

Wasp Venom Peptides may play a role in the Pathogenesis of Acute Disseminated Encephalomyelitis in Humans: A Structural Similarity Analysis

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Abstract—Acute disseminated encephalomyelitis (ADEM) has been reported to develop after a hymenoptera sting, but its pathogenesis is not known in detail. Myelin basic protein (MBP)-specific T cells have been detected in the blood of patients with ADEM, and a proportion of these patients develop multiple sclerosis (MS). In an attempt to understand the mechanisms underlying ADEM, molecular mimicry between hymenoptera venom peptides and the human immunodominant MBP peptide was scrutinized, based on the sequence and structural similarities, whether it was the root of the disease. The results suggest that the three wasp venom peptides have low sequence homology with the human immunodominant MBP residues 85-99. Structural similarity analysis among the three venom peptides and the MS-related HLA-DR2b (DRA, DRB1*1501)-associated immunodominant MHC binding/TCR contact residues 88-93, VVHFFK showed that hyaluronidase residues 7-12, phospholipase A1 residues 98-103, and antigen 5 residues 109-114 showed a high degree of similarity 83.3%, 100%, and 83.3% respectively. In conclusion, some wasp venom peptides, particularly phospholipase A1, may potentially act as the molecular motifs of the human 3HLA-DR2b-associated immunodominant MBP88-93, and possibly present a mechanism for induction of wasp sting-associated ADEM.

Keywords—central nervous system, Hymenoptera, myelin basic protein, molecular mimicry.

I. INTRODUCTION

THE application of bioinformatics technology, including sequence similarity and structural homology analysis, has been increasingly used in evaluating the underlying mechanism of a variety of human autoimmune diseases, and may provide an alternative way to understand and study the pathogenesis of autoimmune neurological diseases, including acute disseminated encephalomyelitis (ADEM) [1], [2].

ADEM or other related acute encephalitis-myelitis has been reported to develop after a hymenoptera sting [3]-[5]. However, there is no evidence demonstrating how hymenoptera venom peptides mediate an inflammatory demyelinating disorder of the central nervous system (CNS). A

previous study has identified T-helper 2 cells reactive to myelin basic protein (MBP) in peripheral blood of patients with ADEM [6]. Although these cells are observed only during the recovery phase of the disease, suggesting that they are not necessarily pathogenic themselves, they might play other roles in the pathogenesis of ADEM, such as regulation rather than induction of the disease. One possible mechanism mediating the pathogenesis of ADEM is a molecular mimicry between antigens of hymenoptera venom peptides and the MBP of the human CNS. Hymenoptera corresponding to the occurrence of ADEM may fall into at least two families, which include *Vespidae* (wasps, hornets, yellow jacket) and *Apidae* (honeybees, bumblebees). Their clinically important immunogens are primarily found among the enzymes. Phospholipase A2 and melittin are found in bee venom, and antigen 5 and phospholipase A1 are found in wasp venom, while both bee and wasp venoms contain hyaluronidase. Among vespids, yellow jacket antigen 5, hyaluronidase, and phospholipase A1 were shown to have high degree of sequence similarities with the homologous proteins of hornets and wasps [7]. Activation by bacterial or viral superantigens that trigger T cells bearing a particular T-cell receptor (TCR) V β segment, and activation by bacterial or viral peptides are the important mechanisms accounting for the activation and clonal expansion of autoreactive T cells in the periphery [8].

Almost one third of patients initially given a diagnosis of ADEM will progress to multiple sclerosis (MS) upon long-term follow-up [9]. It is conceivable that the underlying pathogenic molecular mechanism of relapsing-remitting MS and the recurrent symptoms occurring in some patients with ADEM are similar and referred to as 'epitope spreading', although this phenomenon has not yet been clarified in disease induction. There is no evidence regarding the pathogenic immunodominant MBP peptide in ADEM or whether this peptide is the same as that underlying MS. Two regions of human MBP, residues 84-102 and 143-168, were found to be immunodominant in MS [10]-[12]. HLA-DR2b (DRA, DRB1*1501) was found to be the restriction element of MBP84-102 peptide-specific T-cell clones, and structural characterization of the MBP85-99 peptide identified residues

