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Immunology

Fine specificity of the antibody response to myelin basic protein in the central nervous system in multiple sclerosis: The minimal B-cell epitope and a model of its features

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ABSTRACT **T cells, B cells, and antibody are found in the white matter of the central nervous system in multiple sclerosis. The epitope center for the antibody response to human myelin basic protein (MBP) fits precisely the minimal epitope Pro^{85}-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro^{96} for that reported for HLA DR2b (DRB1*1501)-restricted T cells that recognize MBP [Wucherpfenning, K. W., Sette, A., Southwood, S., Oseroff, C., Matsui, M., Strominger, J. & Hafler, D. (1994) *J. Exp. Med.* 179, 279–290], and overlaps with the reported DR2a-restricted epitope for T cells reactive to MBP [Martin, R., Howell, M. D., Jaraquemada, D., Furlage, M., Richert, J., Brostoff, S., Long, E. O., McFarlin, D. E. & McFarland, H. F. (1991) *J. Exp. Med.* 173, 19–24]. We describe a molecular model of this epitope.**

Myelin basic protein (MBP) is located at the interface of cytoplasm and membranes of central nervous system oligodendrocyte myelin, where it interacts with lipid head groups by hydrogen bonding (1). To date, the secondary and tertiary conformation of the MBP molecule within the myelin membrane is unknown, whereas under physiological conditions soluble MBP is an unstructured, flexible polypeptide chain, endowed with a high degree of segmental mobility and with little α-helical and β-sheet structure (1). The amino acid sequence of human (h) MBP has 170 residues, including 12 lysine and 19 arginine residues, as well as 2 glutamic acid and 9 asparagine residues, thus providing the protein with a net charge of +20 at physiological pH and a high isoelectric point (pI > 10) (2–4). Immune responses to MBP are detectable in patients with multiple sclerosis (MS), and immunization of rodents with MBP induces experimental autoimmune encephalomyelitis with features similar to MS (5, 6).

We previously reported increased levels of free (F) and/or cerebrospinal fluid (CSF)-tissue bound (B) autoantibodies to MBP (anti-MBP) in a large number of patients with MS and optic neuritis (7). Increased anti-MBP levels are highly associated with disease activity, with significantly higher levels observed in patients with active MS. Patients experiencing acute relapses have higher levels of F than B antibody (F:B ratios > 1.0), while the converse profile (lower levels of F than B anti-MBP and F:B ratios ≤ 1.0) is characteristic for patients with insidiously progressing MS. F anti-MBP is usually undetectable in patients in clinical remission, while B antibody may be present in low titers. CSF anti-MBP levels also correlate with disease duration (7). With regards to specificity for MS, anti-MBP levels were measured in CSF of 1195 non-MS patients, and only 36 had increased values, suggesting that anti-MBP is relatively but not absolutely specific for MS. The MBP epitope(s) for non-MS anti-MBP have not yet been determined. Correlation of CSF anti-MBP with the major histocompatibility complex type has not been studied.

F and B anti-MBP can be purified by affinity chromatography from CSF and brain tissue obtained from patients with MS (8–10). On the basis of binding studies with peptides of MBP and competitive inhibition radioimmunoassays, we reported that the MBP epitope for F anti-MBP was located between residues 61 and 106 and that the MBP epitope for B anti-MBP was located between residues 75 and 106 (7, 11, 12). The region of the MBP molecule which reacts strongly with F and B anti-MBP from MS patients defines the B-cell epitope. Synthetic peptides located within this epitope produced either partial (25–75%) or complete (80–100%) inhibition of anti-MBP antibody.

The purpose of these studies was to localize the epitope center for MS anti-MBP, to determine its molecular properties, and to compare the fine structure of the T- and B-cell epitopes for MBP in MS.

