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Structural similarity between the bee venom peptides and the immunodominant human myelin basic proteins: Role for pathogenesis of acute disseminated encephalomyelitis

To the Editor:

Acute disseminated encephalomyelitis (ADEM) or other related acute encephalitis-myelitis has been reported to develop after Hymenoptera sting.¹ There is, however, no evidence demonstrating how Hymenoptera venom peptides mediate an inflammatory demyelinating disorder of the central nervous system (CNS). A recent study identified T_H2 cells reactive to myelin basic protein (MBP) in peripheral blood of patients with ADEM.² 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 of induction of ADEM is a molecular mimicry between the antigens of Hymenoptera venom peptides and the MBP of the human CNS.

Bee venom is a complex mixture of enzymes and peptides that are more well defined than those of wasp venom. Bee venom peptides are composed of many low-molecular-weight peptides. Of these peptides, melittin, apamin, and mast cell degradation peptide 401 (MCD peptide 401) are the major substances, and their amino acid sequences are known, including that of tertiapin, a minor venom peptide. 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 that have sufficient sequence similarity with an immunodominant self-peptide are the important mechanisms accounting for the activation and clonal expansion of autoreactive T cells in the periphery.³ Two recent studies showed that almost one third of patients initially given a diagnosis of ADEM will

progress to multiple sclerosis (MS) on long-term follow-up.⁴ It is conceivable that the underlying pathogenic molecular mechanisms of relapsing-remitting MS and the recurrent symptoms occurring in some patients with ADEM are similar; this is referred to as epitope spreading. There is no evidence regarding the pathogenic immunodominant MBP peptide in ADEM or whether this peptide is the same as that of MS. Two regions of human MBP, residues 84 to 102 and 143 to 168, were found to be immunodominant in MS.⁵⁻⁸ 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 critical for MHC class II binding and for TCR recognition.⁷ Hydrophobic residues (Val-89 and Phe-92) in the MBP88-93 peptide were identified as anchor residues for HLA-DR2b binding, whereas His-90, Phe-91, and Lys-93 were the primary TCR contact residues.⁷ In the present report the MHC class II and TCR contact residues of the immunodominant MBP88-93 peptide were subjected to analysis to define the set of amino acids permitted at each critical position. The structural criteria were then used to search a protein sequence database of melittin, apamin, MCD peptide 401, and tertiapin of a honeybee (*Apis mellifera*), and the structural similarity between these venom peptides and the immunodominant MBP88-93 peptide was analyzed to support the hypothesis of molecular mimicry and their potential role for induction of autoimmunity.

SWISS-PROT release 40.20 protein database was searched for 4 *A. mellifera* venom peptides: 26-amino-acid melittin (accession no. P01503), 18-amino-acid apamin (accession no. P01500), 22-amino-acid MCD peptide 401 (accession no. P01499), and 21-amino-acid tertiapin (accession no. P56587). T-Coffee (Tree-based Consistency Objective Function for alignment Evaluation, version 1.40) was used to investigate global sequence similarity between the 15-amino-acid immunodominant human MBP residues 85 to 99 and residues of *A. mellifera* melittin, apamin, MCD peptide 401, and tertiapin.

For structural similarity analysis, on the basis of the knowledge of properties of amino acid side chains, including hydrophobic or hydrophilic character, polar or nonpolar nature, and presence or absence of ionizable groups, the immunodominant MBP residues 88 to 93 (VVHFFK), which bound with high affinity to the HLA-DR2b molecule, with 2 hydrophobic residues serving as the primary anchors (Val-89 and Phe-92), and which had primary TCR contact residues at His-90, Phe-91, and Lys-93,^{7,8} was used as the structural motif to identify, by means of the local alignment program (SIM) in combination with manual adjustment, *A. mellifera* peptides that structurally fit the MBP88-93 peptide. 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 with some aromatic or aliphatic amino acids, Lys-93 could only be substituted with arginine, and several structurally related amino acids were per-

mitted at Val-88 and His-90. Percentages of structural similarity for each peptide pair compared were calculated.