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critical for MHC class II binding and for TCR recognition [11]. Hydrophobic residues, Val-89 and Phe-92, in the MBP88-93 peptide (VVHFFK) were identified as anchor residues for HLA-DR2b binding, whereas His-90, Phe-91, and Lys-93 were the primary TCR contact residues [11]. Furthermore, the autoantibody epitope shared the same 10-amino acid sequence, VVHFFKIVT (MBP88-97), with the MHC binding/TCR contact residues of the T cell epitope [11]. It has been shown that a cross-reacting epitope between a virus microbe and an endogenous CNS protein need not have identical amino acids in order for T-cell recognition to occur [13]. Structural considerations are increasingly being characterized when explaining antigenic recognition patterns. In the present paper the MHC class II and TCR contact residues of the immunodominant MBP88-93 peptide were subjected to analysis in order to define the set of amino acids permitted at each critical position. The structural criteria were used to search a protein sequence database of hyaluronidase, phospholipase A1, and antigen 5 of a vespid (*Vespula vulgaris*), and the structural similarities were analyzed in order to support the hypothesis of molecular mimicry and their potential roles for regulation of autoimmunity in ADEM.

II. MATERIALS AND METHODS

A. Sequence Alignment

The UniProtKB Swiss-Prot/TrEMBL release 13.3 protein database (<http://expasy.org/sprot>) with annotations last modified in April 2008, was searched for three *V. vulgaris* venom enzymes: 331-amino-acid hyaluronidase (accession no. P49370), 336-amino-acid phospholipase A1 (accession no. P49369), and 227-amino-acid antigen 5 (accession no. Q05110). Two local sequence alignment programs, SIM (<http://expasy.org/tools/sim-prot.html>) and LALIGN (http://www.ch.embnet.org/software/LALIGN_form.html), were used to investigate local sequence identities, including conservative and non-conservative replacements, between the 15-amino-acid immunodominant human MBP residues 85-99 (ENPVVHFFKNIIVTPR) and residues of *V. vulgaris* hyaluronidase, phospholipase A1, and antigen 5 [15].

B. Structural Similarity Analysis

Structural similarity analysis was performed based on knowledge of properties of amino acid side chains including hydrophobic or hydrophilic character, polar or nonpolar nature, and the presence or absence of ionizable groups. The immunodominant MBP residues 88-93 (VVHFFK) was used as the structural motif to identify, by means of the SIM program with some manual adjustments, *V. vulgaris* peptides that structurally fit the MBP88-93. This motif bound with high affinity to the HLA-DR2b molecule, with two hydrophobic residues serving as the primary anchors (Val-89 and Phe-92) and three primary TCR contact residues at His-90, Phe-91, and Lys-93. For the MHC anchor residues, aliphatic amino acids were allowed at the first residue (Val-89), and aliphatic and aromatic residues were permitted at the second residue (Phe-

92). For the TCR contact residues, Phe-91 could be substituted by some aromatic or aliphatic amino acids; Lys-93 could only be substituted by arginine; and several structurally related amino acids were permitted at Val-88 and His-90 [16]. Percentages of structural similarity for each peptide pair compared were calculated.

C. Visualization of the Peptide Structure

To visualize the structure of each peptide for the similarity comparison, their established structures were searched on the RCSB Protein Data Bank website (<http://www.rcsb.org/pdb/home/home.do>). Three protein crystal structures were found: HLA DR2 (DRA*0101, DRB1*1501) complexed with a peptide from human MBP (PDB ID: 1bx2), bee venom hyaluronidase (PDB ID: 1fcu), and *V. vulgaris* antigen 5 (PDB ID: 1qnx) [17]-[19]. The Swiss-PdbViewer software (version 3.7; <http://expasy.org/spdbv>) was used to view and analyze the structures [20].

III. RESULTS

A. Can wasp venom peptides act as molecular mimics of the human HLA-DR2b-associated immunodominant MBP85-99?

The wasp venom peptides were searched on the basis of the structural characterization of the immunodominant MBP85-99 peptide, and focused on the residues 88-93, which are critical for MHC class II binding and for TCR recognition [16]. In addition to these criteria, two local sequence alignment programs were also used to identify local sequence identities, including conservative and non-conservative replacements. The peptides with relatively high sequence similarity compared to other peptides were selected for further structural similarity analysis. There was no obvious sequence similarity between the three hymenoptera peptides and the MBP85-99 (Table I). The greatest sequence identity was 33.3% for one peptide of hyaluronidase, 20.0% for two of phospholipase A1, and 26.7% for two of antigen 5. Most of the peptides, however, showed an increase in the percentage of structural similarity compared to that of sequence identity. These findings indicated that simple alignment may not have predicted that the peptides would activate the MBP85-99-specific T cells. Structural similarity analysis focusing on the similarity of hyaluronidase, phospholipase A1, and antigen 5 with the HLA-DR2b-associated immunodominant MBP residues 88-93 revealed that the greatest structural similarity was 100% for phospholipase A1 residues 98-103, 83.3% for hyaluronidase residues 7-12, and 83.3% for antigen 5 residues 109-114.

