IMMUNOMORPHOLOGIC FEATURES OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS INDUCED BY TRYPTOPHAN PEPTIDE

Yu. L. Zhitnukhin and M. G. Khizhnyak

UDC 616.831/.832-002-056.
3-092.9-091.8

KEY WORDS: experimental allergic encephalomyelitis; tryptophan peptide; delayed-type hypersensitivity; demyelinization; inhibition.

A delayed-type hypersensitivity (DTH) reaction [5, 6] and antibodies to basic myelin protein (BMP) [9, 12] have been found in animals with experimental allergic encephalomyelitis (EAE). However, the role of cellular and humoral immunologic factors in the pathogenesis of the disease has not been finally settled. No convincing illustrations of the demyelinizing process in the CNS of animals with EAE induced by peptides have so far been obtained, so that it is not yet clear whether demyelinization is essential for neuroologic disorders to arise. There is little information on the connection between cellular reactions to peptide fragments of the BMP molecule and the development of EAE [10]. The aim of this investigation was to characterize the encephalitogenic and immunogenic properties of synthetic tryptophan peptide (TP) and its ability to cause the appearance of demyelinization in tissues of the CNS.

EXPERIMENTAL METHOD

EAE was induced in noninbred guinea pigs weighing 350-400 g by a single inoculation (into the footpad) of TP (Free peptide; "Serva," West Germany), together with Freund's complete adjuvant (FCA, "Difco Laboratories," USA). TP with mol. wt. of 1037 and with a particular amino-acid sequence (Phe — Ser — Trp — Cly — Ala — Glu — Gly — Gln — Arg — OH) corresponded to fragment 114-122 of human cationic protein myelin. EAE was evaluated both clinically and histologically. The DTH reaction in the animals was determined by skin tests with 50 μg of TP and the encephalitogenic polypeptide fraction of BMP (FBP) [1]. The guinea pigs' sera were tested for the presence of complement-fixing antibodies to TP and FBP, and also in the indirect hemagglutination test with erythrocytes loaded with TP or FBP. The CNS of animals with neurological symptoms of EAE was studied morphologically. Material (the brain and spinal cord at all levels with the spinal ganglia) was impregnated with osmium by Marchi's method, after which celloidin sections were stained with toluidine blue [2]. The significance of the results was estimated by Student's test.

EXPERIMENTAL RESULTS

Neurological symptoms of EAE were observed in some animals sensitized with TP: muscular weakness, disturbance of movement coordination, motor pareses and paralyses, pelvic disorders.

It will be clear from Table 1 that the frequency of the disease and mortality of the animals depended on the doses of TP and FCA. With the same dose of FCA (0.05 ml) symptoms of EAE were observed only in the group of guinea pigs receiving the largest of the three doses (10 μg), which differed from each other by a factor of 10. Meanwhile with the same dose of TP (10 μg) doubling the dose of FCA led to an increase in the percentage of animals developing the disease from 25 to 45. Meanwhile an increase in the dose of TP from 10 to 20 μg, accompanied by injection of 0.1 ml of FCA, led to a statistically significant increase in the morbidity and mortality and also to a decrease in the duration of the disease in the animals which died. The latent period of development of EAE in animals of the different groups in this series was the same.

Departments of Immunology and Morphology, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 8, pp. 241-244, August, 1989. Original article submitted October 25, 1988.

0007-4888/89/0008-1193$12.50

TABLE 1. Parameters of Development and Course of EAE in Animals Sensitized with TP Mixed with PAF

TP, μg	Dose of FCA, ml	Number of guinea pigs				Time after beginning of illness, days	Duration of EAE, days	
		total	with EAE	dying	recovering		in dying animals	in recovering animals
0,1	0,05	20	0	0	0	—	0	0
1,0	0,05	20	0	0	0	—	0	0
10	0,05	20	5 (25%)	0	5	13,3±0,4	0	8,8±0,4
10	0,1	20	9 (45%)	2 (10%)	7	13,4±0,2	9,5±0,8	10±1,4
20	0,1	20	18 (90%)*	8 (40%)**	10	13,4±0,2	4,1±0,8**	10±0,7

Legend. *$p < 0.001$; **$p < 0.01$ compared with corresponding parameters in penultimate group.

TABLE 2. Skin Reactions of Encephalitogenic Preparations in Guinea Pigs Sensitized with Different Doses of TP Together with 0.1 ml of FCA

TP, μg	Number of guinea pigs		Time after beginning of illness, days	Diameter of skin reactions, mm			
				in unaffected animals		in affected animals	
	total	with EAE		TP	FBP	TP	FBP
0,1	20	0	—	0	0	—	—
20	20	10 (50%)	14,8±0,4	5,6±0,2	0,2±0,4	9,8±0,4*	13,0±0,8*

Legend. *$p < 0.001$ Compared with corresponding parameter in group of unaffected animals.

Sera from animals of the three groups in which cases of EAE were observed to develop were studied in two tests for the presence of circulating antibodies to TP and FBP at different times after inoculation. No such antibodies could be found in any of the sera taken after 10, 14, 21, and 28 days.

