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Research Article

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Murine Vλx and Vλx-containing Antibodies Bind Human Myelin Basic Protein

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Abstract

Myelin basic protein (MBP) is highly immunogenic and a known autoantigen capable of inducing experimental allergic encephalomyelitis (EAE), the animal model of multiple sclerosis. We have previously described a murine monoclonal antibody (mAb), F28C4, directed against the encephalitogenic MBP peptide acetyl (Ac) 1-9, which contains a Vλx light chain. Considering the rarity of Vλx usage, we determined whether other Abs having Vλx light chains shared similar antigen (Ag) specificity. We screened a panel of Vλx-containing monoclonal and polyclonal Abs, of unknown specificity for reactivity with MBP. All such Ab, but not heavy chain isotype matched controls, bound MBP but were not polyreactive with other potential self Ags. The binding of a recombinant form of Vλx alone to MBP demonstrated the important contribution of the Vλx light chain to the reaction. With the exception of mAb F28C4 which recognizes MBP Ac1-9, the epitope specificity of all other Vλx-bearing Abs was localized to MBP residues 25–34. These results demonstrate a unique association between Vλx expression and MBP reactivity. Given that Vλx shares sequence homology with T cell receptors (TCR) from encephalitogenic T lymphocytes, these results imply a potential role for Vλx in the pathogenesis of EAE. (*J. Clin. Invest.* 1996. 97:486–492.) Key words: experimental allergic encephalomyelitis • light chain • multiple sclerosis • B cells • autoimmune disease

Introduction

Experimental allergic encephalomyelitis (EAE)[1] is a $CD4^+$ T cell–mediated inflammatory demyelinating disease of the central nervous system (CNS) that can be induced by immunization with CNS components. EAE is considered to be a prime animal model for the human autoimmune disorder multiple sclerosis (MS) (for review see reference 1). Myelin basic protein (MBP), a molecule of 170 residues which comprises 30% of CNS myelin proteins, is encephalitogenic in actively or passively inducing EAE in a variety of animals. Although T cells clearly play a central role in the pathogenesis of EAE, B cells may be important in modulation of the disease. In both EAE and MS, B cells infiltrate the CNS, transform into plasma cells, and can locally synthesize Ig some of which is anti-MBP (2, 3). Antibodies (Ab) appear after active immunization with CNS antigens (Ag) but titers do not correlate with the presence of EAE. In Lewis rats, Ab is initially detected during the onset of EAE and is maximal after recovery (4). Subsequent to recovery from EAE, Lewis rats are resistant to reinduction of active disease. $CD4^+$ T cells isolated during the recovery phase can transfer resistance only if cotransferred with MBP-primed B cells (5). Such B cell modulation of resistance is paralleled by the finding that sera from rats recovered from EAE passively protect against active EAE (6). Collectively, these data suggest that humoral responses may play an important regulatory role in the pathogenesis of EAE.

Multiple immunogenic and encephalitogenic epitopes reside in MBP (7). For PL/J mice, the $I\text{-}A^u$ restricted, dominant encephalitogenic epitope is MBP peptide acetyl (Ac) 1-9 (8). In PL/J mice and Lewis rats the T cell receptor (TCR) repertoire of the encephalitogenic T cell response to different MBP epitopes is similarly restricted by TCR Vα2.3β8.2 (9). A mAb, F28C4, raised in the PL/J mouse to MBP Ac1-9 shares a cross-reactive idiotope (CRI) with the PL/J $V\beta8.2^+$ TCR recognizing the same MBP peptide (10). This CRI has been defined using the anti-Id mAb, F30C7, which can block both peptide recognition by F28C4 (11) and peptide stimulation of MBP Ac1-9-specific T cells (10). These and other findings suggest that the CRI resides at or near the combining site of F28C4 and the TCR and is not a public Id. A role for this CRI and the related Id network in EAE is suggested by the ability of mAb F30C7 anti-Id to lessen clinical EAE in the adoptive transfer model of EAE in PL/J mice (10), possibly by T cell anergy (12).

The structure and fine specificity of F28C4 appear to be crucial for its paratope and Id characteristics. Similar to the reaction of the encephalitogenic T cell clone, PJR-25, optimal binding of mAb F28C4 requires an extended conformation of the MBP peptide Ac 1-9 so that the central residues are more tightly bound than the terminal residues (13). Furthermore, there is a sequence homology of 75% found between complementarity determining region (CDR) 3 of F28C4 VL and VH and the V-D-J junction of the TCR Vβ8.2 from encephalitogenic T cells. This homology is not shared by other Ig CDR3 regions and arises, in part, because F28C4 uses an unusual L chain V region known as Vλx (14, 15). Before the demonstration that an Ab with Vλx could bind MBP, no Ab with such an L chain had any known specificity. Vλx is an unusual V

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1. *Abbreviations used in this paper:* Ab, antibody; Ac, acetyl; Ag, antigen; CDR, complementarity determining region; CNS, central nervous system; CRI, cross reactive idiotope; EAE, experimental allergic encephalomyelitis; MBP, myelin basic protein; MS, multiple sclerosis; TCR, T cell receptor.

