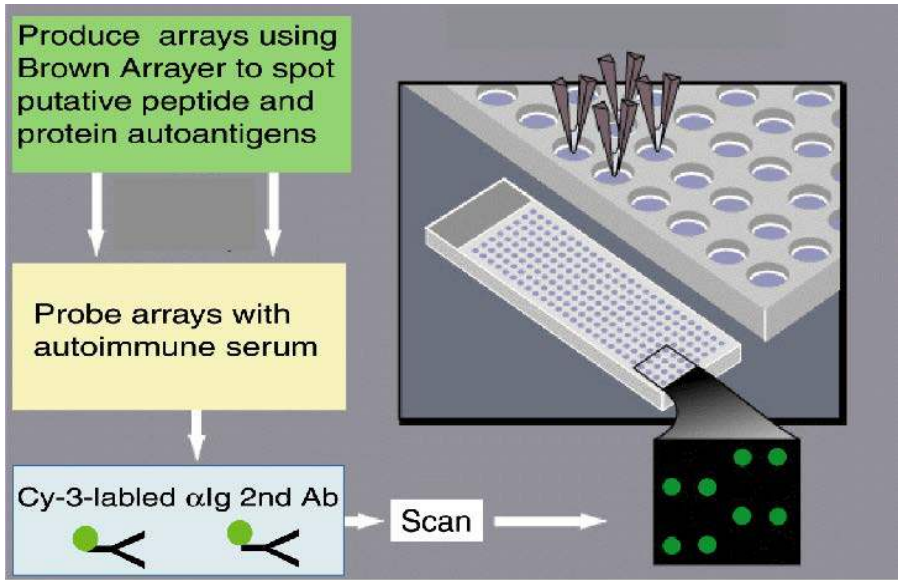
NEWS AND VIEWS

Arraying autoimmunity treatment

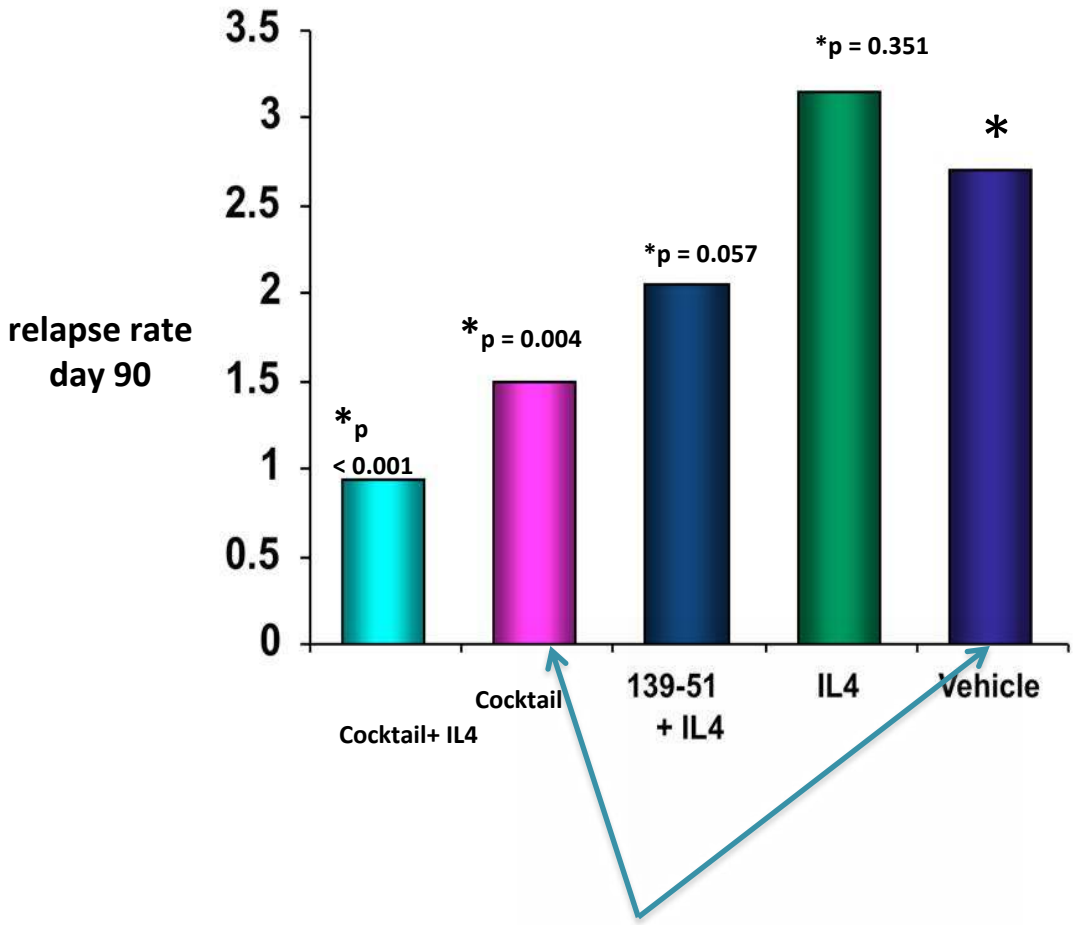
Eli E Sercarz

Protein and peptide arrays that reveal antigens which induce a diverse autoantibody response can be used to guide tolerizing therapy.

