Allergic Encephalomyelitis in Monkeys Induced by a Peptide from the A1 Protein

(clinical signs/peptide fragments/histopathology/encephalitogenic determinants)

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ABSTRACT A major disease-inducing site for induction of experimental allergic encephalomyelitis in monkeys exists in Peptide P 14, the 37-residue segment of the A1 protein comprising its COOH-terminus. The peptide appears to contain the dominant encephalitogenic determinant, since it was as active as the A1 protein on a molar basis. By contrast, the 9-residue tryptophan region and the Peptide R region, active in guinea pigs and rabbits, respectively, were comparatively inactive in monkeys. The clinical and histologic expression of the disease produced by Peptide P 14 appeared identical to that induced by the intact A1 protein.

It is now well established that the basic A1 protein (molecular weight 18,400), a major component of central nervous system myelin, is the agent that induces experimental allergic encephalomyelitis, an autoimmune demyelinating disease of central nervous system tissue (1-3). With the determination of the amino-acid sequences of the human and bovine A1 proteins (4-6), the discrete antigenic regions responsible for disease induction in the guinea pig (7, 8) and rabbit (9, 10) have been characterized. Because of the unusual open conformation of the A1 molecule, recently proposed (11) to exist in an unfolded double-chain conformation, these antigenic regions appear to exist as short linear segments of the polypeptide chain. Identification of the "disease-inducing sites" has thus been simplified, since they can be obtained as isolated or synthesized (12) peptides that are themselves encephalitogenic (1). The use of these small immunogenic peptides should help to clarify the immunologic processes implicated in demyelinating disease in animals and man.

Guinea pigs, rats, or rabbits are usually used for encephalomyelitis assay (13). Kabat and coworkers (14) demonstrated that the monkey is an ideal test animal for encephalomyelitis induced by whole central nervous system tissue. Using the human A1 protein, we (ref. 15 and unpublished data) studied disease induction and suppression in rhesus monkeys. The present work was designed to further clarify the phylogenetic variation in the encephalitogenic response by identification of the major antigenic site responsible for encephalomyelitis in monkeys.

METHODS

Animals. We used rhesus monkeys (young adults, 2-4.5 kg) that were free of tuberculosis, diarrhea, and infection, as shown by frequent periodic examination for appropriate organisms. The diet consisted of Purina monkey chow and fresh fruit. Encephalomyelitis was induced by intradermal injections [0.1 ml each] in both foot pads] of test material

homogenized in saline with an equal volume of Difco complete Freund's adjuvant containing 0.1 mg of *Mycobacterium tuberculosis* (H37Ra). In our laboratory, when 5 mg of human, bovine, or rabbit A1 protein is used, encephalomyelitis is produced in all of the monkeys (15). Sensitized animals were observed daily (2) for clinical signs of encephalomyelitis. Gross and histologic evaluation was performed as described (2).

Peptides. We used peptides derived from homogeneous preparations of either the human or bovine A1 protein (2, 16). Peptide T (residues 116-170) was obtained after treatment with BNPS-skatole* (17); Peptide P 14 (residues 134-170) and Peptide R (residues 44-89) after peptic digestion (5, 10); Peptide T 18 (residues 114-120) after tryptic digestion (5); and synthetic Peptide S3 by the Merrifield solid-state technique (12).

RESULTS

The encephalitogenic activity of various peptides derived from either the human or bovine A1 proteins is shown in Table 1. Two peptides, T and P 14, derived from the COOH-terminal region of the A1 protein, induced encephalomyelitis in all animals tested. The clinical course of encephalomyelitis induced by these peptides was similar to that induced by the A1 protein, both on day of onset and appearance. The clinical signs observed in these animals included loss of appetite, disturbed coordination, lethargy, leg weakness or paralysis, prostration, and death 1 to 3 days after clinical signs were first noted. Blindness was also observed in two animals. Although clinical signs appeared in 80% of the animals injected with these two peptides, all developed histologic lesions characteristic of encephalomyelitis produced by the human A1 protein (unpublished data). Examination of the white matter of the cerebrum, cerebellum, and brain stem revealed lesions ranging from endothelial swelling with mononuclear and polymorphonuclear permeation of the endothelium to extensive perivascular infiltration of polymorphonuclear and mononuclear leukocytes (see Fig. 1). No plasma cells were found. The astrocytes were enlarged and had hyperchromatic nuclei with clumped chromatin. The oligodendroglia were swollen and myelin tracts were prominent. There were areas with neurophagia. Mitotic figures could be found adjacent to areas of infiltration and

