Antibodies to Encephalitogenic Basic Protein in Experimental Allergic Encephalomyelitis (33662)

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Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system (CNS) induced by injection of heterologous, homologous, isologous, or autologous whole CNS (1–4) or the encephalitogenic portion of the CNS, myelin basic protein (BP) (5–7). Emulsification of the antigen(s) in Freund's complete adjuvant (water-in-oil and mycobacteria) is necessary to produce this disease in a rapid and acute form in most species (2, 3). The histological lesion common to all species developing EAE is perivascular accumulation of mononuclear cells, resembling a delayed hypersensitivity reaction (8). This histological feature in conjunction with the ability to transfer disease with sensitized cells (9–11) but not with serum (12) has led most investigators to conclude that the lesions of EAE are due to delayed hypersensitivity to myelin basic protein (13, 14). This conclusion is further strengthened by the fact that skin tests for delayed hypersensitivity to BP correlate with disease induced either actively or passively (11, 13).

The *in vitro* demonstration of demyelination in CNS tissue cultures by serum from animals with EAE (15) and by tissue culture supernatants of "immunocytes" obtained from rabbits just before development of clinical EAE (16) is suggestive evidence that EAE may result from a demyelinating antibody *in vivo* similar to the one demonstrated *in vitro*. The relationship of anti-BP antibody to the demyelinating factor demonstrable in serum and tissue culture supernatant fluid has not been clarified, partly because of the lack of information on the induction of antibodies specifically related to the encephalitogen in EAE. Although antibodies to homologous BP have been demonstrated in sera from both rabbits and guinea pigs during induction of EAE (17, 18), correlation between anti-BP antibody and time of onset or severity of EAE was not convincing.

The present report, based on a specific binding assay suitable for detection of anti-BP antibody in all 3 major guinea pig antibody classes, presents further data on the occurrence of anti-BP antibody in guinea pigs after a single encephalitogenic injection with homologous BP or whole CNS. An overall incidence of anti-BP antibody of about 13% in guinea pigs sensitized with either purified BP or whole CNS casts considerable doubt on the suggested relationship between demyelinating antibody reported in a high percentage of sera from animals sensitized to whole CNS and antibody to myelin basic protein. The role of the latter antibody in the induction of CNS lesions *in vivo* is still open to question.

Methods. Antigens. Guinea pig myelin basic protein (GP-BP) as prepared by Kies (19) was used for all BP injections. Myelin basic proteins of other species (bovine, human, rabbit, rat, and monkey), all known to cause EAE in guinea pigs (4) and to cross react immunologically with GP-BP (13, 20–22), were prepared in a similar manner. Guinea pig spinal cord was employed as the source of "crude white matter" as described by Stone (23).

Clinical-pathologic studies. NIH and Hartley guinea pigs injected with several different preparations of GP-BP or whole CNS at various dose levels were randomly selected

[1] Supported in part by Research Grant 427 from the National Multiple Sclerosis Society and Research Grant NB-03147 from the National Institute of Neurological Diseases and Blindness, United States Public Health Service.

TABLE I. Antibody to Myelin BP in Sera from Guinea Pigs Sensitized with Homologous BP or Whole CNS.

Sensitization dose and route[a]	DI[b]	Clinical incidence[c]	DO_{50}[d]	Antibody incidence[c]
Guinea pig BP with 0.1 mg of *Mycobacterium butyricum*				
20–25 μg, i.d.	7.1	37/40	14	4/40
100–125 μg, i.d.	8.4	13/14	13	1/14
100–125 μg, i.p.	7.0	5/5	10	2/5
625 μg, i.d.	7.4	5/5	14	1/5
Total				8/64 (12.5%)
Guinea pig CNS with 2.5 mg of *Mycobacterium tuberculosis*				
4 mg, i.p.	8.0	3/4	9	0/4
16 mg, i.d.	8.3	13/14	9	1/14
16 mg, i.p.	8.4	5/5	8	2/5
Total				3/23 (13.0%)

[a] i.d. = intradermal; i.p. = intraperitoneal.
[b] Disease index indicating severity of EAE on a scale of 0–10 (24).
[c] No. positive/no. tested.
[d] Day on which 50% of a group had developed clinical signs of EAE.

from groups of animals used for bioassay. Each of the groups represented had a high average disease index although there was considerable variation in the severity of the disease reaction of the individual animals. Sera were obtained at various times during development of EAE. Clinical signs and histologic ratings were recorded routinely in double blind experimental design and this information was used for assignment of a numerical disease index as described earlier (24).