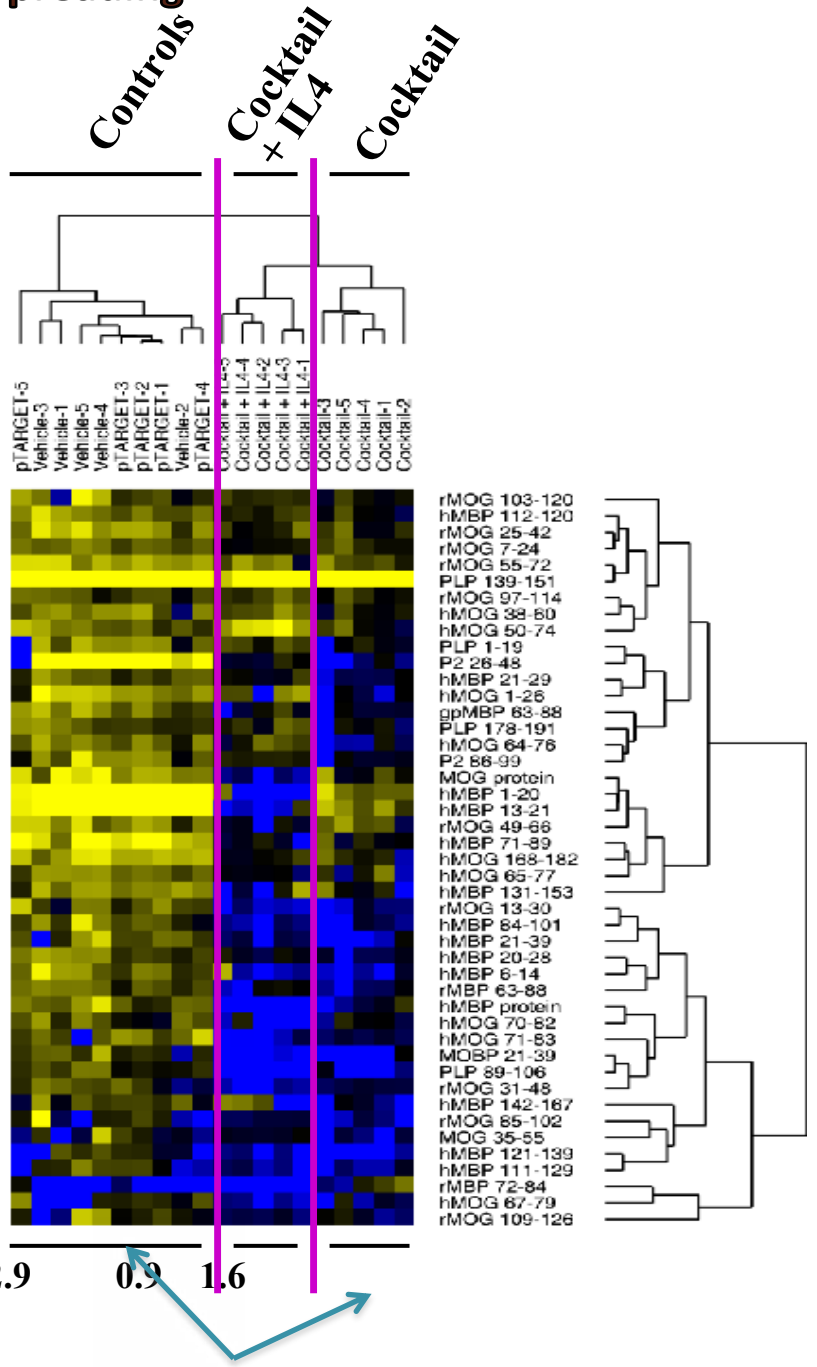




DNA Tolerizing Vaccines Encoding Multiple Array-Identified Targets



DNA Tolerizing Vaccines Reduce Relapses and Epitope Spreading



Relapse rate: **2.9** **0.9** **1.6**

Tolerizing to the Most Abundant Myelin Protein with a Plasmid in Humans- 267 patients

Phase 2 Trial of a DNA Vaccine Encoding Myelin Basic Protein for Multiple Sclerosis

Hideki Garren, MD, PhD,[1,2] William H. Robinson, MD, PhD,[2] Eva Krasulová, MD,[3] Eva Havrdová, MD, PhD,[3] Congor Nadj, MD, PhD,[4] Krzysztof Selmaj, MD, PhD,[5] Jacek Losy, MD,[6] Ilinka Nadj, MD,[4] Ernst-Wilhelm Radue, MD,[7] Brian A. Kidd, MS,[8] Jill Gianettoni, BS,[1] Karen Tersini, BS,[1] Paul J. Utz, MD,[2] Frank Valone, MD,[1] Lawrence Steinman, MD,[2] and the BHT-3009 Study Group

Objective: To evaluate the efficacy and safety of BHT-3009 in relapsing-remitting multiple sclerosis (MS) and to confirm that BHT-3009 causes immune tolerance.