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gene segment that was discovered in 1987 in polyclonally activated B cells (14, 15). Vλx rearranges with Jλ2-Cλ2 and displays at least three unique features: First, its amino acid sequence is only 30–33% homologous to any other known Vλ and/or V-kappa (κ) genes. Second, the last codon of the Vλx gene is a TAA termination codon that must be disrupted by Jλ2 to create a functional codon. Third, the modified joining at the V-J junction results in a CDR3 extended by an additional four amino acids. Homologous sequences have been found in various mammalian species, including man, suggesting that Vλx existed before the speciation of mammals (16). Considering the rarity of Vλx usage, $< 0.5\%$ of all Ig in normal mice sera, it is tempting to speculate that the expression of Vλx in response to an encephalitogenic immunogen was not a coincidental event but represents an important structural component involved in the immunopathogenesis of CNS myelin damage.

We now report on the inherent affinity of Vλx-bearing Abs and Vλx alone for MBP with a preference for its amino-terminal end. The recognition step between Vλx and an encephalitogenic epitope of MBP may reflect the structural features of an Id network that affects both T and B cells in autoimmune demyelination.

Methods

Antigens. Human MBP and its citrullinated isoform, termed C8, were prepared by previously described techniques (17). Briefly, human MBP (1-170) was isolated from human brain by delipidation, acid extraction at pH 3, and carboxymethylcellulose chromatography at pH 10.6. MBP-C8 was purified from the fraction of delipidated brain extract which had not been retained on carboxymethylcellulose at pH 9.6 or pH 10.6. The human MBP peptide acetyl Ac1-9 was synthesized in our laboratory on a Biosearch Peptide Synthesizer (Model 9500; Cambridge, MA) and purified by reverse-phase high-performance liquid chromatography. Human MBP peptides 10–19, 14–24, 25–34, and 80–89 were synthesized by Peninsula Laboratories (San Carlos, CA). Sequences of MBP peptides are shown in Table I.

mAbs. The preparation and characterization of all mAbs used in this study have been previously described (for reference see Table I).

Table I. MBP Peptides and Vλx-containing mAbs

mAb	Isotype	References
F28C4	IgG2a/λ	11
R5B1	IgM/λ	14
L3G3	IgM/λ	14
173E5	IgM/λ	14
IE28	IgM/λ	15, 16
1964	IgM/λ	19

MBP peptide acetyl 1-9
Ac-Ala-Ser-Gln-Lys-Arg-Pro-Ser-Gln-Arg

MBP peptide 10-19
His-Gly-Ser-Lys-Tyr-Leu-Ala-Thr-Ala-Ser

MBP peptide 14-24
Tyr-Leu-Ala-Thr-Ala-Ser-Thr-Asp-His-Ala

MBP peptide 25-34
Arg-His-Gly-Phe-Lys-Pro-Arg-His-Arg-Asp

MBP peptide 80-89
Thr-Gln-Asp-Glu-Asn-Pro-Val-Val-His-Phe

Amino acid sequences of human MBP peptides and characteristics of Vλx-containing mAbs.

The Vλx-containing mAbs R5B1, L3G3, and 173E5 were kindly provided by K. Rajewsky and the Vλx-bearing mAbs IE28 and 1964 were gifts from P. Sanchez and J. Erikson, respectively. The IgM/λ2 (non-Vλx) mAbs A10, A34, LC51, A3L, and B11 were kindly provided by J. Kearney.

Polyclonal monospecific Ab to Vλx. A New Zealand white rabbit (Myrtle Laboratories, Thompson Station, TN) was injected subcutaneously (four sites, every 2 wk over a 6-wk period) with purified F28C4 (50 μg/injection), first in Freund's complete adjuvant and twice in incomplete adjuvant. Serum was obtained before the first injection and 2 wk after each booster to monitor anti-F28C4 titers by ELISA. F28C4 is of the γ2a isotype and, as with all Vλx-bearing Abs, is a λ2 isotype. In order to make the antiserum light chain (i.e., Vλx) specific, it was exhaustively absorbed on a polyclonal IgG2a Sepharose 4B affinity column to deplete heavy chain reactivity. Next, the Jλ2Cλ2 reactivity was removed using a MOPC 315 (IgA, λ2) Sepharose 4B affinity column. Monospecific Ab to Vλx was collected after binding to and elution from an F28C4 affinity column. The specificity of the resulting anti-Vλx Ab was determined by Western analysis (see Results).

Isolation of Vλx-bearing Ig from normal BALB/c mouse serum. Vλx-containing Ig from normal BALB/c mouse sera (Sigma Chemical Co., St. Louis, MO) was purified over a anti-Vλx Ab Sepharose 4B affinity column prepared using the monospecific rabbit anti-Vλx Ab described above. Vλx-containing Abs represented $\sim 0.5\%$ of total Ig as previously described (15).