MATERIALS AND METHODS

Antigens. MBP was isolated and purified from non-MS human brain tissue (10). Only protein preparations with a molecular mass of 18,500 Da were pooled, freeze-dried, and stored at −80°C for further use in radioimmunoassays and affinity chromatography. MBP peptides were synthesized by Procyon Biopharma (London, ON Canada) by using the 9-fluorenylmethoxycarbonyl method; peptide purity was checked by reverse-phase HPLC and amino acid analysis.

Antibody. Anti-MBP in both F and B forms were prepared by affinity chromatography from CSF and brain tissue obtained from MS patients (10–12).

Anti-MBP Radioimmunoassays. F and total (T) anti-MBP levels were determined in CSF or brain tissue extracts before or after acid hydrolysis respectively; B antibody was calculated by subtracting F anti-MBP from the corresponding T antibody value. Briefly, all samples diluted to a final IgG concentration of 0.010 g/liter were incubated in MBP-coated Immulon wells (1 μg of MBP per well) for 1 h. After several buffer washes, the complex was enhanced with anti-human IgG (Fc specific), and after several more buffer washes ^{125}I-labeled protein A(G) (50,000 cpm) was added for 1 h. After five final water washes, wells were individually counted for 10 min or 10,000 counts. Each sample was run in 10 replicates; nonspecific binding (NSB) was determined for each individual sample in uncoated wells. Antibody levels are expressed in radioactivity units (Ru) defined as follows: (sample cpm − NSB cpm) ÷ (total cpm − NSB cpm). Results of less than 4.0 Ru are considered negative. Each assay contains a non-MS negative control (≤1.0 Ru) and one positive control at five different linear dilutions covering the range between 5 and 25 Ru. The variation within an assay is ≤0.04 and variability between assays is 0.05–0.07. In a typical assay, with 50,000 cpm added in each well, CSF antibody

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Abbreviations: MBP, myelin basic protein; hMBP, human MBP; MS, multiple sclerosis; CSF, cerebrospinal fluid; Ru, radioactivity units; TCR, T-cell receptor; F, free; B, bound; T, total.

positive levels fall between 5 and 25 Ru (2500–12,500 cpm); higher Ru values per mg of IgG are observed in MS brain tissue extracts.

Epitope Studies. Constant amounts of anti-MBP (25–35 Ru) were incubated in a liquid-phase assay with increasing amounts of either hMBP (0.01–1.0 μg/ml) or individual synthetic peptides (0.1–10.0 μg/ml). After a 2-h incubation at room temperature, all supernatants were diluted to 0.010 g of IgG per liter, and anti-MBP was determined by radioimmunoassay, as described. Antibody inhibition was calculated as 100 − anti-MBP Ru. In this report F and B anti-MBP were purified from CSF and brain tissue of a single MS patient.

RESULTS

Localization of the B-Cell Epitope. In solid-phase radioimmunoassays using anti-MBP purified from CSF and brain tissue of MS patients and liquid-phase competition with synthetic peptides of hMBP, the B-cell epitope was located between residues 61 and 106 for F antibody and residues 75(85) and 106 for both F and B anti-MBP (7). Similar results have been reported in an animal model system in which a major epitope for rabbit anti-bovine (b) MBP was also associated with residues corresponding to bMBP 81–90 (13, 14).

Localization of the Epitope Center. To localize the epitope center, F and B anti-MBP from MS CSF and brain tissue were mixed in competitive inhibition assays with 41 consecutive MBP synthetic peptides of equal length (each peptide was 10 residues and overlapped the adjacent peptide by 9 residues) covering the area between residues 61 and 110 of hMBP, which included the B-cell epitope (Fig. 1) (15). Inhibition of F anti-MBP progressively increased as residues 84–95 were incorporated at the carboxyl end of the reacting peptides; as residues 87–97 were deleted from the amino end of the reacting peptide, inhibition of F anti-MBP progressively decreased. Complete inhibition (80–100%) of F antibody was produced by 10 decapeptides from MBP83–92 to MBP92–101. B anti-MBP preserved its restricted specificity: inhibition increased with the incorporation of residues 91–95 at the carboxyl end of the reacting peptide, and, as residues 87–90 were deleted from the amino end, inhibition decreased significantly. Complete inhibition (80–100%) of B antibody was produced by four decapeptides: MBP84–93, MBP85–94, MBP86–95, and MBP87–96. These experimental results suggest that the epitope center for F and B anti-MBP is located between residues 84 and 96 of hMBP since peptides corresponding to this area of the molecule produced complete inhibition (80–100%) of both F and B antibody. This location of the epitope center was further confirmed by inhibition results when using purified B anti-MBP and two seven-residue synthetic peptides located within this segment (Fig. 2). Individually, peptides MBP85–91 and MBP89–95 produced 11%