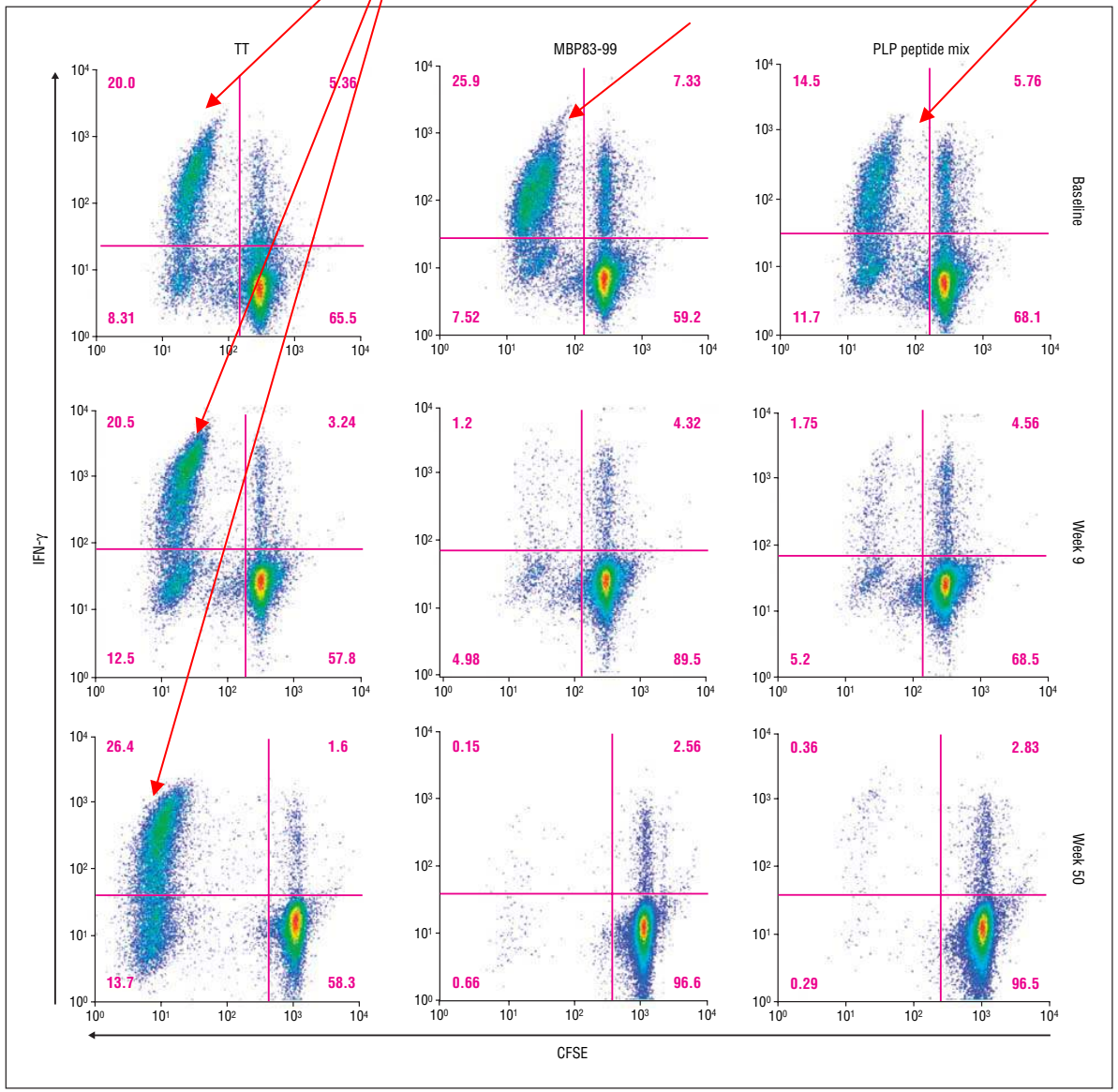
Methods: BHT-3009 is a tolerizing DNA vaccine for MS, encoding full-length human myelin basic protein. Relapsing-remitting MS patients were randomized 1:1:1 into three groups: placebo, 0.5mg BHT-3009, or 1.5mg BHT-3009, given intramuscularly at weeks 0, 2, 4, and every 4 weeks thereafter until week 44. The primary end point was the 4-week rate of occurrence of new gadolinium-enhancing lesions on brain magnetic resonance images from weeks 28 to 48. Protein microarrays were used to measure levels of anti-myelin autoantibodies.

Results: Compared with placebo, in the 267 patient analysis population the median 4-week rate of new enhancing lesions during weeks 28 to 48 was 50% lower with 0.5mg BHT-3009 ($p = 0.07$) and during weeks 8 to 48 was 61% lower with 0.5mg BHT-3009 ($p = 0.05$). The mean volume of enhancing lesions at week 48 was 51% lower on 0.5mg BHT-3009 compared with placebo ($p = 0.02$). No significant improvement in magnetic resonance imaging lesion parameters was observed with 1.5mg BHT-3009. Dramatic reductions in 23 myelin-specific autoantibodies in the 0.5mg BHT-3009 arm were observed, but not with placebo or 1.5mg BHT-3009.

Conclusions: In relapsing-remitting MS patients, treatment with the lower dose (0.5mg) of BHT-3009 for 44 weeks nearly attained the primary end point for reduction of the rate of new enhancing magnetic resonance imaging lesions ($p = 0.07$) and achieved several secondary end points including a reduction of the rate of enhancing magnetic resonance imaging lesions from weeks 8 to 48 ($p = 0.05$). Immunological data in a preselected subgroup of patients also indicated that treatment with 0.5mg induced antigen-specific immune tolerance. The greater dose was ineffective.

