ALLERGIC ENCEPHALOMYELITIS: CHARACTERIZATION OF THE DETERMINANTS FOR DELAYED TYPE HYPERSENSITIVITY

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SUMMARY

Three non-encephalitogenic peptides derived from the encephalitogenic myelin basic protein of the central nervous system, produce delayed type hypersensitivity responses and elicit delayed skin reaction in guinea pigs sensitized with either peptide, the encephalitogenic tryptophan region (peptide E) or the basic protein. The amino acid sequence of the peptides is N-Acetyl-Ala-Ser-Ala-Gln-Lys-OH, forming the N-terminal region of the basic protein molecule, H-Gly-Ser-Leu-Pro-Gln-Lys-OH and H-Gly-Ala-Glu-Gly-Gln-Lys-OH representing residues number 69-74 and 117-122 of the basic protein respectively.

Experimental Allergic Encephalomyelitis (EAE) is an autoimmune disease of the central nervous system associated with an inflammatory reaction of cellular or delayed type hypersensitivity (DTH) (1,2). The experimental disease provides a model for study of normal tissue rejection utilizing pure antigens such as the basic protein (BP) or peptides derived from it which induce immune responses in experimental animals (3-8). Several antigenic determinants have been ascribed to the BP. In addition to the production of circulating antibodies, animals sensitized with BP reveal DTH reactions which correlate with subsequent onset of EAE regardless of the source of the basic protein (9,10). The amino acid regions of the basic protein molecule responsible for induction of EAE in guinea pigs and rabbits were isolated and characterized (11-13). In vitro (14,15) and in vivo (16-19) studies have shown that the encephalitogenic tryptophan peptide derived from bovine or human myelin basic protein induce cellular immunity

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necessary for DTH responses in experimental animals. In this report we describe the amino acid sequence and immunological properties of three peptides derived from the bovine basic protein and responsible for the induction of DTH in guinea pigs.

EXPERIMENTAL

The basic protein was isolated from bovine spinal cords and purified by Cellex-P column chromatography (5). All peptides used in this study were separated by preparative high voltage electrophoresis at pH 4.7 in pyridine: acetic acid: n-butanol: water (1:1:2:36 v/v/v/v) and purified by ascending chromatography on Watman 3MM filter paper using n-butanol: acetic acid: pyridine: water (61:19:90:75 v/v/v/v).

Peptide CTP-1 (45 mg) was isolated from the chymotrypsin digests of the bovine basic (A1) protein (19). Peptide CTP-1 contains 16 amino acid residues lacking tryptophan, tyrosine, methionine and phenylalanine with a calculated molecular weight of 1776 daltons. Peptide CTP-1a and CTP-1b were derived from peptide CTP-1 following hydrolysis of the latter with the enzyme trypsin and purified by high voltage electrophoresis and chromatography on paper (19). Peptide E (the tryptophan containing peptide) was isolated from the pepsin digest of the BP as previously described (11). Peptide CB1-T1 was derived from the trypsin digest of peptide CB1, a cyanogen bromide fragment of the bovine basic protein (20). Synthetic peptides CTP-3S, CB1-T1S and T8 were made by the Merrifield solidphase procedure (21-23). For peptide T8, the modified Merrifield procedure (24) was followed. The EAE assay was performed in outbred guinea pigs following established procedures (1,2,3-8). Saline solutions of the antigen were emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan) and a total of 0.1 ml emulsion was injected in the hind foot pad. The sensitized guinea pigs were examined daily for clinical signs of EAE (1,2). Thirty days following sensitization, the animals were terminated by bleeding under ether anesthesia. The brain and spinal cord tissues were isolated for histological examination

of sections stained with hematoxylin and eosin. The DTH test was performed at 10 or 12 days following sensitization. Sterile saline solutions of the antigens were injected intracutaneously. The skin test site was inspected at 1, 3 and 24 hours and the area of the erythema was recorded.