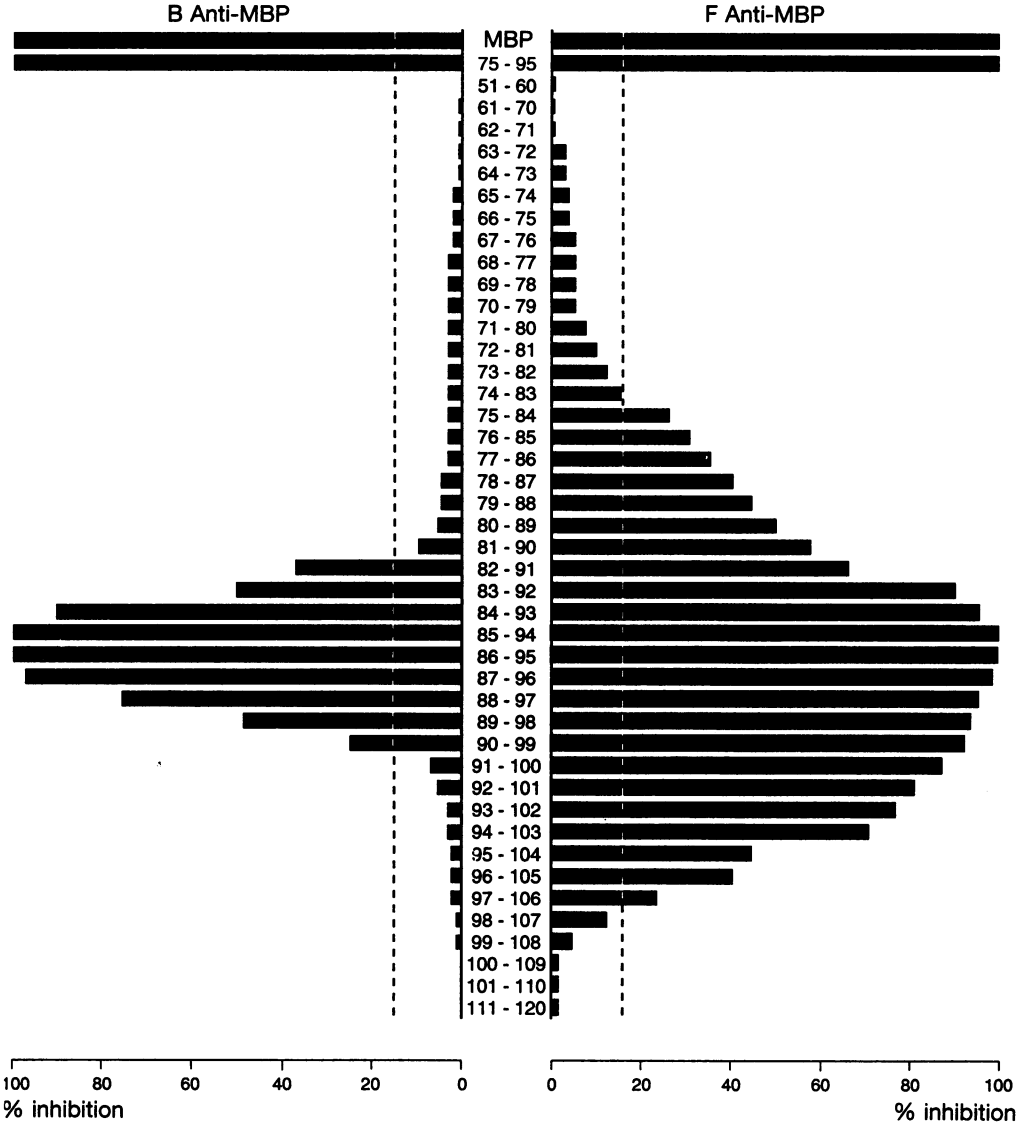

FIG. 1. Localization of the epitope center for MS F and B anti-MBP on hMBP. The epitope center was determined as described in *Materials and Methods*. The black bars represent percent inhibition. Positive controls were complete hMBP and peptide MBP75–95. Negative controls were peptides MBP51-60 and MBP111-120. The dashed line represents the 95% confidence limits of the antibody inhibition assay. This figure is reprinted with permission from ref. 15 (copyright Elsevier Science).

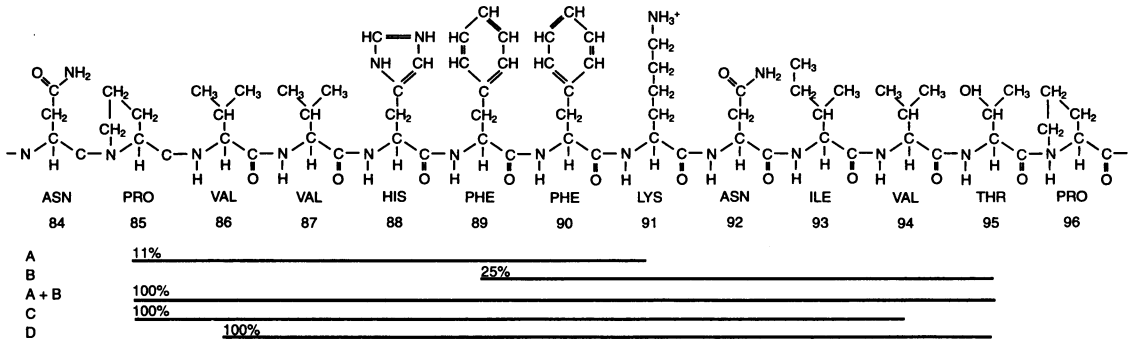

FIG. 2. Chemical structure of the epitope center. Lines below the sequence (labeled A–D) represent peptides used in antibody inhibition studies to further define the epitope center. The percent inhibition results when using B anti-MBP and synthetic peptides is shown at the left of each line. Peptides A (MBP85–91) and B (MBP89–95) produced 11% and 25% inhibition, respectively. Complete inhibition was produced by an equimolar mixture of peptides A and B (MBP85–91 and MBP89–95); peptide C (MBP85–94), a decapeptide obtained by incorporating residues 92–94 at the carboxyl end of MBP85–91; and peptide D (MBP86–95), a decapeptide obtained by incorporating residues 86–88 at the amino end of MBP89–95.

and 25% inhibition, respectively (Fig. 2, peptides A and B); however, when both peptides were simultaneously mixed with purified antibody in an equimolar mixture, complete (100%) inhibition occurred (Fig. 2, peptides A + B), suggesting that the anti-MBP binding site was severely altered when peptide MBP86–95 was fractured between residues 89 and 91. Furthermore, inhibition of antibody was complete when residues 92–94 were incorporated at the carboxyl end of peptide MBP85–91 (Fig. 2, peptide C) or when residues 86–88 were added to the amino end of MBP89–95 (Fig. 2, peptide D). The same major anti-MBP epitope was found regardless of whether the initial source of antibody was from individual MS patients or from a pool of several MS CSF samples.