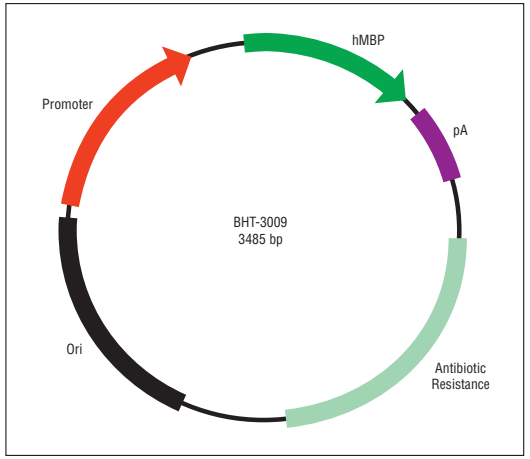
Ann Neurol 2008;63:611–620

Reduced IFN-γ Response in MBP T cells, with some effect on PLP T cells, **but not Tet Tox T cells**



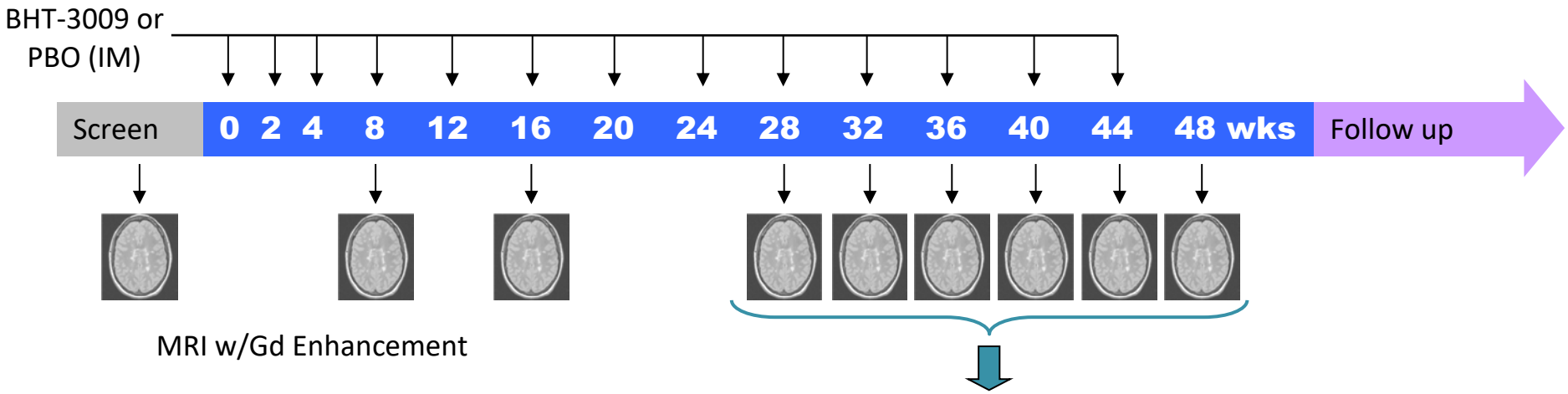
5 of 5 patients with *decreased* MBP T cell response