Enzyme-linked immunosorbent assay (ELISA). Microtiter wells (96-well ELISA plates; Corning Glass Works, Corning, NY) were coated with human MBP (1–170) (10.0 μg/ml in PBS), various other self Ags (concentrations equimolar to MBP), or MBP-related peptides (10.0 μg/ml in PBS) at 4°C for 16 h and plates were blocked with 2% casein in PBS. Washings were performed with PBS containing 0.05% Tween 20. Abs were diluted in PBS containing 1.0% BSA and allowed to bind for 2 h at room temperature. Ab binding was detected with an appropriate alkaline phosphatase conjugated anti-mouse heavy chain specific Ab (Southern Biotechnology, Birmingham, AL) or with rabbit anti-Vλx Ab followed by an alkaline phosphatase conjugated anti–rabbit IgG Ab (Southern Biotechnology, Birmingham, AL). Reactions were developed using *p*-nitrophenyl phosphate (Sigma Chemical Co.) and color development was quantitated as absorbance at 405 nm on a UVmax Kinetic Microplate Reader (Molecular Devices, Menlo Park, CA). Background optical density was the mean of wells where coating Ag was omitted. Controls were also performed with Ag-coated wells and all ELISA components except the primary Ab. These were always negative.

PCR amplification, cloning, and expression of Vλx. Oligonucleotide primers used for cloning Vλx were synthesized on a Dupont Coder 300 DNA Synthesizer (Dupont, Wilmington, DE) and were purified on Nensorb Prep columns (NEN, Boston, MA). Sequences of primers used for specific amplification of Vλx from the F28C4 hybridoma by PCR each contained a restriction site (XhoI and Bpu 1102I for the sense and antisense primers, respectively) at the 5′ ends to facilitate cloning of the PCR products. F28C4 Vλx was amplified using a Vλx-specific sense primer corresponding to nucleotides 229–242 which has the sequence 5′-d(CCG CTC GAG CAA CTT GTG CTC ACT CAG TCA TC) (GenBank Accession No. M34597). The antisense primer is specific for λ2 and is complementary to the J-C junction of F28C4 (13). It has the sequence 5′-d(GGA CTT GGG CTG AGC TAG GAC AGT GAC). Detailed methods used for reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of Ig V regions have been previously provided (18). The resulting double-stranded DNA sequence was then restriction digested and directionally cloned into the expression vector, pET-15b (Novagen, Inc., Madison, WI), and expressed according to the manufacturer's protocol. Purification of the recombinant protein was facilitated by the addition of a polyhistidine tag to the 5′ end of the cloning site. The authenticity of the recombinant protein was verified using Western gel analysis employing rabbit anti-Vλx Ab (see Results).

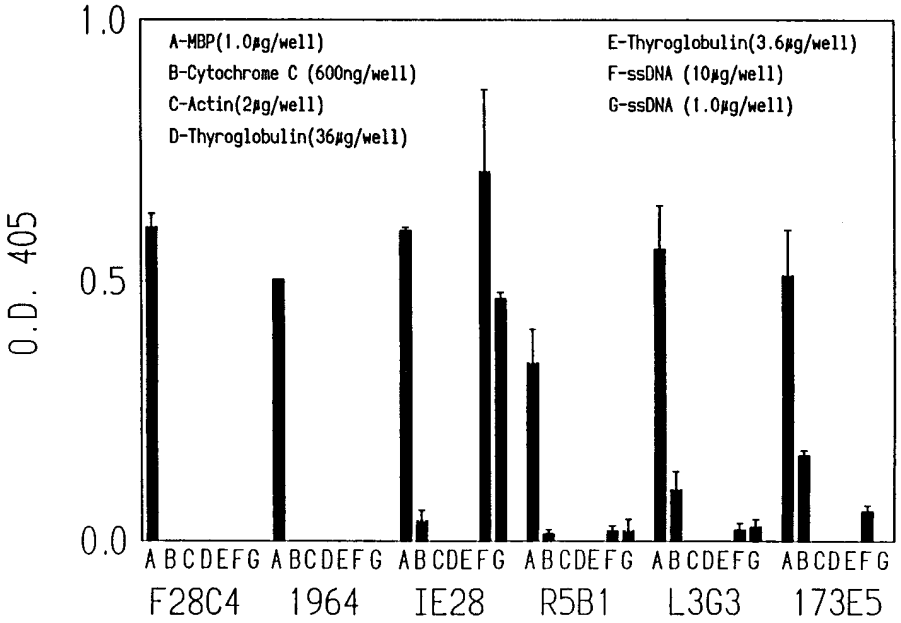

Figure 1. Polyreactivity profile of Vλx-containing mAbs with potential self Ags. ELISA microtiter wells were coated with equimolar concentrations (540 nM) of the indicated Ags. Both thyroglobulin and ss-DNA were also coated at 10-fold lower concentrations. The mAbs were diluted in PBS containing 1.0% BSA to a final concentration of 30 μg/ml, except for F28C4 which was diluted to 2 μg/ml. The results are the mean±SEM of three experiments, each done in duplicate.