RESULTS AND DISCUSSION

The complete amino acid sequence of peptide CTP-1 and those of other natural and synthetic peptides are shown in table I. In the intact basic protein molecule, the N-terminal glycine of peptide CTP-1 is linked to the carboxyl group of tyrosine, residue #68, in the following sequence: -Thr-Thr-His-Tyr-Gly-Ser-Leu-Pro-Gln-Lys-, known to be an encephalitogenic determinant recognized by the rabbit (13). The enzyme chymotrypsin hydrolyzes the C-tyrosyl bond thus destroying the encephalitogenic property of this region of the basic protein molecule. At concentrations of 33 and 500 ug injected into guinea pigs emulsified in CFA, peptide CTP-1 was non-encephalitogenic. The classical EAE symptoms, clinical or histological, normally observed in animals sensitized with peptide E or the basic protein, were not seen in guinea pigs sensitized with peptide CTP-1; however, peptide CTP-1 sensitized animals showed a delayed type response when skin tested with the same antigen (table II). Animals sensitized with basic protein, peptide E, T8, CTP-1, CTP-1a, CTP-3S, CB1-T1 or CB1-T1S and skin tested with either or all antigens separately, a positive skin response was obtained regardless of the antigen used for sensitization suggesting the presence of an antigenic determinant common to all peptides and the basic protein. Peptide CTP-1 was hydrolyzed with trypsin into two portions, peptide CTP-1a and peptide CTP-1b made up of residues number 1 through 6 and 7 through 16 respectively. The skin test reactivity of peptide CTP-1 and the ability to induce a DTH response in guinea pigs were localized in peptide CTP-1a, the N-terminal six amino acid residues of peptide CTP-1. Also, animals sensitized with peptide CTP-1a gave a positive DTH when skin tested with the same peptide (table II). Control animals gave negative skin responses to BP and peptide antigens. Similarly, sensitized animals gave

Table I. The Amino Acid Sequence of Natural and Synthetic Peptides

Peptide	Amino Acid Sequence	Source
	1 5 10 15	
CTP-1	H-Gly-Ser-Leu-Pro-Gln-Lys-Ala-Gln-Gly-His-Arg-Pro-Gln-Asp-Glu-Asn-OH	Natural
CTP-1a	H-Gly-Ser-Leu-Pro-Gln-Lys-OH	Natural
CTP-1b	H-Ala-Gln-Gly-His-Arg-Pro-Gln-Asp-Glu-Asn-OH	Natural
E	H-Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Pro-Gly-Phe-OH	Natural
CTP-3S	H-Gly-Ala-Glu-Gly-Gln-Lys-OH	Synthetic
CB1-T1	N-Ac-Ala-Ser-Ala-Gln-Lys-OH	Natural
CB1-T1S	N-Ala-Ser-Ala-Gln-Lys-OH	Synthetic
T8	H-Thr-Thr-His-Tyr-Gly-Ser-Leu-Pro-Gln-Lys-OH	Synthetic

The natural peptides CTP-1 and peptide E were derived from the bovine basic protein following hydrolysis with pepsin and chymotrypsin respectively (13,19). Peptides CTP-1a and CTP-1b were derived from peptide CTP-1 following hydrolysis with trypsin (19). Peptide CB1-T1 was isolated from the trypsin digest of peptide CB1, a cyanogen bromide fragment of the basic protein (20). The synthetic peptides were prepared by the Merrifield solidphase technique (21-24).