The present findings suggest that some hymenoptera venom peptides, particularly phospholipase A1 residues 98-103, may potentially act as molecular mimics of the human HLA-DR2b-associated immunodominant MBP88-93.

B. Are hymenoptera venom peptides a target for MBP-specific autoantibodies?

TABLE I
SEQUENCE ALIGNMENT OF HYMENOPTERA VENOM PEPTIDES WITH HUMAN HLA-DR2B ASSOCIATED IMMUNODOMINANT MBP 85-99 AND THEIR STRUCTURAL SIMILARITY WITH MHC CLASS II- AND TCR-CONTACT RESIDUES 88-93.

Peptide	Sequence	Sequence identity (%)	Sequence*	Structural similarity (%)
MBP	85ENPVVHFFKNIVTPR99		VVHFFK	
HYA	275ETDVKKTFQEIHING289	33.3	<u>V</u> KKTFQ	33.3
	78GNITIHQLKFIENLD92	26.7	TIHLQK	66.7
	4PKRVFNIYWNVPTFM18	20.0	<u>V</u> FNIYW	83.3
	204ENDKMSWLFNNQNVL218	20.0	<u>K</u> MSWLF	50.0
	55EFPALLSLKDGKYKK69	20.0	<u>A</u> LLSLK	66.7
MBP	85ENPVVHFFKNIVTPR99		VVHFFK	
PHO	238TLGTVDVDFYMNGKNQ252	20.0	<u>T</u> VDFYM	33.3
	82VVFIHGGFTSSASET96	13.3	IHGGFT	50.0
	95ETNFINLAKALVDKD109	20.0	<u>F</u> INLAK	100.0
	153TQKLVKHYKISMANI167	13.3	<u>L</u> VKHYK	66.7
MBP	85ENPVVHFFKNIVTPR99		VVHFFK	
AG5	90QPAPAKNMKNLVWND104	26.7	<u>P</u> AKNMK	50.0
	163YNPKKKFSGNDFLKT174	26.7	KKKFSG	16.7
	106LAYVAQVWANQCQYG120	13.3	<u>V</u> AQVWA	83.3

HYA, hyaluronidase; PHO, phospholipase A1; AG5, antigen 5.

*Amino acids with structural similarity with the human MBP 88-93 are indicated by the underlines.

ADEM resembles experimental autoimmune encephalomyelitis (EAE), based on the histological findings and clinical manifestations. EAE is a CNS disease mediated by activated T cells recognizing several different myelin protein antigens including MBP, and is generally accepted as an animal model of MS. Since local synthesis of MBP-specific autoantibodies in the CNS of MS patients has been demonstrated, and 35% of patients initially diagnosed with ADEM developed MS over a period of 38 months, it was interesting to investigate whether the hymenoptera venom peptides also had the potential to be recognized by MBP-specific autoantibodies [9], [21]. These MBP-specific autoantibodies have been demonstrated to share a similar specificity for the immunodominant MBP peptide with MBP-specific T cell clones from patients with the MS-associated HLA-DR2b haplotype [12]. The 10-amino-acid peptide, VVHFFKNIVT, of the immunodominant MBP85-99 peptide was the specific site for autoantibody binding. The three hymenoptera venom peptides that showed relatively high structural similarity with MHC binding/TCR contact residues of the T cell epitope were then analyzed (Table II and Fig. 1). The residues 98-107 of phospholipase A1 95-109-peptide, which showed high percentages of structural similarity with T cell-contact residues, remained at a rather high degree of structural similarity of 90.0% (Table II). Although the crystallographic structure of phospholipase A1 is not yet established, three-dimensional structural comparison between the peptide VVHFFKNIVT of MBP and the peptide VFNIYWNVPT of hyaluronidase showed that both peptides

are more similar than that compared between the peptide VVHFFKNIVT of MBP and the peptide VAQVWANQCQ of antigen 5 (Fig. 1) [19], [22], [23]. These findings were corresponding to the percentages of structural similarity shown in table 2. However, differences in the conformational epitope of the peptides could affect the accessibility of the antibody to bind its potential binding site on the peptides. Moreover, the antibody-binding motif of MBP has been demonstrated to be located in the epitope center and require sequence identity at four or five contiguous residues, FFKN or FFKNI [12]. These findings imply that these three hymenoptera venom peptides may not have a potential for autoantibody recognition, and that hymenoptera sting-associated ADEM is a T cell-mediated autoimmune disease, like postinfectious ADEM as previously described [24].