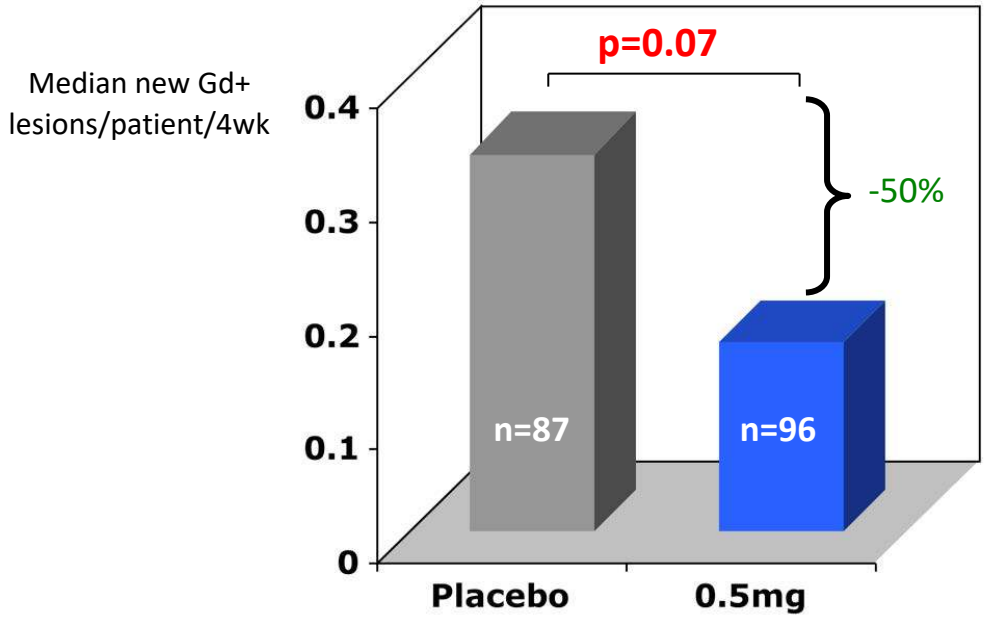
1 patient negative at baseline, and remained negative after treatment



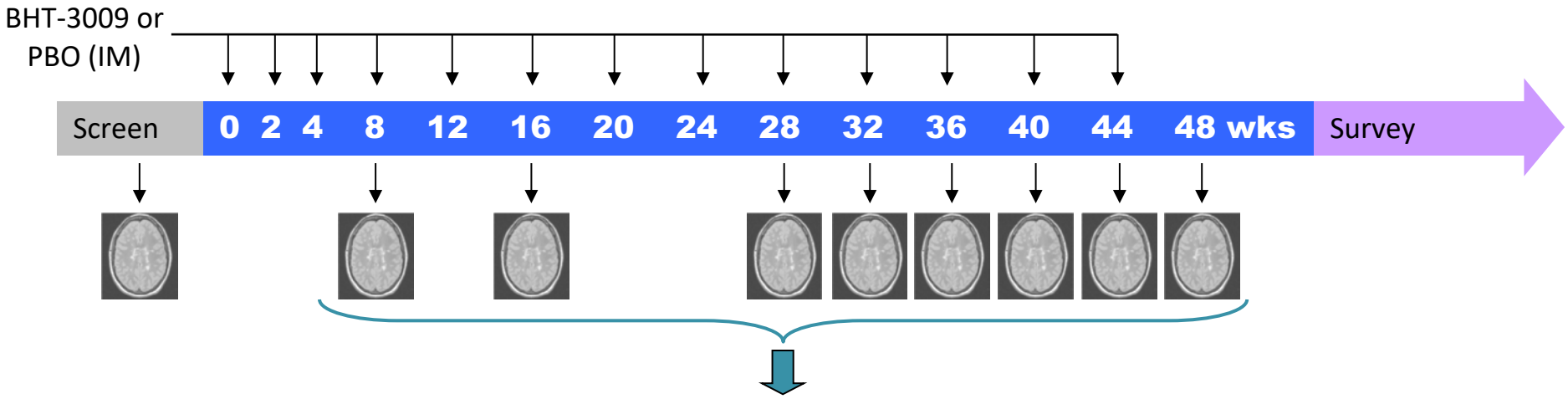
We isolated the full-length complementary DNA encoding the 18.5-kDa isoform of human MBP from a human brain complementary DNA library. This was cloned into a modified expression plasmid driven by a eukaryotic promoter. The expression plasmid was created as a derivative of the pVAX1 plasmid, where **certain immunostimulatory CpG motifs were removed and immuno- inhibitory GpG motifs were included**. We have previously shown that oligonucleotides containing these GpG motifs either alone or combined with DNA vaccine plas- mids were effective in treating animal models of several prototypic autoimmune diseases

