Immunodominant T-cell epitopes of MOG reside in its transmembrane and cytoplasmic domains in EAE

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ABSTRACT

Objective: Studies evaluating T-cell recognition of myelin oligodendrocyte glycoprotein (MOG) in multiple sclerosis (MS) and its model, experimental autoimmune encephalomyelitis (EAE), have focused mostly on its 117 amino acid (aa) extracellular domain, especially peptide (p) 35-55. We characterized T-cell responses to the entire 218 aa MOG sequence, including its transmembrane and cytoplasmic domains.

Methods: T-cell recognition in mice was examined using overlapping peptides and intact full-length mouse MOG. EAE was evaluated by peptide immunization and by adoptive transfer of MOG epitope-specific T cells. Frequency of epitope-specific T cells was examined by ELISPOT.

Results: Three T-cell determinants of MOG were discovered in its transmembrane and cytoplasmic domains, p119–132, p181–195, and p186–200. Transmembrane MOG p119-132 induced clinical EAE, CNS inflammation, and demyelination as potently as p35-55 in C57BL/6 mice and other H-2^b strains. p119-128 contained its minimal encephalitogenic epitope. p119-132 did not cause disease in EAE-susceptible non-H-2^b strains, including Biozzi, NOD, and PL/J. MOG p119-132–specific T cells produced Th1 and Th17 cytokines and transferred EAE to wild-type recipient mice. After immunization with full-length MOG, a significantly higher frequency of MOG-reactive T cells responded to p119-132 than to p35-55, demonstrating that p119-132 is an immunodominant encephalitogenic epitope. MOG p181-195 did not cause EAE, and MOG p181-195–specific T cells could not transfer EAE into wild-type or highly susceptible T- and B-cell–deficient mice.

Conclusions: Transmembrane and cytoplasmic domains of MOG contain immunodominant T-cell epitopes in EAE. A CNS autoantigen can also contain nonpathogenic stimulatory T-cell epitopes. Recognition that a myelin antigen contains multiple encephalitogenic and nonencephalitogenic determinants may have implications for therapeutic development in MS. ***Neurol Neuroimmunol Neuroinflammation* 2014;1:e22; doi: 10.1212/NXI.0000000000000022**

GLOSSARY

aa = amino acid(s); **APC** = antigen-presenting cell; **EAE** = experimental autoimmune encephalomyelitis; **IFN** = interferon; **Ig** = immunoglobulin; **IL** = interleukin; **MHC** = major histocompatibility complex; **MOG** = myelin oligodendrocyte glycoprotein; **MS** = multiple sclerosis; **TCR** = T-cell receptor; **WT** = wild-type.

Myelin oligodendrocyte glycoprotein (MOG) is currently the most commonly studied CNS autoantigen in multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE).[1–4] Most studies in EAE and MS, although not all,[2,5–8] have focused primarily on T-cell recognition of the 117 amino acid (aa) N-terminal extracellular immunoglobulin (Ig) "variable-like" domain of MOG.[9–12] However, native full-length MOG is 218 aa and contains transmembrane and

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cytoplasmic domains.[5] Native MOG requires processing by antigen-presenting cells (APCs) for its presentation to major histocompatibility complex (MHC) II–restricted encephalitogenic $CD4^+$ MOG peptide (p) 35-55–specific T cells.[6] Indeed, susceptibility to MOG-induced EAE is affected by APC expression of invariant chain (Ii) and H-2M (HLA-DM) molecules that participate in MHC II biosynthesis and endocytic processing, which can also influence T-cell epitope selection. Based on those findings and because previous studies of T-cell reactivity did not evaluate intact full-length MOG, we questioned whether undiscovered pathogenic T-cell epitopes of processed native MOG may exist.

In 2011, we reported on our discovery of 3 novel MOG T-cell determinants in C57BL/6 mice: an encephalitogenic epitope, MOG p119-132, located within the transmembrane domain, and 2 determinants, p181-195 and p186-200, which reside within the cytoplasmic domain.[13] In an accompanying report, we have examined T-cell responses to the corresponding MOG determinants in patients with MS and healthy controls.[14] In this study, we define the phenotypic and pathologic characteristics of the T cells that recognize those epitopes in mice. We have examined T-cell reactivity to individual peptides from a library of overlapping 15-mers and 20-mers spanning the aa sequence of full-length MOG, as well as to native MOG. MOG p119-132 induced potent clinical and histologic EAE. Upon recall to immunization with full-length MOG, a higher frequency of T cells responded to p119-132 than to p35-55, suggesting that p119-132 is an immunodominant encephalitogenic MOG determinant. Of interest, although immunization with MOG p181-195 and p186-200 induced robust T-cell proliferative responses, neither of these peptides induced clinical or histologic EAE. T cells specific for MOG p186-200 were incapable of transferring clinical or histologic EAE to wild-type (WT) mice and rarely caused histologic disease in recipient RAG1-deficient ($RAG1^{-/-}$) mice, indicating that this T-cell epitope is only weakly encephalitogenic. Furthermore, MOG p181-195–specific T cells were incapable of inducing clinical or histologic EAE in either WT or $RAG1^{-/-}$ mice. Thus, not all T-cell epitopes of myelin (self) antigens are pathogenic.