IV. DISCUSSION

ADEM is one of the neurological diseases seen after hymenoptera stings and appears to occur after sensitization of peripheral lymphocytes to neural tissue, resulting in an inflammatory response directed against CNS antigens. Hymenoptera venom contains a number of pharmacologically active substances and a number of nonmyelin proteins or peptides that, conceivably, can be encephalitogenic in susceptible individuals by initiating production of specific T cells and antibodies that will immunologically cross-react with MBP [6].

TABLE II.
SEQUENCE ALIGNMENTS OF HYMENOPTERA VENOM PEPTIDES AND THEIR STRUCTURAL SIMILARITY WITH AUTOANTIBODY BINDING RESIDUES, 88-97, OF HUMAN HLA-DR2B-ASSOCIATED IMMUNODOMINANT MBP 85-99.

Peptide	Sequence*	Structural similarity (%)
MBP 85-99	E N P <u>V</u> <u>V</u> <u>H</u> <u>F</u> <u>F</u> <u>K</u> <u>N</u> <u>I</u> <u>V</u> <u>T</u> <u>P</u> <u>R</u>	
HYA 4-18	P K R <u>V</u> <u>F</u> <u>N</u> <u>I</u> <u>Y</u> <u>W</u> <u>N</u> <u>V</u> <u>P</u> <u>T</u> <u>F</u> <u>M</u>	80.0
PHO 95-109	E T N <u>F</u> <u>I</u> <u>N</u> <u>L</u> <u>A</u> <u>K</u> <u>A</u> <u>L</u> <u>V</u> <u>D</u> <u>K</u> <u>D</u>	90.0
AG5 106-120	L A Y <u>V</u> <u>A</u> <u>Q</u> <u>V</u> <u>W</u> <u>A</u> <u>N</u> <u>Q</u> <u>C</u> <u>Q</u> <u>Y</u> <u>G</u>	70.0

*Amino acids with structural similarity with residues of the human MBP 88-93 are indicated by the underlines.

