IMMUNOMORPHOLOGICAL FEATURES OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS INDUCED BY POLYPEPTIDE FRACTION OF BASIC MYELIN PROTEIN

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The encephalitogenic factor responsible for the development of experimental allergic encephalomyelitis (EAE) is known to be a basic myelin protein (BP) [9]. The role and importance of the cellular and humoral components of immunologic reactions responsible for pathomorphological changes in the CNS and neurological disorders still remain subjects for comprehensive study [5, 6]. In recent years reports have appeared that demyelinization does not take place in the nervous system of animals with EAE after inoculation with BP together with Freund's complete adjuvant (FCA) [8, 13, 14]. Meanwhile reports of the presence of demyelinization in the CNS of such animals have been based on the use of inadequate methods of detection of the periaxonal process [4, 7].

In view of the urgent importance of this problem the aim of the present investigation was to compare and analyze the clinical, immunologic, and pathomorphological parameters following injection of a purified encephalitogenic preparation.

EXPERIMENTAL METHOD

EAE was induced in noninbred adult guinea pigs weighing 350-400 g by a single subcutaneous (into the footpad of one forelimb) injection of 1.0 or 0.1 μg of the polypeptide fraction of BP (PBP), mixed with 0.025 ml of FCA (containing 5 mg/ml of tubercle bacilli).

PBP was isolated from bovine spinal cord by column chromatography. CM-Sephadex G-25 (Pharmacia, Sweden), which not only fractionates the mixture of basic proteins on the molecular sieve principle, but also demonstrates ion-exchange properties, was used for preparative isolation of the basic proteins. The yield of protein fractions was monitored by means of a Uvicord system (LKB, Sweden). The homogeneity and protein composition of the fractions were investigated by polyacrylamide gel electrophoresis. The gels were scanned with an Integraph CH densitometer (Leitz, West Germany). The amino-acid composition of the encephalitogenic polypeptides was determined with an amino acid analyzer (Hitachi, Japan). A homogeneous polypeptide fraction with mol. wt. of 3000-5000 daltons and with a definite amino acid composition [2] was used.

EAE was assessed clinically and histologically. Circulating complement-fixing antibodies to PBP were determined and the delayed-type hypersensitivity (DTH) reaction was investigated by skin tests with 20 μg pf PBP [3]. The results were subjected to statistical analysis [1]. The CNS of animals with neurological symptoms of EAE was studied morphologically at the height of the disease. Material from the CNS (brain and spinal cord at all levels, with spinal ganglia) was impregnated with osmium by Marchi's method and celloidin sections were counterstained with toluidine blue.

EXPERIMENTAL RESULTS

Typical neurological symptoms of EAE were observed in 76 and 26% of animals sensitized with 1 and 0.1 μg of PBP, respectively: muscular weakness, loss of movement coordination, motor pareses and paralyses, pelvic disorders, disturbances of balance, and nystagmus. As Table 1 shows, differences in the frequency of the disease, depending on the dose of PBP,

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TABLE 1. Clinical and Pathomorphological Features of EAE in Guinea Pigs Sensitized with Polypeptide Basic Protein Fraction

Dose of PBP, μg	Morbidity		Mortality		Pathological changes in		
	index	day	index	day	brain	spinal cord	brain and spinal cord
1 0,1	23/30* (76 %)	16,7±1,9	18/23 (78 %)	18,7±2,3	5/23 (22 %)	12/23 (52 %)	6/23 (26 %)

Legend. *p < 0.001 (significance of differences between parameters in groups compared), denominator denotes number of animals observed or tested, numerator gives number of animals developing disease or dying, with demylelinization and cellular inflammatory infiltration.

TABLE 2. DTH Reaction in Guinea Pigs Sensitized with Polypeptide Fraction of Basic Myelin Protein

Sensitizing dose of PBP, μg	Total number of animals	Animals with positive skin tests			Animals with negative skin tests		
		number	number of them with EAE	beginning of disease, days	number	number of them with EAE	beginning of disease, days
1 0,1	30 30	20* 9*	18 7	15,8±1,4 15,5±1,5	10 21	5 1	20,2±0,8 21,0

Legend. Significance of differences between parameters in the groups compared at the p < 0.001 level.

were found. Whereas the duration of the latent period and the mortality were similar, the frequency of development of EAE was greater after incoulation with 1 μg of PBP than with a dose of 0.1 μg. CNS lesions in the form of demyelinization and inflammatory infiltration were found in all animals with clinically manifest EAE.

Antibodies to PBP were not found in any of the sera obtained from the animals between 10 and 35 days after inoculation with the encephalitogenic factor.

Skin tests with PBP (20 μg) were carried out on 60 sensitized guinea pigs 12 days after inoculation with the encephalitogenic mixture. The results are given in Table 2.