METHODS Mice. Female 5–8-week-old C57BL/6, B10, 129Sv/J, B10.A, B10.PL, PL/J, SJL/J, BALB/c, $(PL/J \times SJL/J)F_1$, C57BL/6 OVA p257-264–specific T-cell receptor (TCR) transgenic (OT-1) and $RAG1^{-/-}$ mice were purchased from the Jackson Laboratories (Bar Harbor, ME); NOD/MrkTac were purchased from Taconic (Oxnard, CA). BiozziABH/RijHSd mice were purchased from Harlan Laboratories (Blackthorn, United Kingdom). C57BL/6 B $cell^{-/-}$ JHT mice (B6.129P2-Igh-Jtm1Cgn/J) were provided by Mark Shlomchik.[15] C57BL/6 $MOG^{-/-}$ mice were provided by Hugh Reid.[16]

Peptides. Overlapping synthetic MOG peptides spanning the entire 218 aa sequence of mouse MOG and associated truncated peptides were synthesized by Genemed Synthesis (San Antonio, TX). Mouse peptides MOG p35-55 (MEVGWYRSPFSRVVHLYRNGK), MBP peptide Ac1–11 (Ac-ASQKRPSQRHG), PLP p139-151 (HCLGKWLGHPDKF), PLP p180-199 (WTTCQSIAFPSKTSAS IGSL), and OVA p257-264 (SIINFEKL) were purchased from AnaSpec (Fremont, CA). Mouse MOG p119-132 (FYWVNPGVLTLIAL), p119-130 (FYWVNPGVLTLI), p181-195 (TLFVIVPVLGPLVAL), and p186-200 (VPVLGPLV ALIICYN) were synthesized by Genemed Synthesis. Major peaks, analyzed by matrix-assisted laser desorption/ionization time of flight mass spectrometry and high-performance liquid chromatography, contained greater than 95% of the desired product.

Frequency analysis. Mice were immunized with either full-length or recombinant mouse MOG. After 12 days, lymph node cells were isolated and cultured for 12–14 days with the respective antigen used for immunization. Quantification was done upon restimulation using the Mouse IFN-γ ELISPOT Ready-SET-Go! kit (eBioscience, San Diego, CA) according to instructions provided by the manufacturer. Ninety-six-well polyvinylidene fluoride membrane ELISPOT plates (Millipore, Billerica, MA) were coated overnight with interferon (IFN)-γ–specific capture antibodies. After blocking with complete medium, plates were washed and lymphocytes were plated at 1×10^4 cells/well with 2.5×10^5 irradiated splenocytes alone or with various antigens (1–50 μg/mL) and cultured for 24 hours in a 37°C, 5% CO_2 humidified incubator. Plates were washed and incubated overnight with the biotinylated anti-IFN-γ detection antibodies. This plate-bound secondary antibody was visualized by adding avidin-horseradish peroxidase and 3-amino-9-ethylcarbazole substrate (BD Biosciences, San Jose, CA). Image analysis of ELISPOT assays was performed on a Series 2 ImmunoSpot Image Analyzer using 4.0 software (Cellular Technology Limited, Shaker Heights, OH).

Statistics. T-cell proliferation data are presented as medians and means ± SEM, respectively. For EAE clinical scores, significance between groups was examined by Mann–Whitney *U* test. All other statistical comparisons between groups were performed with GraphPad Prism software using analysis of variance where $p < 0.05$ was considered statistically significant.

Information on affinity purification of full-length MOG and rMOG 1-117, myelin isolation, lymphocyte isolation and proliferation, EAE induction and clinical evaluation, isolation of CNS-infiltrating mononuclear cells, flow cytometry analysis, cytokine ELISAs, and histopathology can be found in the e-Methods at Neurology.org/nn.

RESULTS Native MOG contains multiple T-cell determinants in C57BL/6 mice. Overlapping 15 and

20 aa peptides spanning the entire 218 aa sequence of murine MOG were tested for their capability to elicit T-cell proliferative responses in C57BL/6 mice (table 1). In addition to p35-55, p111-130, p181-195, and p186-200 stimulated recall proliferative responses (figure e-1A). Following immunization with full-length MOG, responses could be detected (stimulation index > 2.5) for each of these peptides. The MOG 111-130 sequence contains aa residues from the distal extracellular and proximal transmembrane domains.[2] Immunization with rMOG 1-117 stimulated a recall response to p35-55 but not to 111-130 (figure 1A), indicating that the core of this novel T-cell determinant is located within the transmembrane region. Further, MOG 111-130 and 35-55 sequences are not homologous, and the proliferative responses induced by immunization with either p35-55 or p111-130 were not cross-reactive (figure e-1A). Residues 181-200 correspond to a hydrophobic sequence within the cytoplasmic domain that is thought to be associated with the cell membrane.[17] Although overlapping, T cells primed to p181-195 or p186-200 responded only to the peptide used for immunization (figure e-1A). Thus, our results indicated that each of the 4 MOG peptides contained unique T-cell determinants.

Table 1 Identification of immunogenic and encephalitogenic peptides of MOG

MOG peptides		Antigen for primary immunization		
Residues	Sequence	Peptide[a]	rMOG 1-117[a]	EAE incidence
p1-20	GQFRVIGPRHPIRALVGDEA	–		0/10
p11-30	PIRALVGDEAELPCRISPGK	–		0/10
p21-40	ELPCRISPGKNATGMEVGWY	–		0/10
p31-50	NATGMEVGWYRSPFSRVVHL	+	+	15/15
p41-60	RSPFSRVVHLYRNGKDQDAE	–		0/10
p51-70	YRNGKDQDAEQAPEYRERTE	–		0/10
p61-80	QAPEYRERTELLKETISEGK	–		0/10
p71-90	LLKETISEGKVTLRIQNVRF	–		0/10
p81-100	VTLRIQNVRFSDEGGYTCFF	–		0/10
p91-110	SDEGGYTCFFRDHSYQEEAA	–		0/10
p101-120	RDHSYQEEAAMELKVEDPFY	–		0/10
p111-130	MELKVEDPFYWVNPGVLTLI	+	–	15/15
p121-140	WVNPGVLTLIALVPTILLQV	–		0/10
p131-150	ALVPTILLQVPVGLVFLFLQ	–		0/10
p141-160	VGLVFLFLQHRLRGKLRAE	–		0/10
p151-170	HRLRGKLRAEVENLHRTFDP	–		0/10
p161-180	VENLHRTFDPHFLRVPCWKI	–		0/10
p171-190	HFLRVPCWKITLFVIVPVLG	–		0/10
p181-195	TLFVIVPVLGPLVAL	+	–	0/30
p186-200	VPVLGPLVALIICYN	+	–	0/30
p191-210	PLVALIICYNWLHRRLAGQF	–		0/10
p201-218	WLHRRLAGQFLEELRNPF	–		0/10