Table II. Immunological Properties of Natural and Synthetic Peptides

		Peptides									
Sensitizing Antigen dose ug/CFA		Basic Protein 25 ug	Pep-T8 20	Pep-E 20	CTP 1a 100	CTP 1b 200	CTP 3S 100	CB1 T1 100	CB1 T1S 100	CTP-1 33	CTP-1 500
Skin Testing antigen	dose (ug)	DAYS AFTER SENSITIZATION 10	12	12	12	12	12	12	12	12	12
BP	10	10	10	11	8	2	8	10	12	11	10
CTP-1	20	14	14	13	15	2	9	10	10	12	10
CTP-1a	200	11	10	11	11	2	12	10	10	9	12
CTP-1b	200	2	2	2	2	4	2	2	2	2	2
E	150	12	11	10	8	2	10	10	10	10	10
CTP-3S	200	10	10	12	10	2	10	9	10	10	-
CB1-T1	200	12	10	10	12	2	10	10	10	11	9
EAE (30 D):											
Clinical		35/40	0/40	34/40	0/40	0/40	0/40	0/40	0/40	0/40	0/40
Histological		35/40	15/40	40/40	0/40	0/40	0/40	0/40	0/40	0/40	0/40

Groups of 40 guinea pigs were sensitized with indicated antigens emulsified in equal volume of complete Freund's adjuvant (CFA). The emulsion (0.1 ml) was injected in both hind foot pads. The skin test was done at 10 or 12 days following sensitization using the indicated antigens injected subcutaneously in 0.1 ml sterile saline. The values shown are the average of minimum and maximum diameters (mm) of the erythema formed after 24 hours in 5 guinea pigs.

negative skin responses when tested with ovalbumin and bovine serum albumin.

The cross reactivity among the antigens in the skin test of animals sensitized with either of the three antigens used is readily recognized in view of the structural similarities between them. Studies of the chemical structure of peptide E essential for the induction of EAE revealed the requirement for three amino acid residues in the following structure H-X-X-Trp-X-X-X-X-Gln-Lys-Oh (25). Cleavage of the C-tryptophyl bond or its chemical modification renders the basic protein non-encephalitogenic in the guinea pig (26,27). Similarly, substitution of the glutamine or the lysine with isoleucine renders the resulting peptide E similarly inactive; however, substitution of other residues such as glutamic acid, serine or phenylalanine had no influence on the activity of the peptide (25). In contrast to peptide E, T8 and CB1; the corresponding residues forming the N-terminal region of peptide CTP-1 where all residues except glycine are naturally substituted with the exception of glutamine and lysine at the C-terminal end (table I), peptides CTP-1a, CTP-3S and CB1-T1 induced delayed hypersensitivity in experimental animals without concomitant EAE induction (table II). Indeed the absence of Ser-Arg-Phe-Ser-Trp- and Thr-Thr-His-Tyr sequence from the N-terminus of peptide E and T8 respectively and the absence of the N-Acetyl blocking group from peptide CB1-T1 does not alter the delayed hypersensitivity properties of the corresponding peptides. The DTH determinant, therefore, is defined by 5 amino acid residues or less, examplified by peptide CB1-T1S. Thus, a common relationship exists among the three regions of the basic protein molecule where a glutamine-lysine is found in the following sequence H-X-X-X-Gln-Lys-OH. It is interesting to note that the same sequence is also found in basic proteins isolated from human, monkey, bovine, rabbit and guinea pig CNS tissues. The arginine substituted lysine in the tryptophan region from human myelin BP (28) does not alter the DTH properties of the peptide (7,14,15).

These results clearly show that both encephalitogenic peptides T8, containing tyrosine (13), and peptide E, containing tryptophan (29), have

two overlapping determinants responsible for induction of EAE and delayed type hypersensitivity. The two antigenic determinants were separated; synthetic peptide CTP-3S representing the corresponding portion of peptide E and peptide CTP-1a representing the corresponding region of peptide T8 were non-encephalitogenic but retained full DTH activity. In contrast, peptide CB1 from bovine basic protein, which does not induce EAE in guinea pigs (20), is active in producing DTH. For induction of EAE, both determinants are necessary, in an intact peptide with a specific sequence of amino acids. Antigens having the DTH determinant alone, do not produce hind leg paralysis, incontinence or myelin lesions characteristic of EAE.

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