* BNPS-skatole; a bromine adduct of 2-(2-nitrophenylsulfenyl)-3-methyl indole.

FIG. 1. Three typical sections from the white matter of the centrum semiovale of monkeys with clinical signs of encephalomyelitis induced by Peptide P 14. (A) Extensive cellular infiltration, which consists primarily of mononuclear cells; (B) A late example of perivascular cuffing is shown in longitudinal section, and consists of mononuclear and polymorphonuclear cells. Lysis of inflammatory cells is also seen; (C) An early lesion, showing swelling of endothelial cells and the presence of both polymorphonuclear and mononuclear cells.

within areas of infiltration. Although pronounced histopathology was observed in all cases, only one animal had grossly visible lesions in the brain.

When compared to the A1 protein (170 amino-acid residues), both Peptides T and P 14 have about the same encephalitogenic activity on a molar basis. In four out of six animals, Peptide T induced clinical signs of encephalomyelitis at 2 mg (0.33 μ mol); Peptide P 14 was active clinically at 0.5–1.0 mg (0.13–0.26 μ mol). 5 mg (0.27 μ mol) of A1 protein induces

clinical signs of encephalomyelitis in all of the animals, but it is much less active under our conditions when 3 mg (0.16 μ mol) are used. It is apparent, therefore, that these two peptides are highly encephalitogenic.

Peptides T 18 and synthetic Peptide S3, both containing a single tryptophan residue, were inactive even when tested at 1 mg (about 1 μ mol), a concentration 8-fold greater than active doses of Peptide P 14. In contrast, these tryptophan-containing peptides are highly active in guinea pigs at concentrations 1000-fold less than used here. In addition, prior injection of Peptide T 18 does not lead to unresponsiveness. In two cases, animals sensitized with Peptide T 18 were injected with 5 mg of A1 protein 45 days later; these animals developed encephalomyelitis after 14 days. 1 mg of Peptide R (0.2 μ mol) produced no signs of encephalomyelitis, either clinically or histologically, as noted (10). After injection of 5 mg (1.0 μ mol) of Peptide R, however, clinical signs appeared, but they were later in onset and less severe than with Peptide P 14. The clinical signs developed slowly over a 5-day period; the animals then died. Thus, on a molar basis, Peptide R is about 10-fold less active than Peptide P 14. However, Peptide R may be inactive, since the activity observed at 5 mg might be due to the presence of Peptide P 14 as a trace contaminant. Peptides R and P 14 elute together from the Cellex P column used for their purification, and are difficult to separate (10).

DISCUSSION

We conclude that a chemical grouping exists in the Peptide P 14 region that is the dominant encephalitogenic determinant in the monkey. Peptide P 14, comprising 37 residues of the COOH-terminal region of the A1 protein, appears as encephalitogenic in monkeys on a molar basis as does the A1 protein, and induces histologic and clinical characteristics of encephalomyelitis identical to those induced by A1 protein.