Abbreviations: EAE = experimental autoimmune encephalomyelitis; MOG = myelin oligodendrocyte glycoprotein.
[a] A stimulation index >2.5 was considered positive. Each proliferation assay was performed on 4 mice. Results are representative of 3 independent experiments.

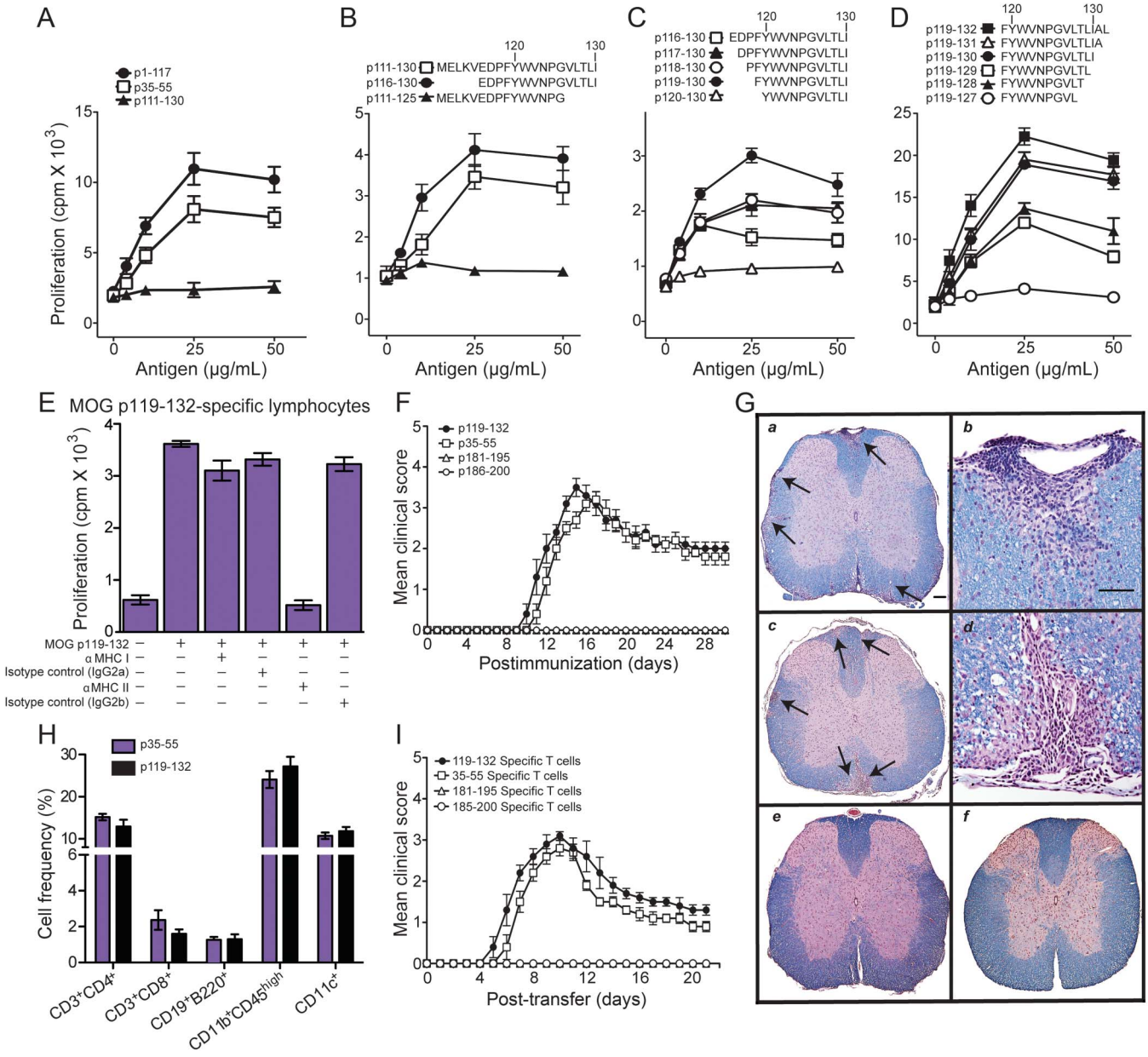
Overlapping synthetic peptides corresponding to the sequence of full-length MOG were tested for EAE induction in C57BL/6 mice (table 1). MOG p111-130 induced EAE in a manner similar to p35-55. In contrast, although MOG p181-195 and p186-200 had induced T-cell proliferative responses, neither clinical (table 1) nor histologic (table e-1) signs of EAE were observed after immunization with any peptide doses tested (data not shown).

Identification of the encephalitogenic MOG T-cell determinant, p119-132. In general, T cells recognize linear peptide determinants containing 9–14 aa.[18,19] Thus, we characterized the encephalitogenic region further by testing shorter overlapping peptides. MOG p116-130 elicited proliferation after immunization with MOG p111-130 (figure 1B) and was encephalitogenic (table 2), whereas MOG p111-125 did not stimulate proliferation or cause EAE.