In the animals of group 2, inoculated with an encephalitogenic and subencephalitogenic dose of TP, DTH reactions were studied in vivo by means of skin tests with TP and FBP on the 11th day of the latent period.

Data on the development of EAE and on DTH reactions, discovered only in the group of animals sensitized with an encephalitogenic dose of TP, and which differed quantitatively within this group in animals with or without neurological symptoms of EAE, are given in Table 2: more marked reactions were observed in animals subsequently developing the disease. Intradermal injection of TP and FBP on the 11th day after inoculation with a mixture of 20 μg TP and 0.1 ml FCA, incidentally, had an inhibitory action on the development of EAE, reducing the morbidity ($p < 0.01$) from 90% (Table 1) to 50% (Table 2).

The inflammatory reaction, discovered mainly in the lumbosacral segments of the spinal cord and in the zone of the pons, consisted of foci of infiltration of lymphocytes and monocytes, accompanied by solitary granulocytes. Signs of demyelinization (Fig. 1a) were particularly marked in the paralyzed animals with pelvic disorders. In guinea pigs with pareses of the hind limbs and with moderate pelvic disorders, breakdown products of myelin were discovered in the form of small clusters of osmiophilic granules, distinguishable only under high power of the light microscope (Fig. 1b). The intensity of demyelinization 1.5 months after the beginning of the disease was very considerable, but the inflammatory changes were clearly subsiding. The foci of demyelinization consisted of aggregations (up to 40-50 μ or more) of strongly osmiophilic granules, located in the white matter of the brain among glial cells (Fig. 1c). No pathological changes were found usually in the neurons.

The results of this investigation indicate that three varieties of EAE can be produced by inoculation with a mixture of TP and FCA: with high morbidity (25%) but without lethal issues, with higher morbidity (45%) and low mortality (10%), and with neurologic symptoms in 90% of the animals and 40% mortality. Meanwhile TP was less active than the polypeptide fraction of BMP which we isolated and tested previously [1], and which induced EAE in 90% of guinea pigs in a dose of 1 μg, in agreement with data on comparison of the encephalitogenic activity of the BMP molecule and of its peptide fragments [4]. It follows from the data given above that after injection of a mixture of TP

