ALLERGIC ENCEPHALOMYELITIS IN GUINEA PIGS AFTER INTRADERMAL AND SUBCUTANEOUS INJECTION OF FRAGMENT 114-122 OF HUMAN BASIC MYELIN PROTEIN

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In order to study and search for methods of treatment of allergic demyelinating human diseases, including disseminated encephalomyelitis, multiple sclerosis, and progressive leukodystrophies, models developed on animals and, in particular, experimental allergic encephalomyelitis (EAE) are used [2, 6-8].

In guinea pigs EAE can be induced [1, 3, 9-15] by injection of a fragment of basic myelin protein containing tryptophan. The presence and position of tryptophan in the amino acid chain are decisive for induction of EAE. The important role of glutathione and lysine also is emphasized, although the latter can be replaced by arginine [15]. The view has been expressed [11] that other amino acid residues of synthetic peptides play an exclusive role, for replacement of any of them leads to loss of the encephalitogenic properties.

Contradictory information is given in the literature on the clinical effect of injection of encephalitogenic peptides, and to some degree this may be due to the conditions of inoculation of the encephalitogenic mixture into the experimental animals.

The aim of this investigation was to discover conditions of injection of peptide 114-122 of human basic myelin protein into guinea pigs, to produce a marked pathogenic effect.

EXPERIMENTAL METHOD

Experiments were carried out on adult male albino guinea pigs weighing 250-300 g. To prepare the encephalitogenic emulsion the peptide (H-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg-OH, from Serva, West Germany) was dissolved in physiological saline, equal volumes of the peptide solution and of Freund's adjuvant (From Difco, USA) were mixed, and they were then ground for a long time in a porcelain mortar until a homogeneous mass of the consistency of whipped cream was formed. A single injection of the mixture in a volume of 0.66 ml was given to each animal.

In preliminary experiments using the same peptide, it was found that doses of 10 and 30 μg are most appropriate, with two methods of injection — intradermal (into the hind footpads) and subcutaneous.

TABLE 1. Prevalence of EAE in Guinea Pigs after Injection of Peptide 114-122 Mixed with Freund's Complete Adjuvant

Group of animals	Time of experiment	Number of animals in group	Dose of peptide, μg	Mode of injection	Number of animals developing disease	
					absolute	%
1	July	11	10	Intradermally	6	54,5
2	»	11	30	»	9	81,8
3	»	11	10	Subcutaneously	6	54,5
4	»	11	30	»	6	54,5
5	October	12	30	Intradermally	10	84,0

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TABLE 2. Severity of Course of EAE after Injection of Peptide 114-122 Mixed with Freund's Complete Adjuvant

Time of experiment	Dose of peptide, μg	Time of onset of disease after injection of peptide, days	Duration of disease, days	Decrease of body weight, g	Clinical manifestations			Recovery	Death
					tremor	pareses	paralyses		
					%				
July	10	9—17	2—14	40—160	45,5	9	13,5	9	9
	30	11—19	3—14	45—170	27	9	23	0	9
October	30	11—17	2—17	100—195	8	25	25	16,5	33

In the course of the experiments the animals were weighed, their temperature taken, and the presence or absence of pareses and paralyses of the limbs noted daily. To detect pareses and to assess their severity quantitatively, the method of passive extension of the hind limbs, developed in G. N. Kryzhanovskii's laboratory [4, 5], was used.

The experiments continued for 29-31 days.

EXPERIMENTAL RESULTS

The disease developed in 80% of cases and followed a severe course (pareses in 75% of cases, paralyses in 58%, death in 33% of cases) after intradermal injection of 30 μg of the peptide with Freund's complete adjuvant (the total volume of the encephalitogenic mixture was 0.66 ml, including 0.33 ml of Freund's complete adjuvant).

The disease was two or three times more severe in the fall than in the summer (Table 1).

The time of onset of the disease and its duration varied considerably and independently of the dose (within the selected limits) or time of conduct of the experiment (Table 2). Conversely, the degree of loss of body weight and also the relative number of pareses and paralyses depended on the dose and were greater after injection of 30 μg of the peptide in the fall, as also was the percentage of animals which dies (Table 2). Incidentally, more of the animals recovered in the fall than in the summer, i.e., it appears that the course of the disease was more severe in the fall.

The method of passive extension of the hind limbs revealed statistically significant differences between the group of control and the group of experimental animals (Fig. 1).

Comparison of the results with data in the literature is rather difficult because of the absence of information on the pathogenic activity of the peptide used in these experiments.

The 11-member peptide 112-122, which like the nine-member peptide which we used, was synthesized from human basic myelin protein, possesses high pathogenic activity [12]. Injection of that peptide in a dose of 0.1 μg induces EAE in guinea pigs. The authors cited obtained the maximal clinical effect with doses five to 10 times greater. With a further increase in dose the pathological changes were weaker. Thus, the 11-member peptide (112-122), according to data in the literature [12], was significantly more effective than that used in the present investigation.

The nine-member peptide differing from that used in the present investigation by having lysine in position 122, in a dose of 20 μg, induced EAE in guinea pigs with a lethal outcome if injected subcutaneously and intradermally simultaneously [15]. Unfortunately, the authors cited do not describe in detail the development of the disease or the tests used to assess the clinical picture, which once again makes comparison with our own data difficult. On the whole, however, the results of the present investigation did not contradict data in the literature.

Of the tests which we used the time course of the body weight is the one which gives an early indication of the development of EAE. The body temperature in the early period of the disease is characterized by considerable fluctuations. To test for disturbances of muscle tone developing after injection of the encephalitogenic mixture in small animals, passive extension of the hind limbs proved useful. The results obtained by means of this test can be expressed quantitatively.

Thus, the peptide containing fragment 114-122 of human basic myelin protein (H-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg-OH), injected simultaneously together with Freund's adjuvant,

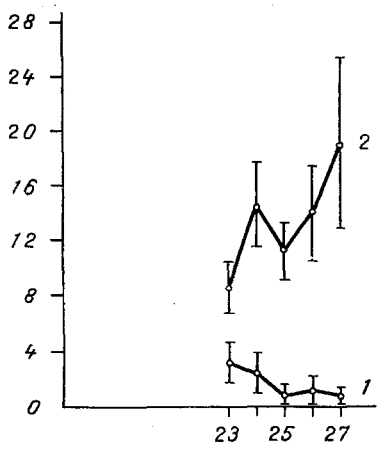

Fig. 1. Time of passive extension of hind limb of a quinea pig during development of EAE. Abscissa, Days of experiment; ordinate, time of extension (in sec). 1) Control; 2) experiment. At all points $P < 0.05$ compared with control.

gives rise to the most marked pathogenic effect in guinea pigs (albino males weighing 250-300 g) in a dose of 30 μg per animal when injected subcutaneously into the hind footpads.

The disease develops on the 9th-19th day after injection, and the source of EAE is more severe in the fall than in the summer, and is fatal in 33% of cases. The model thus developed can be used in the search for methods of treatment of demyelinating diseases.

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