The minimal encephalitogenic T-cell determinant within MOG p116-130 was identified using nested peptides truncated by individual residues from its N-terminus or C-terminus. While MOG p119-130 stimulated proliferation (figure 1C) and caused EAE (table 2), p120-130 did not stimulate proliferation or cause EAE, suggesting that the Phe at residue 119 is critical for recognition of this determinant. As the inability to stimulate proliferation with p120-130 could simply have reflected a requirement for length of this determinant, we immunized mice with MOG p120-131 and p120-132. These 2 peptides did not stimulate proliferation (figure e-1A) or cause EAE (table 2), confirming the importance of hydrophobic residue, Phe, at position 119 of this T-cell epitope. Peptide 119-128, which was the shortest peptide to stimulate proliferation (figure 1D), was also the smallest one to cause EAE (table 2). Peptide 119-130 induced clinical and histologic EAE as potently as p35-55. The addition of residues 131 and 132 to this sequence created peptides that had slightly greater stimulatory capacity. Increasing length at the C-terminus beyond residue 132 (e.g., p119-133) did not increase the stimulatory capacity (figure e-1A) or encephalitogenic potential (table 2). Collectively, these results indicated that p119-132 contains the complete encephalitogenic T-cell epitope of this region.

MOG p119-132–specific T cells are $CD4^+$ and MHC II ($I\text{-}A^b$)–restricted, produce proinflammatory T cells, and transfer EAE to naive recipient mice. In most EAE models, encephalitogenic myelin autoantigen-specific T cells are $CD4^+$ and MHC II–restricted.[20] In this

Figure 1 **Identification of the encephalitogenic MOG determinant, p119-132**



(A) Immunization with rMOG 1-117 elicited a recall proliferative response to p35-55 but not to p111-130. (B) Proliferation was detected to MOG p116-130 but not to p111-125 in mice immunized with p111-130. (C) Testing proliferative responses to truncated peptides after immunization with MOG p116-130 identified the core N-terminal boundary, F^{119}. (D) Recall proliferative responses to MOG p119-132 and truncated peptides after immunization with MOG p119-132 identified the core C-terminal boundary, T^{128}. The proliferative response was maximal for p119-132. Lymph node cells were harvested 12 days after immunization. Results shown in panels A–D are representative of 3 separate experiments with 4 mice/group. (E) Mice were primed with MOG p119-132. Lymph node cells were isolated on day 10 and restimulated with MOG p119-132 in the presence of anti-MHC class II (M5/114), anti-MHC class I (28-14-8), or isotype control antibodies. Proliferation was evaluated after 72 hours by thymidine incorporation. (F, G) Mice were immunized with MOG p35-55, MOG p119-132, p181-195, and p186-200 for experimental autoimmune encephalomyelitis (EAE) induction. (F) EAE clinical course was similar after immunization with p35-55 and p119-132, but no signs of disease were observed with MOG p181-195 and p186-200. Data are representative of 5 separate experiments (5 mice/group) and represent mean clinical scores ± SEM. (G) Histologic analysis was performed on mice 14 days after immunization. Mice immunized with p35-55 (a, b) and p119-132 (c, d) developed EAE lesions in spinal cord white matter (arrows in a and c). (b, d) Meningeal and parenchymal mononuclear cell inflammation and demyelination were observed at higher magnifications. No evidence of histologic disease was observed in spinal cords of mice immunized with MOG p181-195 (e) or p186-200 (f). Luxol fast blue-hematoxylin & eosin; scale bars 100 μm (a, c, e, and f), 50 μm (b and d). (H) Mice were immunized with p35-55 or p119-132 and CNS-infiltrating cells were isolated 4 days after disease onset. Cells were stained with markers specific for $CD4^+$ T cells, $CD8^+$ T cells, B cells ($CD19^+B220^+$), monocytes ($CD11b^+$, $CD45^{high}$), and dendritic cells ($CD11c^+$). (I) Adoptive transfer EAE was induced by MOG p119-132–specific and p35-55–specific $CD4^+$ T cells but not by p181-195–specific and p186-200–specific T cells. MOG epitope-specific $CD4^+$ T cells were isolated from mice primed with individual MOG peptides and adoptively transferred into naïve recipient mice by intraperitoneal injection. Data shown represent mean clinical scores ± SEM of 5 recipient mice/group. EAE incidence was 100% in recipients of MOG p119-132–specific or p35-55–specific T cells. No clinical EAE was detected in recipient mice that received donor MOG p181-195–specific or p186-200–specific T cells. Results are representative of 3 independent experiments. MHC = major histocompatibility complex; MOG = myelin oligodendrocyte glycoprotein.

Table 2 Identification of the minimal encephalitogenic sequence, p119-128, within the transmembrane region of myelin oligodendrocyte glycoprotein (MOG)

MOG peptide		Disease incidence	Mean day of disease onset[a]	Mean maximal severity[a]
Residue	Sequence			
p35-55	EVGWYRSPFSRVVHLY	15/15	11.2 ± 0.3	3.5 ± 0.4
p111-130	MELKVEDPFYWVNPGVLTLI	10/10	12.7 ± 0.4	2.9 ± 0.4
p111-125	MELKVEDPFYWVNPG	0/10	—	—
p116-130	EDPFYWVNPGVLTLI	10/10	12.5 ± 0.5	3.5 ± 0.3
p117-130	DPFYWVNPGVLTLI	10/10	12.5 ± 0.2	3.5 ± 0.1
p118-130	PFYWVNPGVLTLI	10/10	11.7 ± 0.4	3.2 ± 0.2
p119-130	FYWVNPGVLTLI	15/15	11.2 ± 0.5	3.6 ± 0.3
p120-130	YWVNPGVLTLI	0/10	—	—
p120-131	YWVNPGVLTLIA	0/10	—	—
p120-132	YWVNPGVLTLIAL	0/10	—	—
p119-133	FYWVNPGVLTLIALV	4/5	10.3 ± 0.6	3.0 ± 0.3
p119-132	FYWVNPGVLTLIAL	15/15	10.2 ± 0.7	3.8 ± 0.4
p119-131	FYWVNPGVLTLIA	10/10	11.0 ± 0.4	3.4 ± 0.2
p119-129	FYWVNPGVLTL	6/10	12.0 ± 0.5	1.6 ± 0.3
p119-128	FYWVNPGVLT	7/10	12.7 ± 0.2	1.7 ± 0.2
p119-127	FYWVNPGVL	0/10	—	—