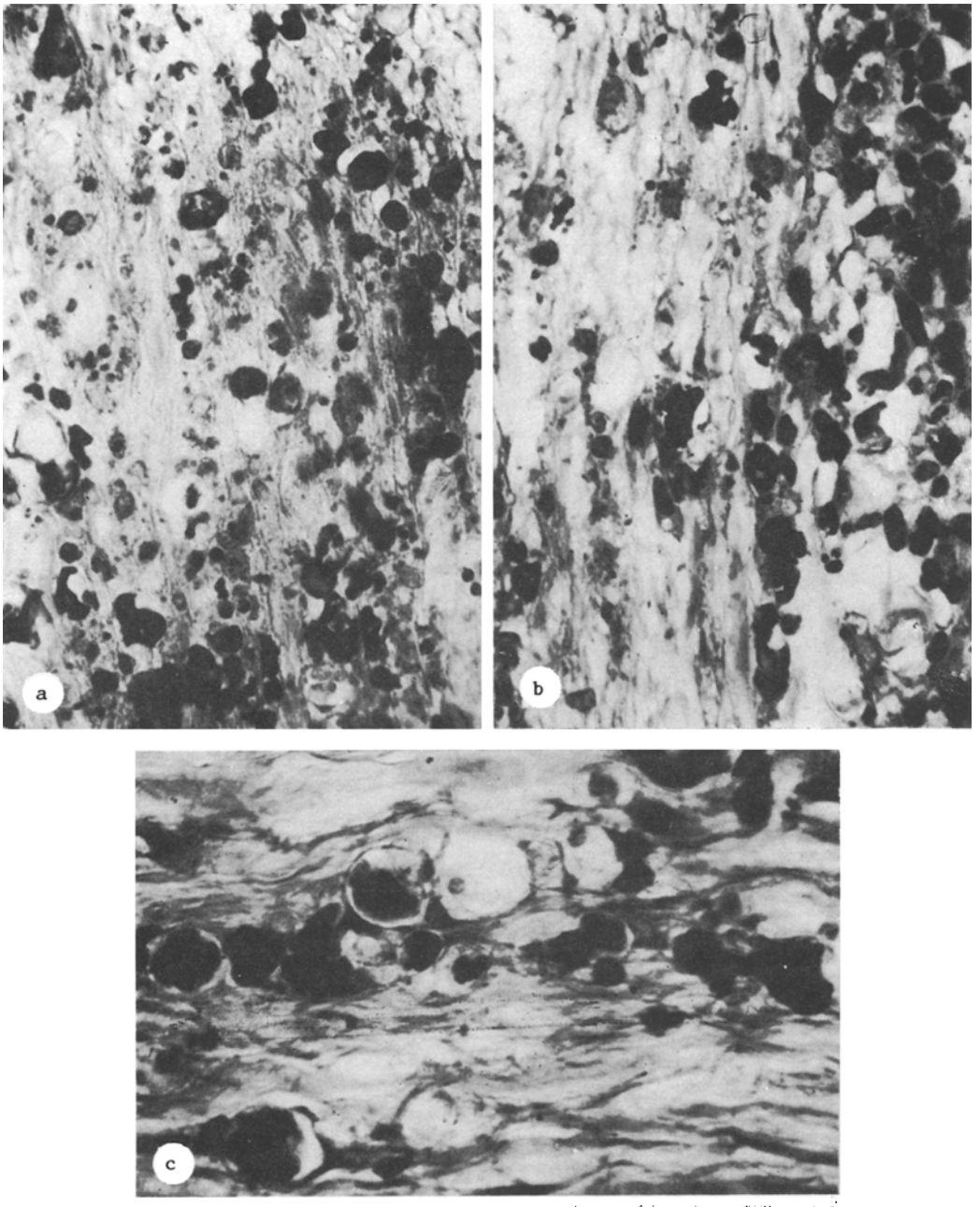

Fig. 1. White matter of lumbar segment of spinal cord of guniea pigs with EAE after inoculation with TP and FCA. Modified Marchi method, stained with toluidine blue. a) Focal aggregations of myelin breakdown products in a paralyzed guinea pig. 450 ×; b) Small aggregations of myelin breakdown products among lymphocytes, macrophages, and solitary granulocytes in an animal with pareses of the hind limbs and with moderate pelvic disorders. 450 ×; c) Myelin breakdown products undergoing phagocytosis and aggregated by microglial cells. Recovery period. 580 ×.

and FCA, no antibodies could be found in the sera of animals with EAE. Similar results were obtained in experiments on rats [8]. Meanwhile skin tests with TP and FBP were positive in all animals inoculated with an encephalitogenic dose of TP; stronger reactions were observed, moreover, in animals which developed the disease. It is important to note that intradermal injection of TP and FBP at the end of the latent period had an inhibitory action on the development of EAE. Thus the encephalitogenicity of TP is associated with its ability to induce a DTH reaction in animals without forming antibodies, indicating that these reactions play a leading role in the genesis of EAE after inoculation with TP. According to some investigators, demyelinization accompanying EAE is caused by antibodies to lipid haptens [13] or to the oligodendrocytic glycoprotein myelin [7]. Reports of the absence of demyelinization in the CNS of animals sensitized with BMP [3, 11] and the absence of data on demyelinization after inoculation with synthetic encephalitogenic peptides

leave the question of the direct cause of the neurologic disorders unanswered. The present investigation showed for the first time that demyelinization occurs in animals sensitized with TP. DTH reactions to TP were found in these animals in the absence of circulating antibodies. There is thus good reason to consider that the periaxonal process may be due to a cellular immunologic reaction to encephalitogenic fragments of the BMP molecule.

LITERATURE CITED

1. Yu. L. Zhitnukhin and V. M. Pleskov, Vopr. Med. Khimii, No. 1, 57 (1978).
2. M. G. Khizhnyak, Yu. L. Zhitnukhin, and G. V. Konovalov, Arkh. Patol., No. 3, 54 (1987).
3. J. Colover, Br. J. Exp. Pathol., 61, 390 (1980).
4. G. A. Hashim, Gerontology, 33, No. 3, 181 (1987).
5. P. J. Higgins and H. L. Weiner, J. Immunol., 140, No. 2, 440 (1988).
6. M. K. Kennedy, R. J. Clath, M. C. Dal Canto, et al., J. Neuroimmunol., 16, No. 3, 345 (1987).
7. C. Linington and H. Lassman, J. Neuroimmunol., 17, No. 1, 61 (1987).
8. J. A. M. McPhee and D. W. Mason, J. Neuroimmunol., 16, No. 1, 113 (1987).
9. E. Mitsuzawa, T. Yasuda, N. Tamura, and S. Ohtani, J. Neurol. Sci., 52, No. 1, 123 (1981).
10. L. L. Perry and M. E. Barzage, J. Immunol., 138, No. 5, 1434 (1987).
11. C. Raine and U. Traugott, J. Neuroimmunol., 2, No. 1, 83 (1982).
12. O. Ryskova, Yu. L. Zhitnukhin (Y. L. Zhitnuhin), and M. G. Khizhnyak (M. G. Chizhnyak), Zh. Gig. Epidemiol. (Prague), 32, No. 1, 105 (1988).
13. T. Saida, D. H. Silberberg, J. M. Fry, and M. C. Manning, J. Neuropathol. Exp. Neurol., 36, No. 3, 627 (1977).
14. F. C. Westall, A. B. Borinson, J. Caccam, et al., Nature, 229, 22 (1971).
15. S. S. Zamvill, D. J. Mitchell, A. C. Moore, et al., J. Immunol., 139, No. 4, 1075 (1987).