Results

To investigate whether Vλx could confer MBP reactivity to an Ig, we collected and screened the known Vλx-containing mAbs for binding to MBP. Of six such mAbs that are known, five were of previously unknown Ag specificity while F28C4, which was raised against MBP Ac 1-9, served as a positive control. By ELISA, all Vλx-bearing mAbs bound human MBP (1-170) but, with the one exception of IE28, did not bind other common auto Ags including cytochrome c, thyroglobulin, actin, or single-stranded DNA (Fig. 1). IE28 bound single-stranded DNA. This reactivity with MBP was not observed with other IgM/λ2 (non Vλx) mAbs (Fig. 2). These results suggest that Vλx-containing IgMs are not polyreactive but rather have a measure of specificity for MBP. The lack of reactivity with cytochrome c is notable since it, like MBP, carries a basic charge. This suggests that the interaction is not merely electrostatic. The Vλx-bearing IgMs displayed a spectrum of K_as, ranging from at least 10^6 to 10^7 l/m, which likely reflects differences in VH usage (Fig. 3). The much higher affinity of F28C4 ($K_a \sim 10^9$) undoubtedly results from its being selected for by MBP immunization. However, considering that Vλx is the common denominator shared between these mAbs, the results suggest that their inherent ability to bind MBP may largely reside with the light chain while the affinity is governed by the heavy chain.

Four out of the six mAbs used in these studies were derived from polyclonally activated B cells whereas mAb 1964 was obtained from a mouse transgenic for the VH3H9 chain previously described in the autoimmune MRL/lpr mouse (19). Thus, in a sense, these mAbs were somewhat artificial in their selection and might not occur in a more natural setting. To ad-

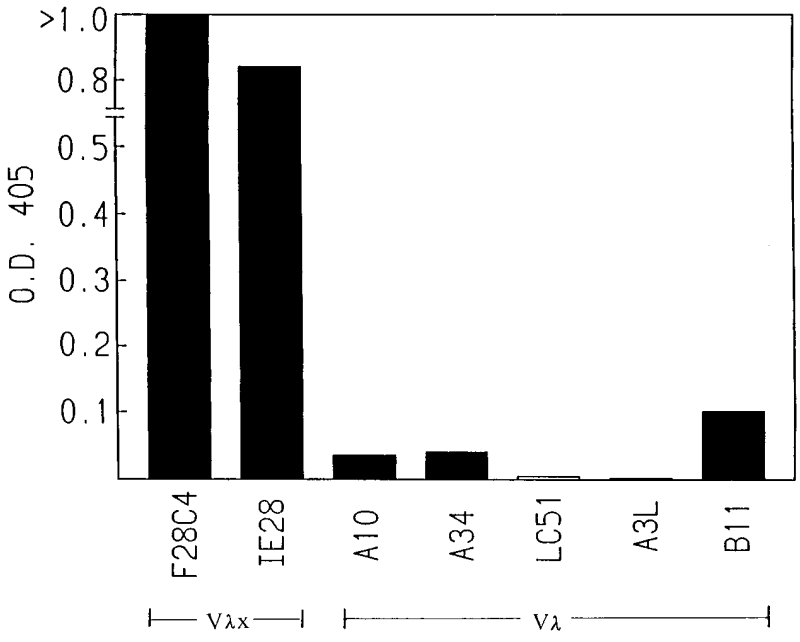

Figure 2. Reactivity of a Vλx-containing IgM mAb, IE28, with human MBP (1-170), as compared with other IgM/λ2 (non Vλx) controls. The IgM mAbs were diluted in PBS containing 1.0% BSA to a final concentration of 30 μg/ml whereas mAb F28C4, which served as a positive control, was diluted to a final concentration of 2 μg/ml. The results are presented as the optical density at 405 nm.