It will be clear from Table 2 that positive skin tests were found in 20 of the 30 guinea pigs sensitized with 1 μg PBP and in 9 of the 30 animals sensitized with 0.1 μg PBP. Most of the animals in which a DTH reaction was observed to PBP fell ill with EAE irrespective of the dose of the encephalitogen. Neurological disorders appeared in only 6 of the 31 guinea pigs which did not respond to PBP in the skin test, and they began to appear later.

Morphological investigation showed that the demyelinizing process was localized in the lumbosacral portion of the spinal cord more frequently than in thoracic and cervical segments (Fig. 1a). Myelin breakdown products were usually located at the periphery of the white matter of the spinal cord in the region of the anterior median fissure and posterior median sulcus. In the brain osmiophilic granules were usually distributed around the 3rd ventricle, in the zone of the striopallidal system, in the roots of the oculomotor nerve, the mesencephalic tract of the trigeminal nerve, and the basal zones of the pons (Fig. 1b). Cellular inflammatory infiltration, which is always observed in zones of perivascular demyelinization, consisted mainly of hematogenous macrophages and neutrophilic granulocytes (Fig. 2). The most marked periaxonal changes took place in animals inoculated with 0.1 μg of PBP, whereas induction of EAE with 1 μg of PBP usually caused more severe and widespread decomposition of myelin.

Since some investigators [12, 14] are of the opinion that besides BP, other components of myelin must be injected into animals for demyelinization to occur during EAE, special importance was attached to the purity of the encephalitogenic preparation. In this investigation a polypeptide fraction of basic myelin protein, described previously [2], was used. The low molecular weight, the presence of a single peak on rechromatography on Sephadex G-250 and of a single stained zone after polyacrylamide gel electrophoresis, indicate that this fraction is homogeneous, and this was confirmed by its high encephalitogenicity. The

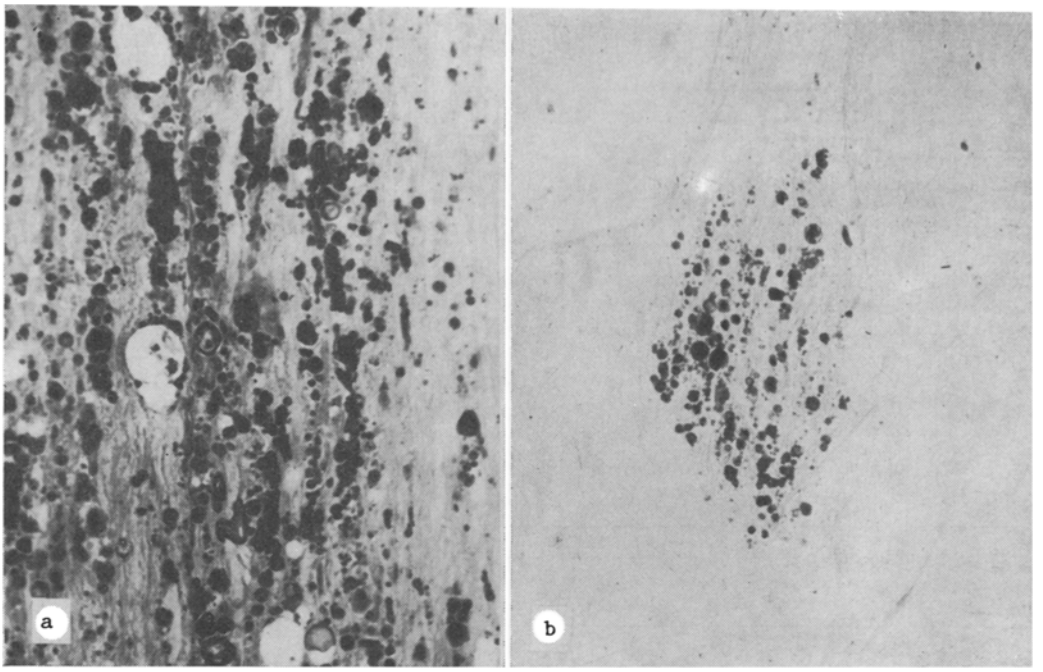

Fig. 1. Demyelinization in white matter of lumbar segment of spinal cord (a) and destruction of myelin in mesencephalic tract of trigeminal nerve (b). Marchi's method. Magnification: a) 160, b) 110.