[a] All values are shown as mean ± SEM.

regard, T cells that proliferated to MOG p119-132 or p119-130 expressed CD4 but not CD8 molecules. A limited repertoire of TCR genes may be utilized for recognition of certain encephalitogenic myelin epitopes.[21] For example, nearly 80% of MBP Ac-11–specific T clones use the same TCR Vβ gene. Although there was heterogeneity within the population of MOG p119-132–specific T cells as measured by TCR Vβ usage, 40% of $CD4^+$ T cells for this determinant utilized Vβ8.3, a Vβ that was rarely used by p35-55–specific $CD4^+$ T cells (table e-2). Proliferation to MOG p119-132 was inhibited by anti–MHC II but not anti–MHC I antibodies (figure 1E), indicating that T-cell recognition of MOG p119-132 is restricted by $I\text{-}A^b$ MHC II molecules. Besides inducing encephalitogenic responses in C57BL/6 mice, MOG peptides p119-130 and p119-132 also caused EAE in C57BL/10 and 129/Sv, 2 other $H\text{-}2^b$ ($I\text{-}A^b$) mouse strains (table 3). However, MOG p119-132, like p35-55, did not induce EAE in C57BL/6 $MOG^{-/-}$ mice,[22] indicating that p119-132 did not elicit encephalitogenic responses through cross-reactivity with another CNS autoantigen (table e-3). In contrast to recombinant human MOG, which is a T-cell and B-cell dependent autoantigen and does not cause EAE in B-cell deficient (JHT) mice,[15] p119-132 caused clinical and histologic EAE in JHT mice (data not shown). MOG p119-132 did not induce clinical or histologic EAE in NOD ($H\text{-}2^{g7}$), Biozzi ($H\text{-}2^{dq1}$), or PL/J ($H\text{-}2^u$) mouse strains that are susceptible to EAE induced by MOG p35-55,[2,23] or in strains of other MHC (*H-2*) haplotype genes that are susceptible to EAE induced by different myelin antigens.[20]

The clinical course of EAE induced by either p119-132 or p35-55 in C57BL/6 mice was similar at all equivalent peptide doses tested (figure 1F, table e-4). Like MOG p35-55, p119-132 induced parenchymal and meningeal inflammation as well as demyelination. Optic neuritis was also observed. Composition and distribution of the CNS inflammatory lesions, as well as the frequencies of CNS-infiltrating $CD4^+$ and $CD8^+$ T cells, B cells, monocytes, and dendritic cells, were similar in EAE induced by p119-132 and p35-55 (figure 1, G and H).

Encephalitogenic myelin-specific T cells produce proinflammatory cytokines.[3] Therefore, we examined various cytokines produced by T cells following immunization with MOG p119-132 and during acute EAE. T cells primed to p119-132 in complete Freund's adjuvant produced IFN-γ and interleukin (IL)-17 in a manner that was similar to mice immunized with p35-55 (figure e-1B). Percentages of Th1 and Th17 cells in the periphery and in CNS-infiltrating cells were similar when EAE was induced by MOG p119-132 or p35-55 (figure 2A, figure e-1B). When used as donor cells for adoptive transfer, proinflammatory MOG p119-132–specific T cells induced EAE in naive recipient C57BL/6 mice,

Table 3 MOG p119-132 induces EAE in H-2^b mouse strains

Strain	MHC II haplotype	Antigenic peptide	Incidence	Mean day of onset[a]	Mean maximal severity[a]	Histologic disease[b]	Recall proliferative response[c]
C57BL/6	H-2^b	MOG p119-132	10/10	11.5 ± 0.3	2.9 ± 0.3	+	+
		MOG p35-55	5/5	12.0 ± 0.5	2.6 ± 0.4	+	+
Sv129	H-2^b	MOG p119-132	10/10	10.5 ± 0.3	3.0 ± 0.2	+	+
		MOG p35-55	5/5	11.5 ± 0.5	2.8 ± 0.2	+	+
B10	H-2^b	MOG p119-132	10/10	10.0 ± 0.5	3.2 ± 0.3	+	+
		MOG p35-55	5/5	11.0 ± 0.4	2.9 ± 0.2	+	+
BALB/c	H-2^d	MOG p119-132	0/5	—	—	–	–
		PLP p139-151	3/5	17.0 ± 0.7	2.2 ± 0.5	+	+
B10.A	H-2^k	MOG p119-132	0/10	—	—	–	–
		MBP Ac1-11	5/5	21.0 ± 0.5	2.7 ± 0.4	+	+
A/J	H-2^k	MOG p119-132	0/10	—	—	–	–
		MBP Ac1-11	3/5	20.3 ± 0.5	2.2 ± 0.2	+	+
B10.PL	H-2^u	MOG p119-132	0/5	—	—	–	–
		MBP Ac1-11	3/5	15.0 ± 0.6	2.0 ± 0.3	+	+
PL/J	H-2^u	MOG p119-132	0/10	—	—	–	–
		MBP Ac1-11	4/5	18.0 ± 0.4	2.3 ± 0.3	+	+
SJL/J	H-2^s	MOG p119-132	0/10	—	—	–	–
		PLP p139-151	5/5	16.0 ± 0.5	2.6 ± 0.2	+	+
(PL/J × SJL/J)F$_1$	H-2$^{u/s}$	MOG p119-132	0/5	—	—	–	–
		MBP Ac1-11	5/5	13.2 ± 0.2	3.2 ± 0.1	+	+
NOD	H-2^{g7}	MOG p119-132	0/10	—	—	–	–
		MOG p35-55	5/5	14.4 ± 0.4	2.0 ± 0.2	+	+
Biozzi	H-2^{dq1}	MOG p119-132	0/10	—	—	–	–
		MOG p35-55	7/7	20.6 ± 0.9	3.6 ± 0.5	+	+