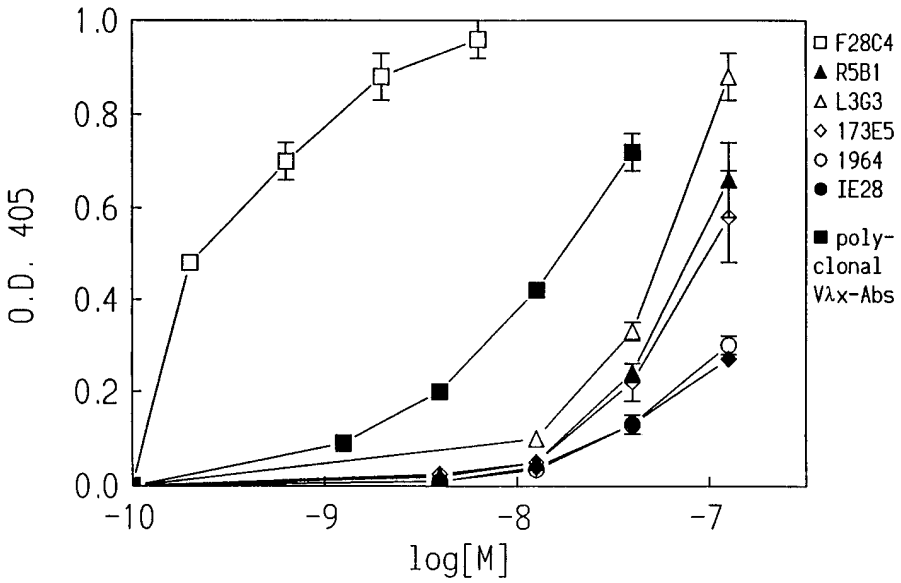

Figure 3. Reactivity of Vλx-containing mAbs and purified polyclonal Vλx-bearing Abs from normal mouse sera with human MBP (1-170). The Vλx-containing IgM mAbs, mAb F28C4, which served as a positive control, and the purified Vλx-containing Ab fraction from normal BALB/c mouse serum were diluted in PBS containing 1% BSA at the indicated concentrations. Reactivity of the Vλx-containing mAbs with human MBP was detected using an appropriate goat anti–mouse H chain-specific Ab (Southern Biotechnology) whereas reactivity of the Vλx-bearing Abs purified from normal mouse sera was monitored using rabbit polyclonal monospecific Ab to Vλx (diluted in PBS containing 1% BSA to a final concentration of 1 μg/ml) and detected with an alkaline phosphatase conjugated goat anti–rabbit IgG-specific Ab (Southern Biotechnology). The results are the mean±SEM of three experiments, each done in duplicate.

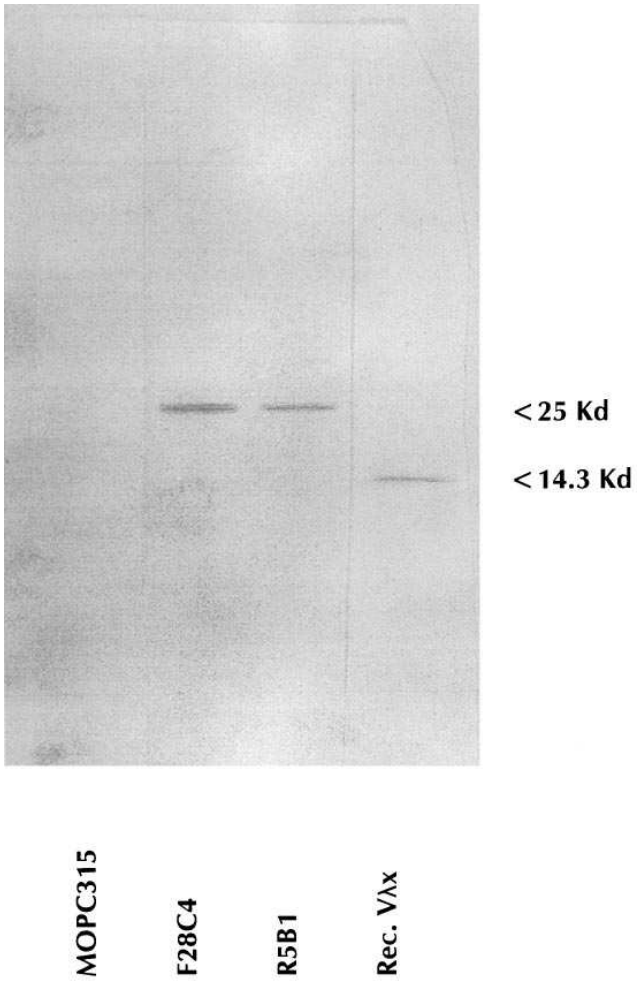

Figure 4. Western gel analysis verifying the specificity of rabbit anti-Vλx Ab and the authenticity of recombinant Vλx. Purified recombinant Vλx, F28C4 (γ2a, Vλx), MOPC 315 (α, λ2), and mAb R5B1 (μ, Vλx) were separated by 20% SDS-PAGE and electrophoretically transferred onto nitrocellulose. After blocking for 16 h at 25°C with 5% casein in PBS, rabbit polyclonal monospecific anti-Vλx Ab (1 μg/ml in PBS containing 5% casein) was added and incubation proceeded for 2 h at 25°C. Finally, alkaline phosphatase-conjugated goat anti–rabbit IgG (Southern Biotechnology), diluted 1:1000 in PBS containing 5% casein, was added and the color developed. Molecular weights of markers are indicated at the right. We detected the expected 14.3 Kd fragment which is consistent with the molecular weight of Vλx alone (i.e., no C region) as compared to the full length L chain (e.g., F28C4).

dress this possibility and to determine whether MBP reactive Vλx-containing Abs occur naturally, we purified Vλx-containing Ig from normal BALB/c sera. Fig. 3 shows that Vλx-bearing Abs from normal mouse sera bind human MBP. Interestingly, the average affinity of these Abs is much higher (∼ 10-fold) than that of the IgM mAbs. Since other studies have demonstrated that virtually all of the Vλx-bearing polyclonal murine Abs are IgG (Galin et al., manuscript submitted for publication), the results suggest that Vλx association with γ isotypes results in tighter binding than those with μ (data not shown). To assure that we were only measuring Vλx-bearing Abs, Fig. 4 shows by Western analysis that the polyclonal rabbit anti-Vλx Abs used for purification were monospecific for Vλx and did not react with λ2 light chains or γ2a heavy chains. These data implied that Vλx-containing Abs bound MBP by virtue of having Vλx light chains. In order to examine the MBP binding of Vλx alone, Vλx from mAb F28C4 was cloned and expressed. The recombinant Vλx reacted with the rabbit monospecific Ab to Vλx (Fig. 4). It also bound human MBP (Fig. 5) indicating that it is the light chain of Vλx-containing Abs which confers affinity for human MBP. Unlike most isolated light chains, recombinant Vλx interacts with MBP at concentrations similar to that of intact IgM/Vλx. Further studies will be required to determine whether the recombinant protein is reacting as a multimer.