DISCUSSION

We further analyzed the hMBP molecule in terms of position and/or number of "molecular spacers" and "molecular bends" (Fig. 3). We defined molecular spacers as short-chain residues (side chains of one carbon or less, namely Gly, Ala, and Ser) that could provide molecular space for adjacent long-chain amino acids, and molecular bends as disruptions in secondary structure(s) which tend to occur in association with proline residues (16, 17). Within the hMBP molecule, the largest series of 10 consecutive long-chain residues (side chains of two carbons or more) located between molecular spacers and/or molecular bends corresponds to the segment between Pro^{85} and Pro^{96}, the precise location of the B-cell epitope center; outside this segment, a total of 57 molecular spacers (12 Ala, 19 Ser, and 26 Gly) and 12 molecular bends (proline) reduce the probability of other such large stretches of contiguous long-chain residues, emphasizing the distinct properties of the epitope center. To provide a theoretical model of the secondary structure for the epitope center and its adjacent areas, the MBP segment between residues 85 and 96 was studied with regard to the distribution of polar and nonpolar residues (18). The epitope center contains six hydrophobic residues (positions 86 and 87, 89 and 90, and 93 and 94) and four hydrophilic

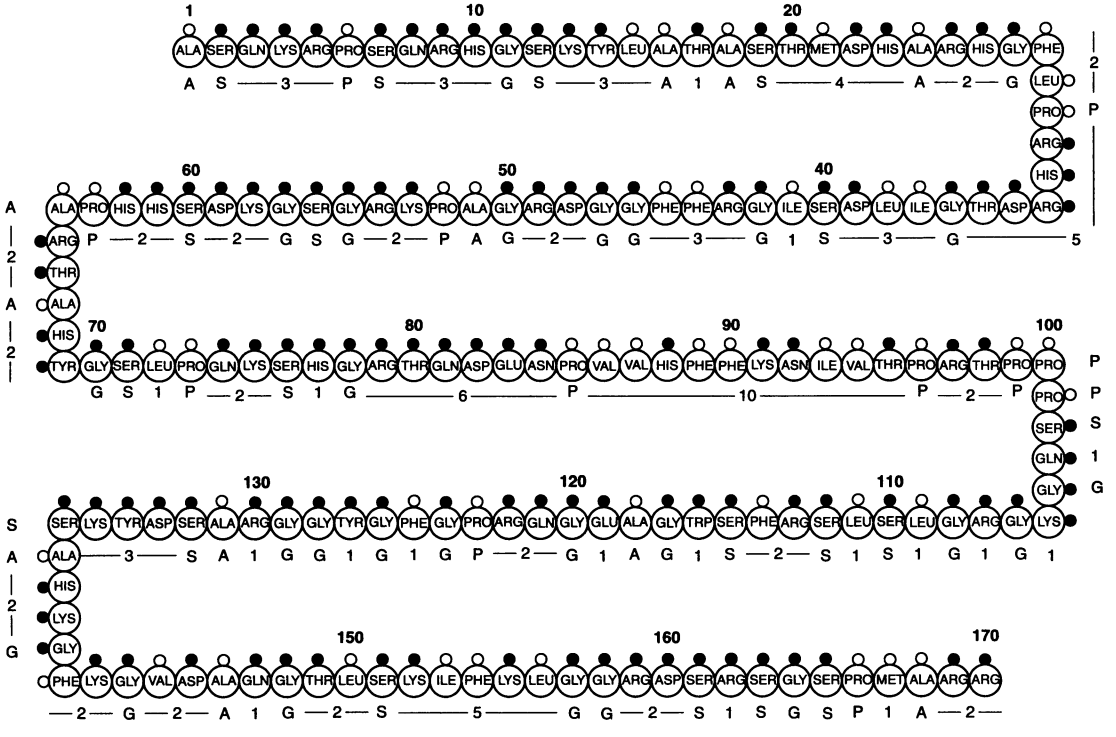

FIG. 3. Primary structure of the hMBP molecule containing 170 residues. The positions of molecular spacers [Gly (G), Ala (A), and Ser (S)] and molecular bends [Pro (P)] and the number of consecutive long-chain residues located between them are given under the sequence. ●, Polar residues; ○, nonpolar residues.