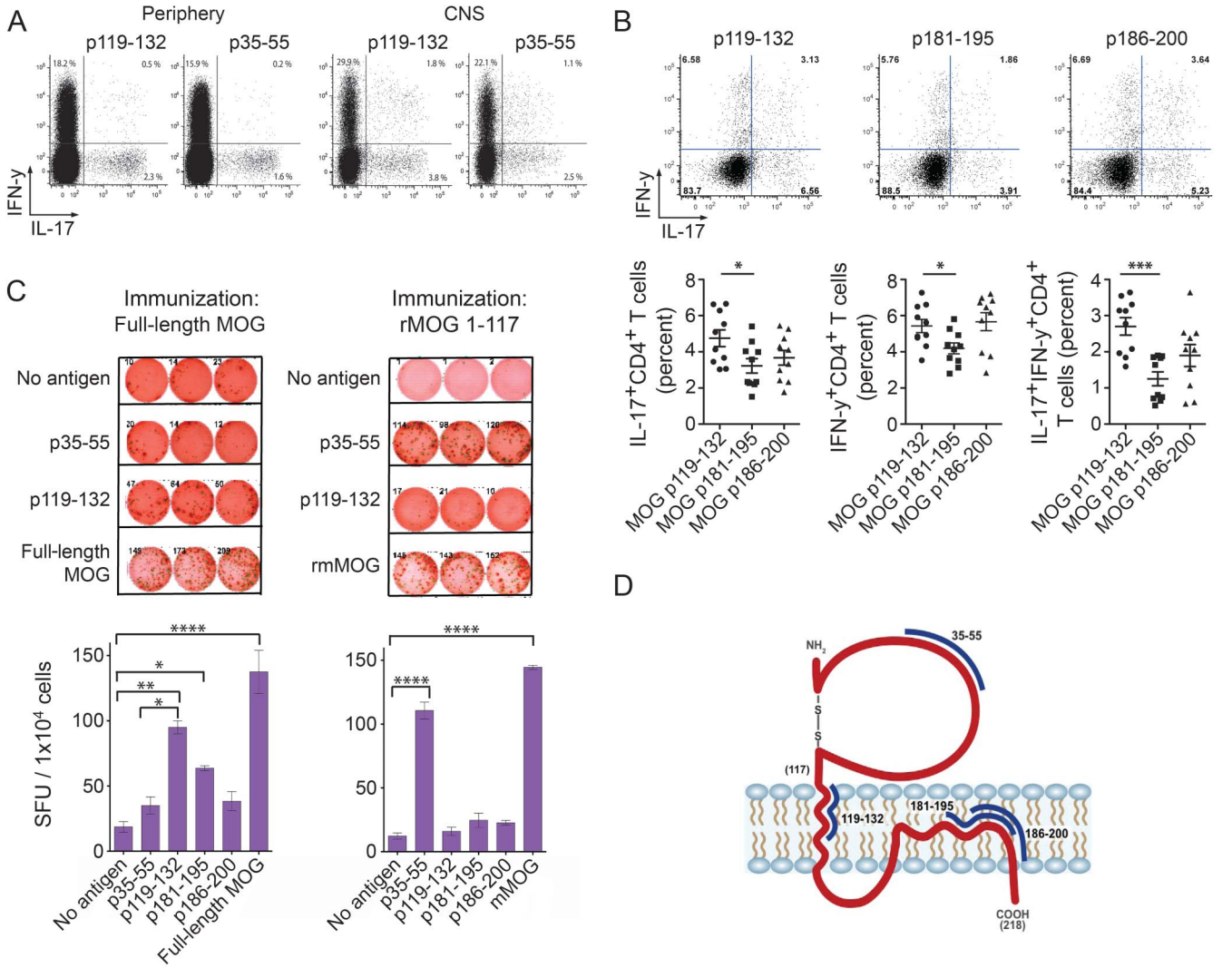
Abbreviations: EAE = experimental autoimmune encephalomyelitis; MBP = myelin basic protein; MHC = major histocompatibility complex; MOG = myelin oligodendrocyte glycoprotein; PLP = proteolipid protein.
[a] EAE results are shown as mean ± SEM.
[b] Three mice per group were examined for CNS inflammation and demyelination.
[c] Recall proliferative responses with a stimulation index >2.5 were considered positive. Each proliferation assay was set up on 5 mice and is representative of 2 independent experiments.

demonstrating the pathogenicity of those T cells (figure 1I). Incidence, onset, and severity of acute and chronic EAE were similar when induced by MOG p119-132–specific T cells or p35-55–specific T cells.