Human MBP exists in five major isoforms that represent differential splicing of the MBP gene. Post-translational modifications of MBP result in a microheterogeneous population of the five differentially charged isomers. Among the modified

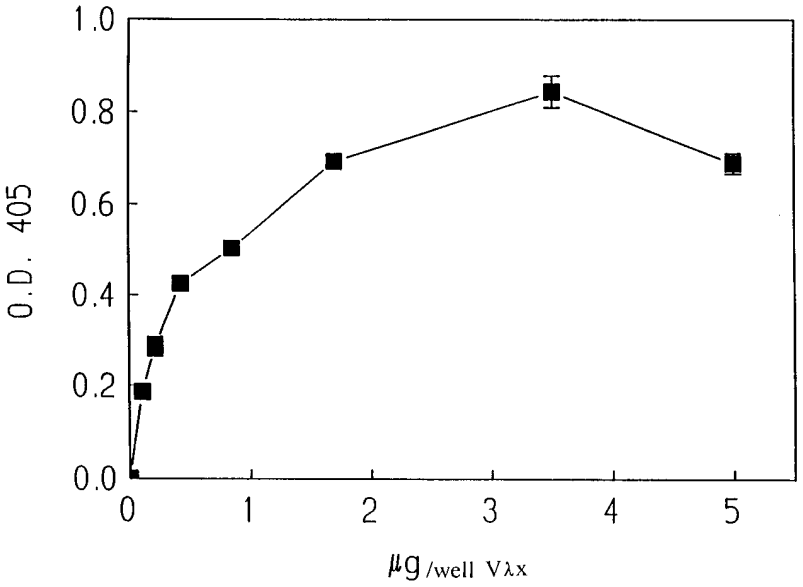

Figure 5. Binding of recombinant Vλx to human MBP (1–170) as determined by ELISA. The recombinant Vλx was diluted at various concentrations ranging from 50 μg/ml to 1 μg/ml in PBS and was allowed to bind for 2 h at 25°C. Reactivity was monitored using rabbit polyclonal monospecific Ab to Vλx (1 μg/ml in PBS containing 1% BSA) and detected following incubation with alkaline phosphatase labeled goat anti–rabbit IgG-specific Ab (Southern Biotechnology, Birmingham, AL). The results are the mean±SEM of three experiments each done in duplicate.

isomers, one termed C8 has undergone deimidation of at least six of the arginyl residues (positions 25, 31, 122, 130, 159, and 170) generating citrulline in these positions (20). Because of the number of altered residues, reactivity with C8 was tested in an attempt to restrict the interactive site of the aforementioned Vλx-containing Abs. We have found that, with the exception of F28C4, the Vλx-bearing Abs do not recognize the C8 isomer of MBP (Fig. 6). Thus, citrullination may disrupt or mask specific epitopes on MBP. Since F28C4 is specific for MBP Ac 1-9, and no citrullination occurs within this region, it is not surprising that F28C4 recognizes C8 MBP. In order to further localize potential epitopes within human MBP for Vλx and Vλx-containing Abs, we assayed binding to synthetic peptides representing human MBP residues Ac 1-9, 10-19, 14-24, 25-34, and 80-89 of the MBP molecule (Fig. 7). With the exception of F28C4, all Vλx-bearing mAbs as well as recombinant Vλx showed preferential binding to MBP peptide 25-34 as compared with the other peptides. Interestingly, mAbs 173E5 and 1964, as well as the recombinant protein, seem to be polyreactive with different regions of MBP. On the other hand, F28C4 only shows reactivity with Ac 1-9. Thus, the heavy chain seems to contribute to epitope specificity as well as affinity for MBP. These data also demonstrate that the dominant epitope recognized by recombinant Vλx and most Vλx-containing IgMs can, in part, be defined by a particular region in MBP which includes residues 25–34. This result is, of course, consistent with the loss of binding that occurs when Arg_{25} and Arg_{31} are deimidated (Fig. 6).