T-Coffee is more accurate than the conventional ClustalW program for sequences with less than 30% identity. When analyzed by using this program, sequence identity decreased in the order of MBP-MCD peptide 401 (22.7%), MBP-apamin (22.2%), MBP-tertiapin (19.0%), and MBP-melittin (15.4%; data not shown).

When aligned to MBP residues 85 to 99 and analyzed with a local similarity program, the greatest sequence identity was 33.3% for one peptide of MCD peptide 401 (Table I). Structural similarity analysis between 4 *A mellifera* peptides and the HLA-DR2b-associated immunodominant MBP residues 88 to 93 revealed that the greatest structural similarity was 83.3% for melittin residues 16 to 21. Comparison of other peptides of MCD peptide 401 and tertiaryapin with MBP residues 88 to 93 showed no more than 16.7% sequence identity and 50% structural similarity. This preliminary report suggests that some peptides of bee melittin might act as molecular mimics of the human HLA-DR2b-associated immunodominant MBP88-93 peptide.

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.⁹ Sharing of 6 amino acids is sufficient to produce immunologic cross-reactivity, and a 6-amino-acid peptide can sensitize MBP-specific T cells to cause experimental allergic-autoimmune encephalomyelitis (EAE).¹⁰ ADEM is one of the neurologic 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. Bee 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 cross-react immunologically with MBP.²

The novel point of this letter is the result of structural similarity analysis in which the amino acid sequence of the HLA-DR2b-associated immunodominant MBP88-93 peptide, which contains the primary TCR and MHC class II contact residues, was used as the molecular recognition motif. Then molecular mimicry motifs were defined by searching a protein database for *A mellifera* melittin, apamin, MCD peptide 401, and tertiaryapin sequences that fit these regions. Melittin residues 16 to 21 (LISWIK) contained 5 amino acids that fit the criteria, except for residue 18, which did not match His-90 TCR contact residue of MBP. Interestingly, simple alignment would not have predicted them to be efficient stimulators of MBP88-93 peptide-specific T cells, as shown

TABLE I. Sequence alignment of *Apis mellifera* melittin, apamin, MCD peptide 401, and tertiaryapin with human HLA-DR2b-associated immunodominant MBP88-93 peptide and their structural similarities with MHC class II and TCR contact residues 88-93

Peptide	Sequence*	Sequence identity (%)	Structural similarity (%)
MBP	85ENPVVHFFKNIVTPR99		
Melittin	1GIGAILKVLSTGL13	16.7	66.7
	2IGAILKVLSTGLPAL16	0	66.7
	13LPALISWIKRKRQE26	16.7	83.3
	14PALISWIKRKRQE26	0	66.7
MBP	85ENPVVHFFKNIVTPR99		
Apamin	6PETALCARRCQQH18	0	66.7
MBP	85ENPVVHFFKNIVTPR99		
MCD401	3CNCKRHVIKPHICRK17	33.3	66.7
	8HVIKPHICRKICGKN22	16.7	50.0
MBP	85ENPVVHFFKNIVTPR99		
Tertiaryapin	1ALCNCNRRIIPHM13	0	50.0
	6NRIIPHMCWKKCGK20	0	50.0
	7RIIPHMCWKKCGKK21	16.7	50.0
	9IIPHMCWKKCGKK21	16.7	50.0

*Amino acid sequences compared with the human MBP88-93 peptide are underlined, and amino acids with structural similarity with those of the human MBP88-93 peptide are in bold.

by the very low percentages of sequence similarity. Like the melittin16-21 peptide, adenovirus type 12 residues (VVTFLK) and *Pseudomonas aeruginosa* residues (LMLFAK) contained unmatched residue at a position equivalent to His-90 of the MBP88-93 peptide; however, these 2 residues could efficiently stimulate the MBP88-93 peptide-specific T-cell clones.⁹

The present study suggests that bee melittin16-21 peptide might act as a molecular mimic of the immunodominant MBP88-93 peptide and therefore possibly present a mechanism for induction of EAE or even ADEM. Further studies are necessary to confirm the immunodominant properties of bee venom peptides. In vitro studies are needed to test their ability 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 bee 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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Treatment with omalizumab normalizes the number of myeloid dendritic cells during the grass pollen season

To the Editor:

Humanized anti-IgE antibodies are safe and effective in treating patients with allergic rhinitis and asthma.¹ Therapeutic anti-IgE antibodies such as omalizumab have a unique binding specificity in that they bind to soluble IgE and to membrane-bound IgE on IgE-expressing B cells but not to IgE already bound by high-affinity receptors (FcεRI) on mast cells and basophils or by low-affinity IgE Fc receptors (FcεRII) on other cell types.² In addition to neutralizing free IgE and inhibiting IgE production by B cells,³ omalizumab might have additional immunomodulating effects on antigen-presenting cells.⁴ Recent research indicates that dendritic cells (DCs) are specialized for antigen uptake, processing, and presentation to T cells. Two subsets of DCs were identified in the blood: CD11⁺ myeloid DCs and CD123⁺ plasmacytoid DCs. Each represented a small fraction (0.3%) of mononuclear cells, and both subtypes differ in origin, in the route of activation, and in immunologic activity.⁵ A flow cytometric assay was designed to detect the 2 different subsets of peripheral blood dendritic cells.⁶ In this report, this assay was used to determine the frequency of DC subpopulations in children and adolescents treated with omalizumab.

Forty-nine patients were enrolled in this substudy. They were treated at 2 centers (Bochum and Wesel)

located in the western region of Germany as part of a phase IIIb, multicenter, randomized, double-blind, placebo-controlled, multiple-dose, parallel-group rhinitis study investigating the safety and efficacy of subcutaneously administered omalizumab in children and adolescents with seasonal allergic rhinitis (SAR). The whole study population consisted of 221 children and adolescents (6 to 17 years of age), each having a clinical history of birch pollen-induced and grass pollen-induced SAR within the previous 2 years. Signed informed consent was obtained from all subjects and their guardians, and the study design was approved by the ethics committees of the participating centers. The details of the core study are described elsewhere.⁷

Four treatment arms used omalizumab and specific immunotherapy (SIT): SIT/birch + omalizumab; SIT/birch + placebo; SIT/grass + omalizumab; and SIT/grass + placebo. SIT for birch or for grass (ALK-Scherax/Abello, Berlin, Germany) was administered with increasing doses with 12 weekly injections.⁷ Thereafter, patients were switched to 5 maintenance injections of SIT in a 4-week interval; placebo or omalizumab was added to the SIT for 24 weeks. Omalizumab was administered on the basis of body weight and serum total IgE, providing a dose of at least 0.016 mg/kg per IU/mL of IgE every 4 weeks.

During the birch season (>10 grains/m³ per 24 hours; April 3 to May 1) and the grass season (> 5 grains/m³ per 24 hours; May 11 to June 23), blood was obtained and stained for DCs. The respective mean cumulative doses at the time of blood collection during the relevant pollen season did not differ between patients treated with omalizumab and patients given placebo (SIT birch, 494630 versus 496335 SQ units; SIT grass, 614722 versus 634461 SQ units). Daily symptom severity scores (eyes and nose) and rescue medication usage (for definitions, see Kuehr et al⁷) were assessed during the pollen seasons.

Staining procedures were performed as follows: 100 μL of whole blood was incubated with 20 μL of the lineage cocktail fluorescein isothiocyanate, 10 μL anti-human leukocyte antigen D-related (HLA-DR) peridinin chlorophyll protein (PerCP), and either 5 μL CD11c PE, 5 μL CD123 PE, or the appropriate isotype controls (all from Becton Dickinson, Heidelberg, Germany). Measurements were performed on a FACScan flow cytometer (Becton Dickinson) equipped with a Power Macintosh G3 computer and CellQuest software. DCs were identified as showing no labeling for the lineage cocktail and strong labeling for HLA-DR. Through the use of 3-color analysis, the proportion of strongly CD123-labeled plasmacytoid cells and of CD11c⁺ myeloid cells among DCs was determined. The absolute number of blood DCs was calculated on the basis of the percentage of DCs and the absolute number of mononuclear cells, as determined by the blood count.⁶

The results are shown in Fig 1. DC numbers did not differ between patients with SIT birch and patients with SIT grass, and there was no difference between placebo-treated and omalizumab-treated patients in the birch season. Indeed, DC numbers in healthy age-matched control sub-