Of interest, donor T cells specific for p181-195 or p186-200 that produced proinflammatory cytokines did not induce clinical (figure 1I) or histologic (table 4) EAE in recipient WT mice. RAG1$^{-/-}$ mice, which do not contain either mature T or B cells, are more susceptible to EAE induction by donor encephalitogenic T cells than WT recipient mice.[24,25] When using this sensitive EAE measure, MOG p186-200–specific T cells rarely caused clinical (1/20 mice tested) or histologic (2/20 mice tested) EAE. Donor T cells specific for p181-195 did not induce clinical or histologic signs of EAE (table 4), confirming that p181-195 is indeed a nonencephalitogenic MOG T-cell determinant. Notably, frequencies of IL-17$^+$, IFN-γ$^+$, and IL-17$^+$IFN-γ$^+$ T cells were statistically lower after immunization with the nonencephalitogenic T-cell determinant MOG p181-195 (figure 2B).

p119-132 is an immunodominant T-cell epitope of native MOG. In general, multideterminant protein antigens, like native MOG, are processed by APC.[6,15] By definition, immunodominant T-cell epitopes are those that are recognized more frequently among all T cells responding to the naturally processed protein.[26] Even though MOG p119-132 caused EAE as severe as MOG p35-55, it was possible that p119-132 represented a subdominant or cryptic determinant. Thus, we immunized mice with native MOG and examined recall to itself, p35-55, p119-132, p181-195,

Figure 2 MOG p119-132 is the immunodominant T-cell determinant of full-length mouse MOG and elicits a proinflammatory bias



(A, B) Mice were immunized with myelin oligodendrocyte glycoprotein (MOG) p35-55, p119-132, p181-195, and p186-200. (A) Fourteen days after immunization with p35-55 and p119-132, spleen cells and CNS-infiltrating cells were isolated and analyzed for interleukin (IL)-17 and interferon (IFN)-γ production by intracellular staining after stimulation with phorbol 12-myristate 13-acetate (PMA)/ionomycin for 5 hours. Results are representative of 2 experiments that tested 4 individual mice for each peptide immunization. (B) Ten days after immunization with p119-132, p181-195, and p185-200, spleen cells were isolated and analyzed for IL-17 and IFN-γ production by intracellular staining after stimulation with PMA/ionomycin for 5 hours. Data summarize 2 experiments that tested 5 individual mice for each peptide immunization. (C) Frequencies of MOG peptide-specific IFN-γ–secreting cells were determined by ELISPOT as described in the methods. Lymph node cells from mice immunized with full-length MOG (left panel) or the extracellular domain of MOG (rMOG 1-117) (right panel) were cultured with the respective antigen used for immunization. Quantification of MOG peptide-specific T cells was performed at the time of restimulation with MOG peptides (25 μg/mL). Results are representative of 3 separate experiments (3 mice/group). (D) Schematic anatomic localization of T-cell epitopes within full-length (218 amino acid) mouse MOG (indicated in red).[41] Amino acid 117, the last residue of extracellular domain, is indicated. T-cell determinants are highlighted in blue.

and p186-200. ELISPOT analysis of IFN-γ–secreting cells, a highly sensitive measure to examine the repertoire of antigen-specific T cells,[27,28] was used to quantitate the frequency of MOG-specific T cells. Of responses to the peptide determinants, the frequency of IFN-γ–secreting T cells was highest for MOG p119-132 (figure 2C) and was significantly greater than for MOG p35-55. These results indicated that p35-55 is not the immunodominant T-cell epitope of MOG. In parallel, we examined the response to these peptides after immunization with the extracellular MOG domain. Immunization with MOG 1-117 elicited a robust response to MOG p35-55 (figure 2C, left panel) but not to the novel determinants located in the transmembrane and cytoplasmic domains (figure 2C, right panel). Similar results were obtained when testing a different concentration of MOG peptides in recall to immunization with full-length MOG or rMOG (figure e-1C).

DISCUSSION MOG, a minor component of myelin protein, was first recognized as a CNS autoantigen in

Table 4 Development of experimental autoimmune encephalomyelitis (EAE) in recipient mice after adoptive transfer of myelin oligodendrocyte glycoprotein (MOG) peptide-specific T cells

	Clinical EAE			No. of inflammatory foci		
MOG peptide	Incidence, %	Mean day of onset[a]	Mean score at peak of disease[a]	Meninges[a]	Parenchyma[a]	Total
Wild-type						
119-132	100 (15/15)	6.5 ± 0.7	3.2 ± 0.2	113 ± 7.7	138 ± 9.9	244.3 ± 13.2
35-55	100 (15/15)	7.4 ± 0.5	2.8 ± 0.5	100.3 ± 17.0	85 ± 7.0	185.3 ± 22.3
181-195	0 (0/15)	0	0	0	0	0
186-200	0 (0/15)	0	0	0	0	0
RAG1$^{-/-}$						
119-132	100 (14/14)	18.5 ± 0.9	2.75 ± 0.3	121 ± 19.3	132 ± 22.8	255.3 ± 39.2
35-55	100 (5/5)	16.8 ± 1.2	2.4 ± 0.4	83.2 ± 17.5	120.2 ± 22.7	203.4 ± 37.8
181-195	0 (0/15)	0	0	0	0	0
186-200	5 (1/20)	19[b]	3[b]	36.5 ± 2.84[c]	41 ± 1.89[c]	77.5 ± 4.74[c]

Results are representative of 3 independent experiments (4 mice/group/experiment).
[a] Results represent mean ± SEM. Donor mice were primed by being injected subcutaneously with 50 μg/mouse of relevant MOG peptide.
[b] Results represent actual values (i.e., not mean).
[c] Results represent mean ± SEM of the 2 mice with inflammatory loci (i.e., 18/20 mice examined did not have detectable CNS inflammatory loci).