Discussion

Evidence for the role of an Id network in EAE rests on the demonstration of a CRI on the TCR of encephalitogenic T cells and modulation of disease by anti-Id (10, 12). The reagent central to the recognition of these immune events is mAb F28C4, an IgG2a/λ anti-MBP Ac 1-9 (10–12). The realization that the L chain of mAb F28C4 was Vλx, an unusual and phylogenetically old protein, led to the investigations reported herein. In brief, Vλx confers binding to MBP whether in normal poyclonal murine Ig, a mAb, or as a recombinant product. It's binding to MBP is not a polyreactive phenomenon, but it can be modified by the heavy chain type. The MBP site prefer-

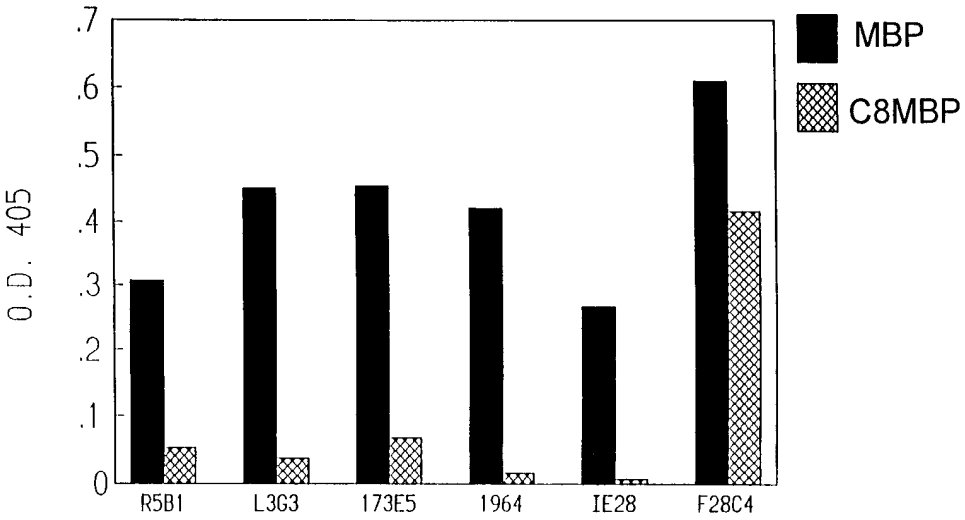

Figure 6. Lack of binding of Vλx-containing mAbs to the C8 isoform of human MBP (1–170). MBP and C8 MBP were coated onto ELISA microtiter wells at equal concentrations (10 μg/ml in PBS) at 4°C for 16 h. The mAbs were diluted in PBS containing 1% BSA to a final concentration of 30 μg/ml for the IgM mAbs and 2 μg/ml for mAb F28C4 and allowed to bind for 2 h at 25°C. Ab binding was detected with an appropriate alkaline phosphatase conjugated anti–mouse heavy chain specific Ab (Southern Biotechnology). The results are presented as the optical density at 405 nm.

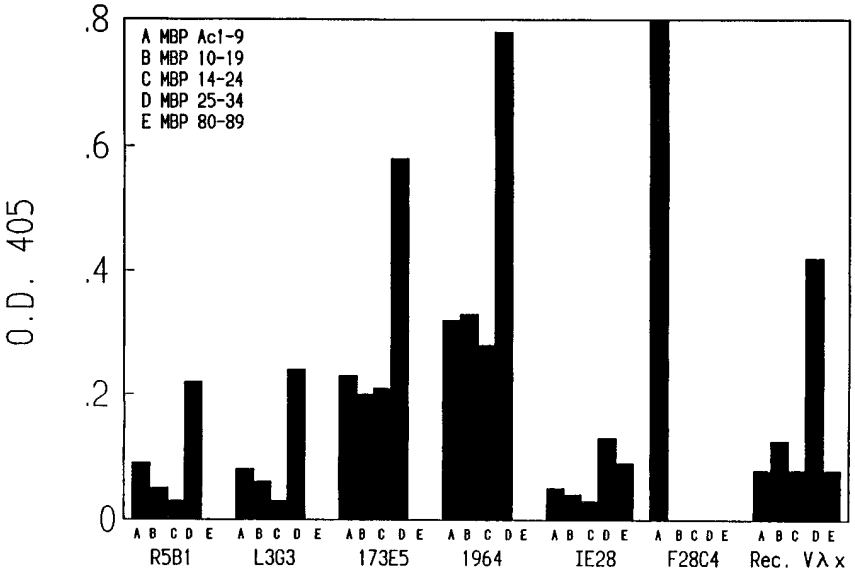

Figure 7. Binding of recombinant Vλx and Vλx-containing mAbs to various human MBP peptides. The indicated MBP peptides were coated onto ELISA microtiter wells at equal concentrations (10 μg/ml in PBS) at 4°C for 16 h. The mAbs and recombinant Vλx were diluted in PBS containing 1% BSA to a final concentration of 30 μg/ml. Ab binding was detected with an appropriate alkaline phosphatase conjugated anti-mouse heavy chain specific Ab (Southern Biotechnology). The binding of recombinant Vλx was monitored with rabbit anti-Vλx monospecific Ab (1 μg/ml in PBS containing 1% BSA) followed by an alkaline phosphatase conjugated anti–rabbit IgG-specific Ab (Southern Biotechnology). The results are presented by the optical density at 405 nm.

entially bound is the amino terminus itself or in the region encompassing residues 25–34. Until the present report nothing was known about the specificity of Vλx bearing Abs because they were originally described in polyclonally activated B cells.