TABLE 1. *Peptides tested for encephalitogenic activity in monkeys*

Number of monkeys	Amount tested, mg	Peptide	Day of onset	Result	
				Clinical	Histology
3	2	T (bovine)	14, 25, 26	+	+
3	2	T (human)	18, none, none	+, 0, 0	+, +, +
1	0.5	P 14	13	+	+
1	0.9	P 14	18	+	+
2	1.0	P 14	13, 16	+	+
3	0.1, 0.5, 1.0	T 18	—	0	0
3	1.0	S 3 (synthetic)	—	0	0
2	1.0	R (bovine)	—	0	0
3	5.0	R	20, 25, 26	+	+

Encephalitogenic activity was determined by clinical and histologic criteria described in the text. Under the same conditions, the bovine and human A1 proteins were encephalitogenic (by clinical evaluation) at 5 mg in all of the monkeys; at 1 mg only 10–20% of the monkeys showed clinical signs of encephalomyelitis.

Peptide	No. of Residues	Sequence
T	54	Gly-Ala-Glu-Gly-Gln-Lys-Pro-Gly-Phe-Gly-Tyr-Gly-Gly-Arg-Ala-Ser-Asp-Tyr-Lys-Ser-Ala-His-Lys-Gly-Leu-Lys-Gly-His-Asp-Ala-Gln-Gly-Thr-Leu-Ser-Lys-Ile-Phe-Lys-Leu-Gly-Gly-Arg-Asp-Ser-Arg-Ser-Gly-Ser-Pro-Met-Ala-Arg-Arg
P 14	37	Tyr-Lys-Ser-Ala-His-Lys-Gly-Leu-Lys-Gly-His-Asp-Ala-Gln-Gly-Thr-Leu-Ser-Lys-Ile-Phe-Lys-Leu-Gly-Gly-Arg-Asp-Ser-Arg-Ser-Gly-Ser-Pro-Met-Ala-Arg-Arg
T 18	9	Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys
S 3	11	Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg
R	46	Phe-Gly-Ser-Asp-Arg-Gly-Ala-Pro-Lys-Arg-Gly-Ser-Gly-Lys-Asp-Gly-His-His-Ala-Ala-Arg-Thr-Thr-His-Tyr-Gly-Ser-Leu-Pro-Gln-Lys-Ala-Gln-Gly-His-Arg-Pro-Gln-Asp-Glu-Asn-Pro-Val-Val-His-Phe

FIG. 2. The amino-acid sequences of peptides tested for encephalitogenic activity in monkeys.

The sequence of Peptide P 14 (human and bovine), shown in Fig. 2, bears no obvious similarity to either Peptide R or the tryptophan region. Apparently, the Phe to Leu and Val to His substitutions (4), the only changes between the human and bovine sequences in the Peptide P 14 region, are not critical. By analogy to the 9-residue tryptophan determinant, the essential residues may span only a small segment within the 37 residues of Peptide P 14. It is evident that the disease-inducing site in Peptide T must be contained within the segment defined by Peptide P 14.

The 9-residue tryptophan region (see Fig. 2), which is the dominant encephalitogenic determinant in guinea pigs and rabbits, is not active in monkeys. Even when tested at 10-fold greater doses than Peptide P 14 and Peptide T, the tryptophan-containing peptides were inactive. These data are consistent with our results that the HNB-A1 protein (unpublished data), which is inactive in guinea pigs because the tryptophan residue is modified by 2-hydroxy-5-nitrobenzyl bromide, is fully active in monkeys. Therefore, the tryptophan region plays a minor, perhaps insignificant, role in disease induction in the monkey.

The results with Peptide R are inconclusive, and suggest that this region may contain a minor determinant, at least

10-fold less active than the Peptide P 14 region. However, with peptides of low activity the question of possible contamination, in this case with Peptide P 14, must be resolved.

We showed elsewhere (15) that encephalomyelitis induced in monkeys with purified A1 protein is useful experimentally because of the reproducible and predictable expression of clinical signs. Because of the similarity between poststrabismic encephalomyelitis in man, after vaccination with rabies virus, and allergic encephalomyelitis in monkeys (18), our results have relevance to human demyelinating diseases. The encephalitogenic site found in Peptide P 14 assumes major interest because of the ability of the A1 protein to suppress encephalomyelitis in monkeys after clinical signs have developed (15).

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