1993.[7] Since then, most studies evaluating immune responses to MOG have focused on its extracellular Ig-like domain.[11,29–31] In C57BL/6 mice, the encephalitogenic T-cell determinant of the extracellular MOG domain, rMOG, was mapped to residues 35-55.[32] Subsequently, p35-55 has emerged as a widely used encephalitogenic myelin antigen for EAE investigations.[33,34] Although separate encephalitogenic determinants of rMOG have been identified in other mouse strains with different *H-2* haplotypes,[12,29] in C57BL/6 mice (H-2^b) p35-55 is the widely recognized encephalitogenic T-cell epitope of MOG.[32] In contrast, MBP and PLP, 2 other CNS autoantigens, contain multiple pathogenic T-cell epitopes in H-2^u as well as H-2^s strains.[35,36] Because the extracellular domain represents only 54% of the MOG aa sequence and earlier studies suggested intact MOG protein might contain additional T-cell determinants, we investigated this possibility. We discovered 3 discrete T-cell determinants, one located within the transmembrane domain and 2 within the cytoplasmic domain (figure 2D).[13] A more recent report also described T-cell determinants within these 2 regions in C57BL/6 mice.[8] Here, we demonstrate the transmembrane MOG determinant, p119-132, caused EAE as potently as MOG 35-55 in 3 H-2^b strains. By examining individual responses to MOG p35-55, p119-132, and the 2 epitopes within the cytoplasmic domain following immunization with intact full-length MOG, we were capable of evaluating the physiologic T-cell repertoire to MOG determinants generated in vivo. To our surprise, a higher frequency of MOG protein-specific T cells recognized p119-132 than p35-55. Thus, p35-55 is not the immunodominant encephalitogenic T-cell determinant of native MOG. Whether the immunodominance of p119-132 reflects greater affinity for I-A^b than other MOG determinants or is due to distinct processing requirements is not clear.[6] Recognizing that MOG contains multiple pathogenic T-cell epitopes in this widely studied model and that p119-132 is one of the immunodominant T-cell epitopes will permit evaluation of potential changes in the MOG-specific T-cell repertoire during EAE pathogenesis. Of interest, it is recognized that the pathogenic humoral response targets the extracellular Ig MOG domain, which contains the aa sequence 35–55 recognized by pathogenic T cells. Identification of the encephalitogenic MOG transmembrane T-cell epitope, p119-132, should permit examination of whether T cells targeting this distinct anatomic domain participate in T-B collaboration and the MOG-specific humoral response in a different manner than when both T and B cells target the same region.

Many mouse strains, representing different haplotypes, are susceptible to EAE induced by various myelin proteins.[20] MOG p35-55 induces EAE in H-2^b strains as well as in mice that express H-2^u (e.g., PL/J), H-2^{g7} (e.g., NOD), and H-2^{dq1} (e.g., Biozzi) haplotypes.[2,23] It is interesting that although we evaluated EAE induction by MOG p119-132 in all of those strains, T-cell proliferative responses, CNS inflammation, and clinical EAE were induced only in H-2^b strains. These observations suggest that residues in MOG p119-132 and p35-55 utilize distinct agretopes and do not contact the MHC II (I-A^b) peptide-binding

groove in precisely the same manner. The lack of immunogenicity is unlikely to be due to potential differences in TCR repertoire, as all of these particular strains contain intact TCR α and β gene repertoires. In future studies, it may be important to define the physical MHC II binding characteristics of p119-132.

In 1985 it was demonstrated for the first time that autoantigen-specific T-cell clones could mediate autoimmune disease.[37] At that time we also showed that only T-cell clones that recognized shared determinants of mouse (self) MBP, but not foreign determinants of heterologous (e.g., human or guinea pig) MBP alone, were capable of causing CNS inflammation and clinical disease in recipient mice, establishing the importance of self-nonself discrimination in CNS autoimmunity[37,38] Since that time, it was observed that like dominant T-cell determinants of CNS autoantigens,[19] peptides corresponding to subdominant or cryptic T-cell determinants of self-myelin antigens in general also cause EAE, sometimes as potently as the immunodominant epitope.[35] In contrast to T-cell recognition of autoantigens, humoral responses to self-antigens can be pathogenic or nonpathogenic.[39,40] It is interesting that here we did not observe clinical EAE or histologic evidence of CNS inflammation either by direct immunization with mouse MOG p181-195 or p186-200, or by adoptive transfer of proinflammatory-polarized T cells to these determinants, even when testing excessively large amounts of peptides or numbers of T cells. Further, T cells specific for MOG p181-195 did not induce clinical or histologic evidence of EAE even when transferred into T- and B-cell–deficient RAG1$^{-/-}$ mice, which are more susceptible to EAE than WT recipient mice. A lower frequency of proinflammatory T cells was elicited by immunization with p181-195 than p119-132, which could be one of the factors contributing to its inability to cause EAE. Although "absence of proof is not proof of absence," with these experimental paradigms, we have now identified a stimulatory T-cell epitope of a self CNS autoantigen that is not encephalitogenic. Thus, like for antibody responses, pathogenic and nonpathogenic T-cell determinants of CNS autoantigens may exist. Identification of a nonpathogenic determinant of MOG should now permit investigators to test whether therapeutics targeting a nonencephalitogenic T-cell epitope of this multideterminant autoantigen can regulate pathogenic responses. In our accompanying report, we demonstrated that T cells from patients with MS also recognized the corresponding novel determinants within transmembrane and cytoplasmic domains of human MOG.[14] Both pathogenic and nonpathogenic T-cell epitopes may also exist within CNS autoantigens in humans.

AUTHOR CONTRIBUTIONS

A.S., S.G.G., M.V.-D., and S.S.Z. designed the study, analyzed data, and wrote the paper. A.S., S.G.G., M.V.-D., M.S.W., T.P., N.M., and U.S.-T. performed the experiments. J.C.P. and N.J. assisted in the experiments. P.A.N., S.E.F., and A.J.S. gave conceptual advice and discussed the results. R.A.S. performed the histologic analysis. T.F. and C.C.A.B. contributed new reagents. S.S.Z. supervised the study.

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