Current dogma associated with EAE, and perhaps applying to MS, essentially dictates that the encephalitogenic response to MBP, particularly with regard to T cell activation, is restricted to certain TCR usage and may be predominantly directed against limited immunodominant epitopes. Here we have shown that the reactivity of Vλx-containing Abs with MBP can be largely localized to the amino-terminal end, particularly in the region of residues 25–34. Although no formal studies were done using peptides representing the carboxy-terminal half of the MBP molecule, it seems unlikely that this region of the molecule is involved in the binding activity of these mAbs since, quantitatively, the reactivity of these mAbs with peptide 25–34 accounts for that seen with the intact molecule. This is further supported by the lack of binding seen with the most amino-terminal peptide of the carboxy-terminal half of MBP, peptide 80–89. Since it is known that Vλx and Vλx-bearing mAb share sequence homology and specificity with encephalitogenic TCRs, it is tempting to speculate that peptide 25–34 may represent yet another encephalitogenic epitope. MBP 25–34 has, to our knowledge, never been shown to be encephalitogenic, thus this possibility is intriguing, especially since we now know that multiple MBP peptides can be encephalitogenic even within an inbred strain of mice (21). Furthermore, new MBP epitopes are emerging as encephalitogenic even in the context of multiple MHC molecules (22). Thus, there is still the potential for identification of novel encephalitogenic peptides that may have been previously overlooked. On the other hand, Vλx may have a protective role with MBP 25–34, evoking a beneficial B cell response. This is particularly intriguing since MBP 21–40 has been shown to trigger TGF-β release and can suppress active induction of EAE (23). In addition, recent data suggests that virulent, restricted T cell responses predominate only during active disease whereas the initial anti-MBP and recovery responses are marked by non-pathogenic heterogeneity (24). Thus, a Vλx response to MBP 25–34 could perhaps alter or reduce the encephalitogenic T cell response through the induction of anti-idiotypic antibodies or T cells responding to Vλx-idiopeptides (25).

Although the specificity of most Abs results from a precise match between H and L chains, cooperation between the two chains is not always necessary for tight and specific Ag binding. Particular VH domains have now been shown to modulate binding to DNA, cardiolipin (26), and lysozyme (27). However, in some of these cases, different L chains could dramatically alter binding specificities (26). These results suggest that, as in the case for Vλx, a single V region may contain most or all of the necessary determinants required for efficient binding, but that certain H or L association can either enhance or abolish such Ag recognition or change specificity. The results of our investigation support this notion in that there is clearly an inherent or intrinsic quality associated with Abs bearing Vλx that results in MBP binding and the MBP reactivity resides mostly with Vλx. Moreover, the H chain seems responsible for apparent differences in affinity between the Vλx-containing Abs for MBP and could certainly account for the shift in epitope specificity for F28C4. It is important to note that F28C4 was originally selected for Ac1-9 specificity and, thus, it is not surprising that F28C4 does not bind peptide 25–34. On the other hand, the various Vλx-containing IgM's were not initially generated to exhibit MBP reactivity, therefore these mAbs may not have both H and L chain contributions to MBP specificity and, based on the findings presented here, bind MBP and MBP peptide 25–34 solely by virtue of bearing a Vλx L chain. This is consistent with the ability of recombinant Vλx alone to preferentially bind peptide 25–34. Considering this, clonal expansion of certain MBP-specific B cells could be initiated via a threshold stimulation of surface Igs with Vλx light chains by MBP. The exact MBP specificity and affinity would then be determined by the particular VH and heavy chain isotype. This type of scenario would seem to explain why many MBP reactive Igs in normal mouse sera have Vλx light chains. If this idea is correct, it suggests that a skewed distribution of Vλx containing MBP specific antibodies may eventually be observed in EAE. With regard to MS, the observation of a human equivalent to Vλx suggests that our findings in the mouse

may in fact apply to humans (16). If found, such Abs could be markers for MS and EAE much like certain Ig V regions are associated with SLE and rheumatoid arthritis. Indeed, a human Vλx-like light chain could, in part, explain certain oligoclonal light chain bands in the cerebral spinal fluid of MS patients (28, 29).

Although it is often assumed that Ab responses to self Ags result only in pathogenicity, there is also evidence to the contrary. For instance, sera obtained from animals that have recovered from EAE are able to suppress EAE when given to naive recipients at the time of active disease induction (30). This is entirely consistent with the ability of antiserum from animals immunized with MBP or its encephalitogenic peptides to inhibit the disease (6). Considering that mAbs specific for MBP can, in vitro, inhibit the proliferation and cytotoxicity of human MBP-specific T cell clones (31), these data suggest that the humoral response to MBP, in vivo, can potentially modulate the T cell-mediated immune response through a T cell/B cell network. Thus, we cannot overlook the possibility that Vλx may play a role in such regulation. In fact, our preliminary results suggest that the Vλx containing Ab F28C4 is a very potent idiotypic vaccine against adoptively transferred EAE in the Lewis rat model of disease (Galin et al., manuscript in preparation). If extended to humans, such results could suggest a new therapeutic regimen for MS. In any event, further investigation into the regulation of Vλx expression, particularly in response to MBP, may provide critical insight into the potential function of this unique V region and its role in EAE and MS.

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