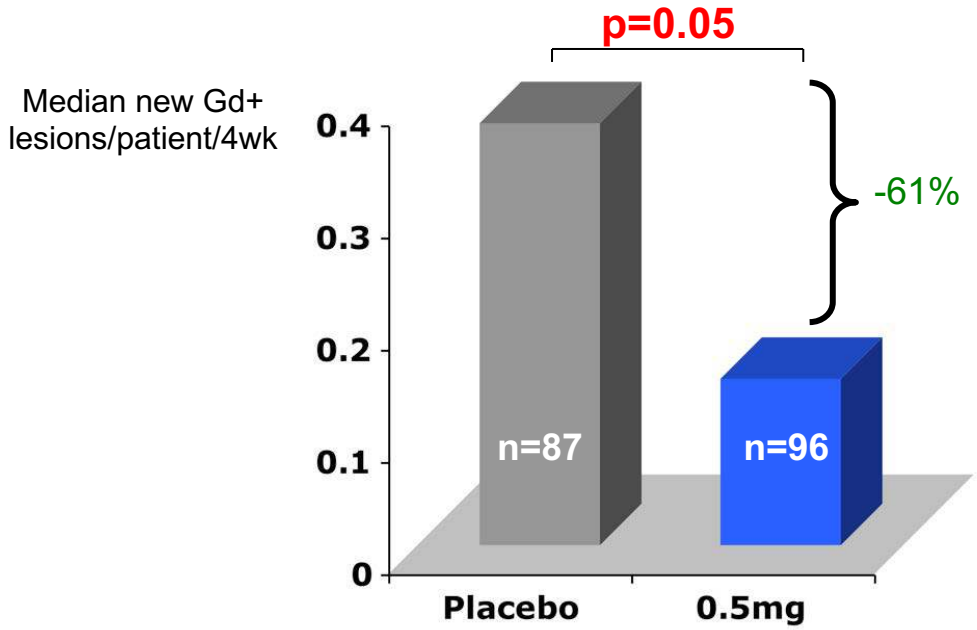
Gadolinium-Enhancing (Gd+) Lesions





Phase II:
Gadolinium-Enhancing (Gd+) Lesions





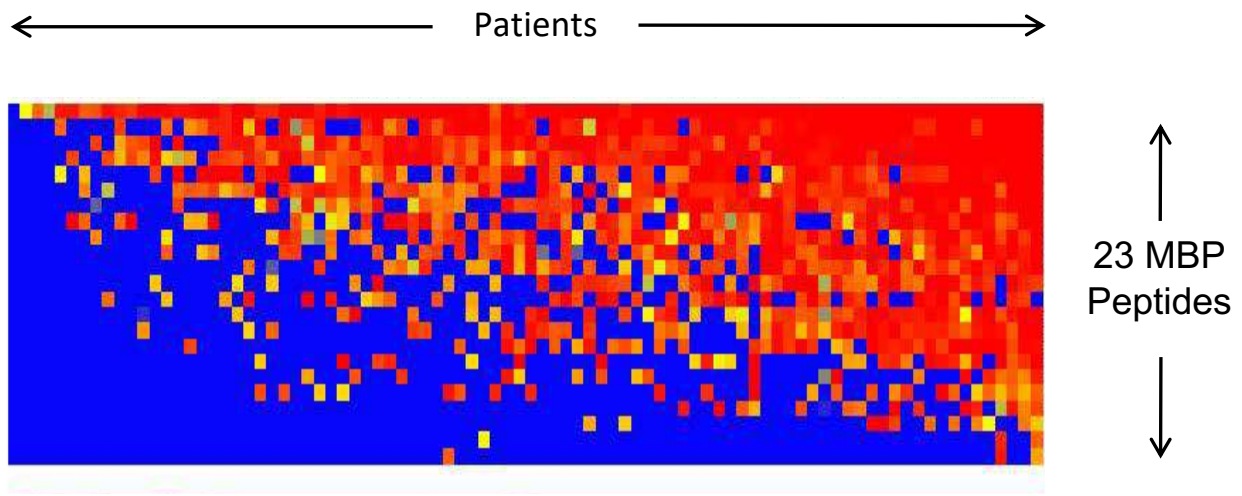
PP population

Patients with Highest MBP Antibody in CSF had Best Response, leading to a conjecture:

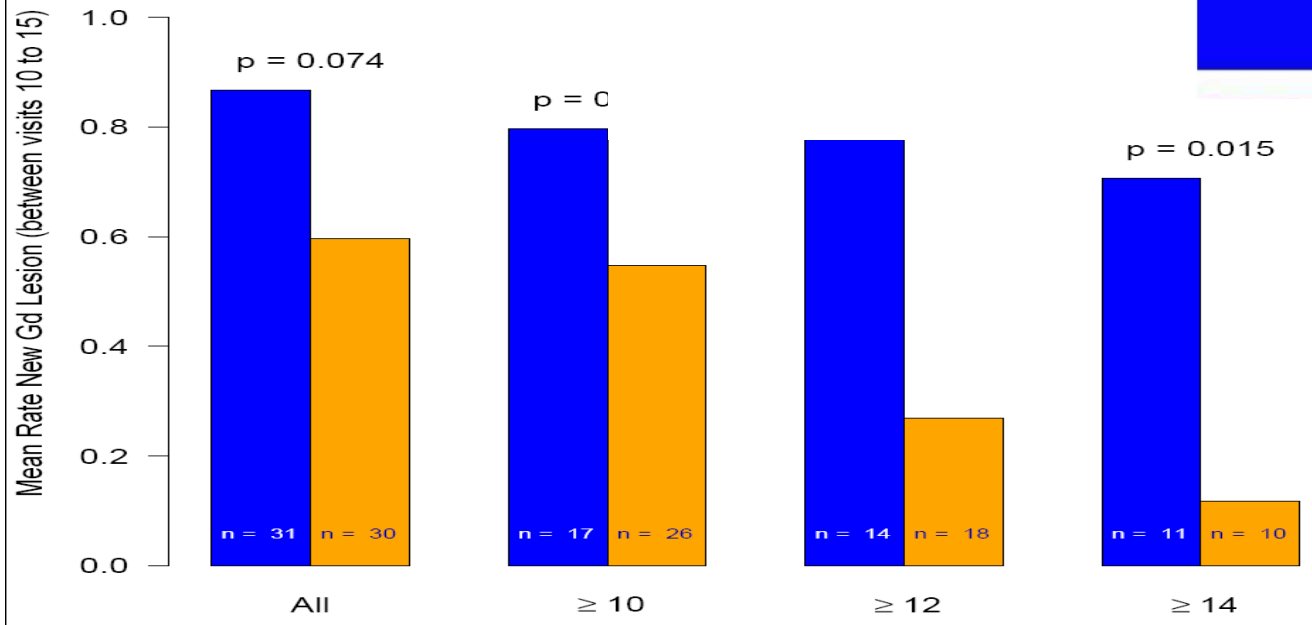
Conjecture: Tolerization works best when autoreactivity is highest.

Why capturing the cells to tolerize makes sense.

Num MBP Ag
w/ reactivity > 10,000



Mean New Gd+ Lesion Rate (0.5 mg).



In the upper half of the anti-MBP reactive patients, there was a significantly lower number of new Gd-enhancing lesions per patient in week 28 to 48 MRIs with 0.5mg BHT-3009 compared with placebo (mean ± standard deviation, 2.5 ± 4.03 on 0.5mg BHT-3009 vs 3.3 ± 4.59 on placebo; $p < 0.02$).

p-values from one-sided Wicoxon test

Does Molecular Mimicry Explain Epidemiology Linking EBV & MS?
Oligoclonal Antibody in MS Cerebrospinal Fluid Binds EBNA-1 & GlialCAM
EBNA-1--> Molecular Mimicry “Hot Zone”

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