residues (positions 88, 91, 92, and 95). Although the secondary structure of hMBP within the myelin sheath has not been elucidated to date, it has been proposed that hMBP may contain as much as 10.6% α-helix and 17–20% β-sheet (19, 20) and that the MBP domain from Pro^{85}–Pro^{96} is a β-structure (21). If the MBP epitope center is located at the interface between the oligodendrocyte cytoplasm and the lipid membrane, the suggested β-structure cannot accommodate molecular interactions between the side chains and the lipid head groups; however, an α-helical model can accommodate this sequence so that the nonpolar side chains project into the hydrophobic oligodendrocyte membrane and the polar residues remain in the hydrophilic oligodendrocyte cytoplasm (Fig. 4). We are, therefore, proposing that the hMBP epitope center (Pro^{85}–Pro^{96}) is an α-helix located at the myelin interface with the oligodendrocyte cytoplasm.

Structural elements consisting of two β-strands connected by an α-helix and called beta-alpha-beta domains are thought to be present in numerous proteins (17, 18). A structural feature of this kind may be present within the B-cell epitope (between residues Gln^{74} and Gly^{108}) (Fig. 5) where two series of consecutive polar residues (74–84 and 102–108) are located on each side of the α-helix (85–96), creating a beta-alpha-beta domain. These domains are highly associated with the location of binding sites, such as antigen–antibody bonds (18).

Alternatively, this response may be directed to a processed epitope of MBP presented on B cells with anti-MBP specificity, and thus able to trigger T cells reactive to the same epitope. In this way, the antibody need not be directed to MBP in its native conformation in the myelin membrane but may be directed to a processed epitope of MBP. Given the immunodominance of the T-cell response to the MBP fragment 86–99 in MS (22–24), and the capacity of HLA DR molecules to bind peptide MBP86–99 preferentially, such an alternative is possible. Indeed, the binding of peptide MBP86–99 to the HLA cleft may protect this MBP fragment from proteolytic cleavage directed to the Phe^{89}-Phe^{90} bond by brain proteases like cathepsin D.

Analysis of the T-cell receptor (TCR) gene rearrangements in the MS lesion has indicated that there are a restricted group of complementarity-determining region 3 (CDR3) motifs which are identical to those found in several human and rodent T-cell clones that react to peptide MBP86–99 (24). A T-cell clone from an MS patient who was HLA DR2 with specificity for peptide MBP86–105 shares the identical Vβ5.2 gene segment and CDR3 variable/diversity/joining β sequence with a common TCR gene rearrangement in MS brain lesions from HLA DR2 patients (23, 24). This clone is cytotoxic for peptide MBP86–95 (Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr) bound to HLA DR2b (DRB5*0101) but not to peptide MBP87–95 or peptide MBP86–94 (24). Peptide MBP85–98 is the minimum residue necessary for optimal binding to both HLA DR2a and 2b (22, 23, 25).

The HLA DR2 haplotype is associated with 75% of MS cases. The B-cell epitope center fits precisely the minimal epitope Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro

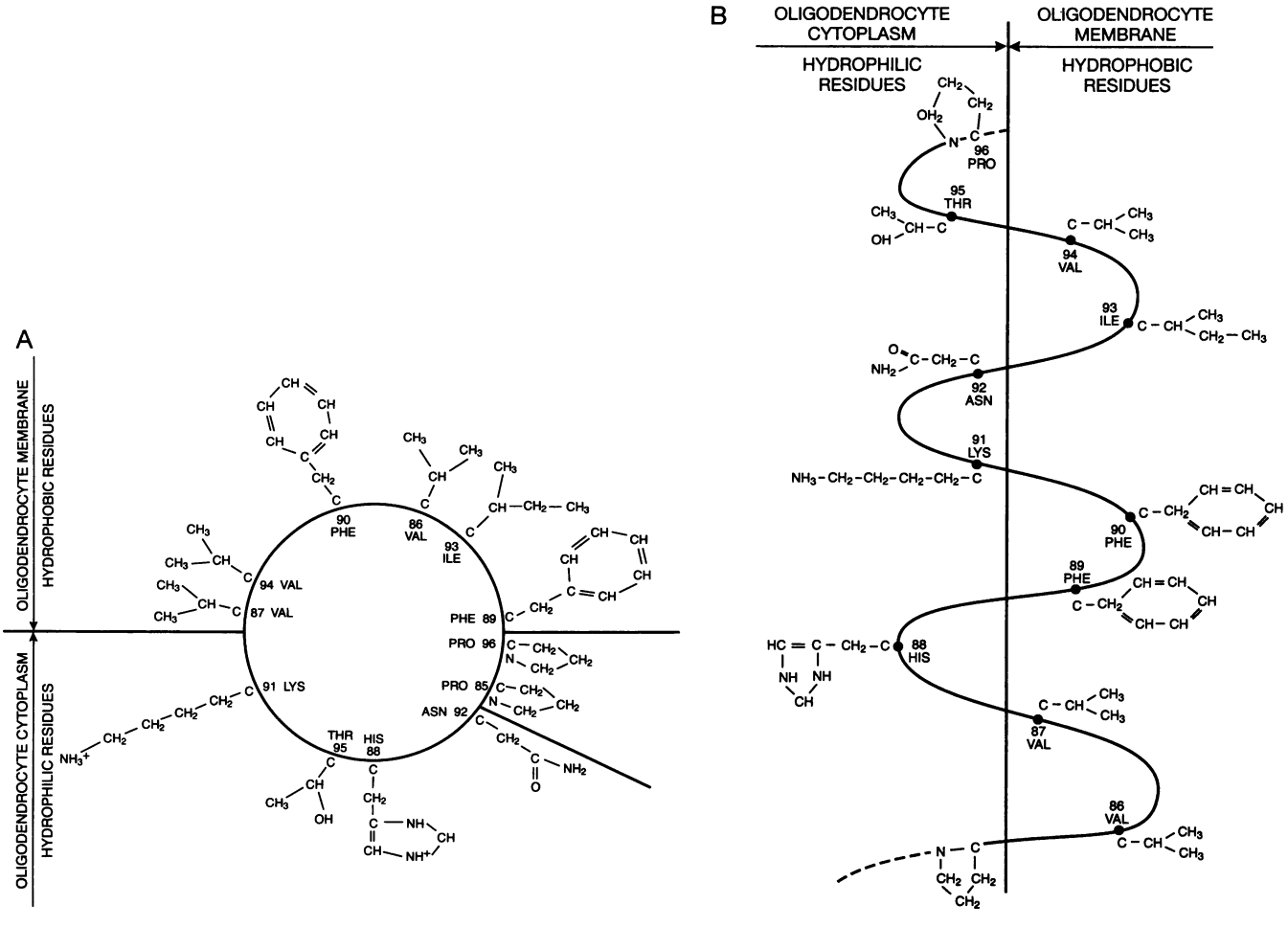

FIG. 4. Theoretical α-helical model of the hMBP epitope center (Pro^{85}–Pro^{96}). (*A*) Cross-sectional view. When residues located between Pro^{85} and Pro^{96} are consecutively plotted every 100° (3.6 residues per turn) on the circle representing the projection of an α-helix, the amino acids segregate into a hydrophilic cluster on one side and a hydrophobic cluster on the opposite side. In this model, the hydrophilic side chains would extend into the polar medium of the oligodendrocyte cytoplasm, and the hydrophobic side chains would extend into the nonpolar membrane. Since proline residues are not adverse to exposure to water, they may be accommodated in either polar or nonpolar environments. (*B*) Longitudinal view.