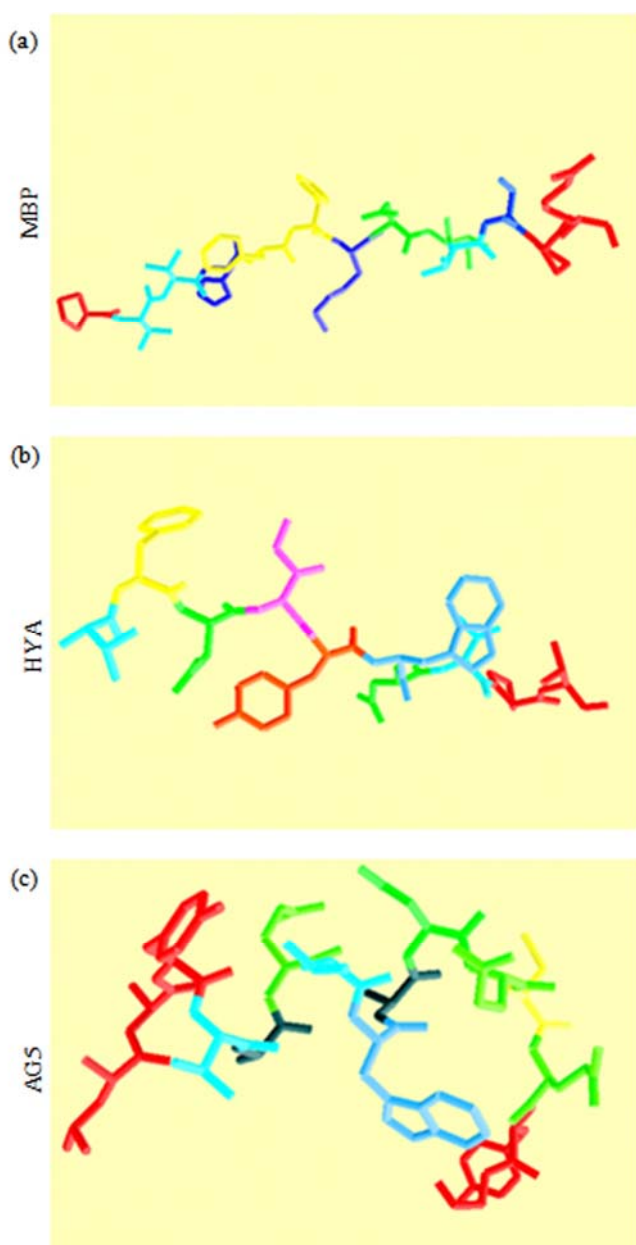


Fig. 1 Magnified views of three-dimensional structures the peptide VVHFFKNIIVT of MBP (a), the peptide VFNIYWNVPT of hyaluronidase (b), and the peptide VAQVWANQCQ of antigen 5 (c), visualized using the Swiss-PdbViewer.

The sharing of a linear amino acid sequence from different genes or a conformation fit between a microbial or environmental determinant and a host self-antigen is the basic concept of molecular mimicry, which has been proposed as a pathogenic mechanism for several autoimmune diseases, including MS. A single TCR can recognize several distinct but structurally related peptides from multiple pathogens, suggesting that T-cell mimicry is not the result of a minor degree of cross-reactivity but rather the result of structural similarity sufficient for potent T-cell activation [16]. Sharing of six amino acids is sufficient to produce immunologic cross-reactivity, and a six-amino-acid peptide can sensitize MBP-

specific T cells to cause EAE [25]. The present bioinformatic analysis study was based on the hypothesis that small motif sequence and structural similarities between hymenoptera venom peptides and human HLA-DR2b-associated immunodominant MBP88-93 peptide, which contains the primary TCR and MHC class II contact residues, could have an important role in the pathogenesis of wasp sting-associated ADEM.

The molecular mimicry motifs were defined by searching a protein database for *V. vulgaris* hyaluronidase, phospholipase A1, and antigen 5. The hyaluronidase peptide residues 7-12 (VFNIYW) and antigen 5 peptide residues 109-114 (VAQVWA) contained five amino acids that fit the criteria except its last amino acid that did not match Lys-93 TCR contact residue of MBP. The phospholipase A1 peptide residues 98-103 (FINLAK) showed 100% structural similarity with the MBP residues 88-93. Although substitution of Phe-91 of MBP by alanine abolished TCR recognition, it did not affect other positions substituted by alanine, including Ala-102 of phospholipase A1 and Ala-114 of antigen 5 [16]. Interestingly, for all the peptides, based on simple alignment, I would not have predicted them to be efficient stimulators of MBP88-93 peptide-specific T cells, as shown by the very low percentage of sequence similarity. Like hyaluronidase residues 7-12 and antigen 5 residues 109-114, adenovirus type 12 residues (VVTFLK) and *Pseudomonas aeruginosa* residues (LMLFAK) contained an unmatched residue at a position equivalent to His-90 of the MBP88-93 peptide; however, these two peptides could efficiently stimulate the MBP88-93 peptide-specific T-cell clones [16]. In MS, the MHC/TCR contact residues for HLA-DR2b-restricted T cell clones were located in the same 10-amino acid segment that represented the autoantibody epitope. The autoantibody binding, however, required sequence identity at four or five contiguous residues in the epitope center [12]. Autoantibodies to MBP have not yet been identified in either patients with ADEM or in experimental animals with EAE. The present paper also showed that the molecular mimicry motifs from three wasp venom peptides were not a recognizable site for binding of the MBP88-97 peptide-specific autoantibodies on the linear peptide segment of MBP despite their rather high degree of structural similarities.

The novel point of this bioinformatic study is the result of the structural similarity analysis, which suggests that the three hymenoptera venom peptides, particularly phospholipase A1 residues 98-103, may potentially act as the molecular motifs of the immunodominant MBP88-93 peptide and be 'probable epitopes' for binding of MBP-specific T cells, and therefore possibly present a mechanism for induction of EAE or even ADEM. Further studies are necessary to confirm the immunodominant properties of wasp venom peptides. *In vitro* studies are needed to test the ability of the peptides to activate human MBP85-99 peptide-specific T-cell clones established from blood T cells of patients with MS or MBP-specific T-cell clones from patients with ADEM in animals. This could be

achieved by means of immunization with wasp venom peptides that structurally mimic human MBP88-93 peptide to determine the ability to produce EAE. Studies are also needed to examine patients who have disease after sting compared with those who do not to determine whether there is a HLA-DR2b (DRA, DRB1*1501) specificity for recognition and disease development.

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