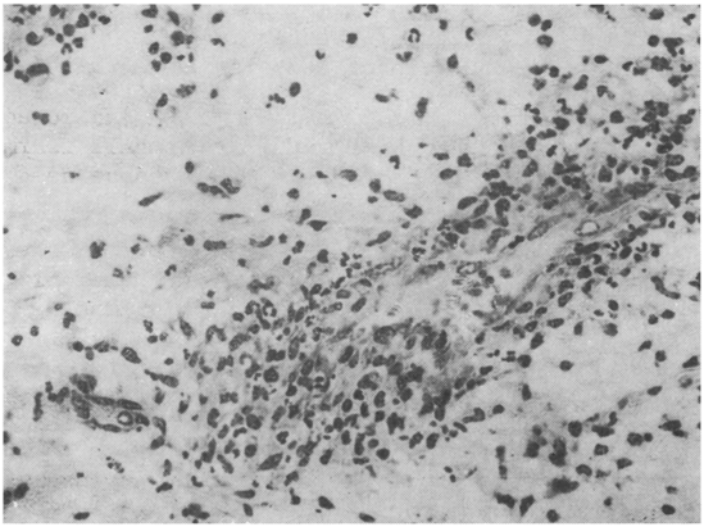

Fig. 2. Perivascular cellular inflammatory infiltration in white matter of basal zone of pons. Nissl's method. 220 ×.

appearance of demyelinization in animals sensitized by this fraction leads to the conclusion that cellular immunologic reactions directed toward BP are sufficient for demyelinization to develop in the CNS. No circulating antibodies to PBP were found in sensitized animals, but DTH reactions correlating with the development of EAE and with the times of appearance of neurological symptoms were observed. These tests are of prognostic value, for they enable the appearance of neurological symptoms to be predicted in the majority of animals on average after 3-4 days. The results are in agreement with the concept of BP as a weak inducer of antibody formation, possessing marked sensitizing properties [10]. We also know that sera of animals inoculated with BP do not possess demyelinizing activity in vitro [15]. It is therefore quite probable that during induction of EAE with the aid of PBP the demyelinization observed is the result of a cell-mediated immune response.

During immunization of animals with BP in combination with myelin lipids, other mechanisms of demyelinization, including humoral immune reactions directed against components of the myelin sheath other than BP, such as galactocerebrosides [11], or anti-BP-antibodies of a different class from those responsible for complement-dependent demyelinization in vitro [12], may also take place. The combined action of several mechanisms may lead to the appearance of more widespread and more numerous foci of myelin destruction, more varied in their distribution. Meanwhile the indications of the absence of demyelinization in animals sensitized with BP are not convincing, for they are based on methods with a resolving power that is lower than that of the Marchi method, so that it is difficult to find small foci of demyelinization. It follows from the data given above that the use of adequate morphological methods enables the presence of demyelinization to be demonstrated both in the brain and in the spinal cord of animals with EAE, induced by PBP. The experimental results are thus evidence of the importance of the allergic components (factor) and, in particular, of the DTH to PBP, in the development of EAE and they indicate that PBP-induced EAE can be regarded as a model of the demyelinizing process in the CNS.

LITERATURE CITED

1. V. S. Genes, Tables of Significant Differences between Groups of Observations Based on Qualitative Parameters [in Russian], Moscow (1964).
2. Yu. L. Zhitnukhin and V. M. Pleskov, Vopr. Med. Khimii, No. 1, 57 (1978).
3. Yu. L. Zhitnukhin, B. M. Sofronov, D. Kh. Bëme, and M. N. Baikovskaya, Patol. Fiziol., No. 5, 56 (1978).
4. S. K. Ainsworth, S. Brostoff, R. I. Sharbaug, and C. T. Fitts, Patholgoy, 11, 457 (1979).
5. A. Ben-Nan and L. Lando, J. Immunol., 130, 1205 (1983).
6. C. F. Brosnan, U. Traugott, and C. S. Raine, Acta Neuropathol. (Berlin), 9, Suppl., 59 (1983).
7. S. W. Brostoff and J. M. Powers, Brain Res., 93, 175 (1975).
8. J. Colover, Br. J. Exp. Pathol., 61, 390 (1980).
9. E. H. Eylar, J. Salk, G. C. Beverige, and L. V. Brawn, Arch. Biochem., 34, 132 (1969).
10. E. J. Field, Clinical Aspects of Immunology, Oxford (1975), p. 1545.
11. A. B. Johnson, M. B. Bornstein, and C. S. Raine, J. Neuropathol. Exp. Neurol., 39, 364 (1980).
12. R. Lebar, J. M. Boutry, C. Vincent, et al., J. Immunol., 116, 1439 (1976).
13. C. S. Raine, U. Traugott, M. Faroog, et al., Lab. Invest., 45, 174 (1981).
14. C. S. Raine and U. Traugott, J. Neuroimmunol., 2, 83 (1982).
15. T. J. Seil, M. W. Kies, and M. Bacon, J. Immunol., 114, 630 (1975).