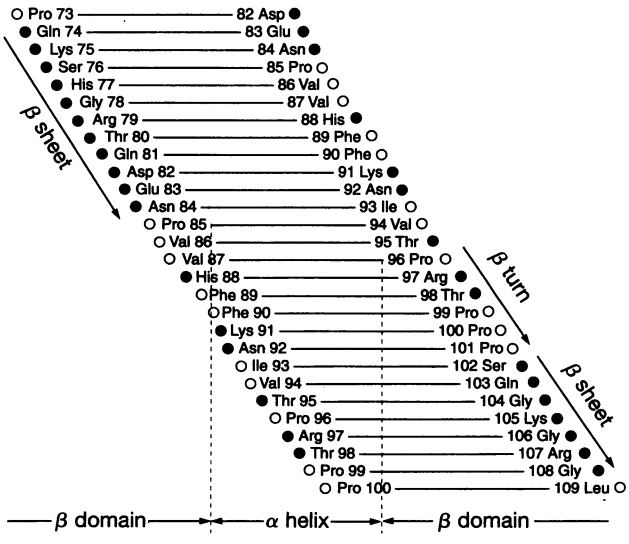

FIG. 5. Beta-alpha-beta domain present within the MBP B-cell epitope (between Gln^{74} and Gly^{108}) is highly associated with the location of binding sites.

for HLA DR2b (DRB1*1501)-restricted T cells (24). The minimal epitope for HLA DR2a (DRB5*0101)-restricted MBP-reactive T cells is shifted by three amino acids at the amino terminus and two amino acids at the carboxyl terminus to Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr (24).

For this critical fragment of MBP, similarities in the amino acid residues which interact with major histocompatability complex or TCR are conserved across species. The main anchor residue for both RT D1 in rats (homologous to HLA DR in man), and HLA DR2a and -2b in man is Phe^{90}, and the main TCR interaction residue is Lys^{91} (22, 23, 25, 26). A large number of different HLA DR antigens bind this peptide with high affinity, though the highest affinity binding is to HLA DR2a and -2b (27). In rodents, T cells directed to peptide MBP86-95, which share TCR variable gene CDR3 sequences with rearrangements found in MS brain, are capable of inducing paralysis and inflammation in the central nervous system, supporting a pathogenic role for the T cells reacting to MBP peptide 86–99 in MS (6, 24). Moreover, peptide MBP84–103 bound DR molecules associated with increased susceptibility to MS particularly well, explaining, in part, why these subtypes of HLA-DR2 confer a higher risk for development of MS than subtypes associated with weak binding (22, 27, 28).

Non-MS patients, even normal healthy individuals, possess T cells reactive with MBP (5). It is noteworthy that MBP is present in thymus during the development of the immune system, and that T cells with specificity for peptide MBP84-103 can be isolated easily from peripheral blood of healthy individuals. In contrast, the spinal fluid of healthy individuals does not have anti-MBP antibody, and brains of individuals who died from non-neurologic diseases, as well as inflammatory diseases other than MS, do not have the TCR variable/diversity/joining sequences found in T cells reactive to peptide MBP84–103 (24). These observations reinforce the concept that the anti-MBP antibody and the MBP-specific T cells which are found in central nervous system lesions are critical in the pathogenesis of MS.

The discovery that both B cells and T cells found in the central nervous system of MS patients react to the same area of the MBP molecule emphasizes the involvement of this MBP segment in the pathogenesis of the disease. In another autoimmune disease, myasthenia gravis, the main immunogenic region for T cells and antibody responses is in close proximity on the acetylcholine receptor, the pathogenic autoantigen in this condition (29). Whether neighboring or overlapping T- and B-cell epitopes is a general feature of autoimmunity warrants further investigation.

Antibody responses to other myelin antigens like MOG (30), PLP (5, 7), and transaldolase (31) are seen in the spinal fluid of some MS patients. Nevertheless, the widespread antibody activity directed to MBP86–99 in >90% of MS patients is noteworthy (7). Wucherpfenning and Strominger (32) have described several microbial peptides which mimic the minimal MBP epitope for HLA DR2. Molecular mimicry for the ligand recognized by immunoglobulin on B cells may lead to the breakdown of self-tolerance at the T-cell level since B cells may present antigen to T cells in the periphery or even in MS lesions.

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