Antibody assays. All sera were incubated with GP-BP-^{125}I, and then subjected to immunodiffusion and immunoelectrophoresis (25, 26) for detection of soluble antigen–antibody complexes containing BP-^{125}I (27, 28). Immunodiffusion was carried out in agar (pH 7.4, ionic strength 0.15) and immunoelectrophoresis in agarose (pH 8.6, ionic strength 0.075). Normal guinea pig serum and serum from guinea pigs hyperimmunized by multiple injections of GP-BP in incomplete adjuvant (water-in-oil *without* mycobacteria) were run as negative and positive controls. The hyperimmune sera had previously contained high levels of anti-BP antibodies (18, 21, 22, 27). Precipitin arcs were developed with rabbit or goat antiguinea pig serum and rabbit antiguinea pig 7S-γ_1, 7S-γ_2, and 19S-γ_1 globulin antisera (kindly provided by Dr. Richard Asofsky, National Institute of Allergy and Infectious Diseases). After the slides were stained, autoradiographs were made. Presence of isotopically labeled lines as well as their identification with a specific immunoglobulin class was determined by at least 2 observers. All slides were coded so that the identity of the experimental sera was not known by the investigators at the time the autoradiographs were examined. Sera giving a positive reaction when incubated with GP-BP-^{125}I were tested for their ability to bind the myelin BP from at least 2 other species.

Results. The data in Table I were obtained from guinea pigs injected with encephalitogenic emulsions capable of inducing EAE of near maximal severity, generally of early onset. Since the sera were selected at random from guinea pigs being used for routine bioassay, the dose of encephalitogen varied over a wide range. In Table I the results were combined in groups on the basis of the dose of encephalitogen used. Values for disease index, clinical incidence and day of onset are therefore averages of groups of ani-

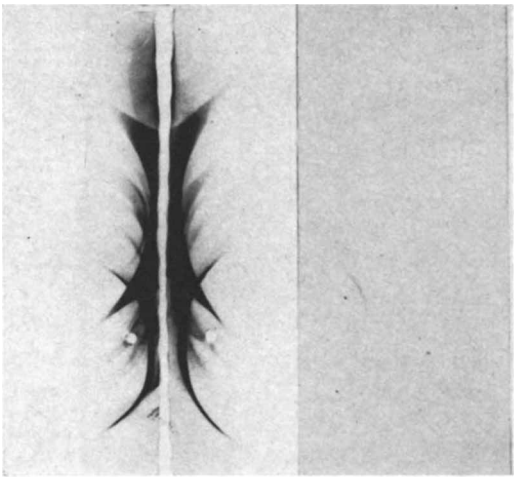

FIG. 1. Radioimmunoelectrophoresis of guinea pig serum: (left) stained immunoelectrophoretic pattern; (right) autoradiograph; (left well) EAE serum + GP-BP-^{125}I; (right well) normal guinea pig serum + GP-BP-^{125}I; (trough) rabbit anti guinea pig serum; anode at top of figure.

mals which were unrelated except for the dose and encephalitogenic fraction each received. Five of the 87 guinea pigs whose sera were studied never showed any clinical signs of disease.

When antibody was found in sera from animals with EAE, it was usually detected in the final bleeding, either at time of sacrifice when clinical signs were severe or at the end of the experiment (25–30 days after challenge) if clinical signs were mild or absent. Antibody was never found in the first bleeding (5 days after challenge) and rarely in the second bleeding (10–14 days after challenge). The antibody found in animals with EAE was always 7S-γ_2 by radioimmunoelectrophoresis (Fig. 1). Hyperimmune guinea pig sera (18), which served as positive controls, contained anti-BP activity in all 3 immunoglobulin classes (7S-γ_1, 7S-γ_2, and 19S-γ_1). The intensity of the isotopically labeled 7S-γ_2 line in EAE sera never approached the intensity of 7S-γ_2 lines in sera obtained late in the course of hyperimmunization (28, 29).

Incidence of antibody was the same whether the guinea pigs had been sensitized with whole CNS or GP-BP and was not influenced by route of administration of the emulsions. Sixty-five of the guinea pigs studied had a disease course of less than 6 days; of these, 8% had sera with detectable anti-BP antibodies. Thirty-five per cent of 17 animals having milder clinical disease and a longer disease course (6 or more days) had demonstrable antibodies to BP. The difference between these two groups is highly significant ($p<0.001$). Similarly reflecting the same temporal events, the longer an animal lived after challenge the more likely it was that antibody would be detected. The incidence of antibody in those animals sacrificed 19 or more days after challenge was 6 out of 20 (30%) as opposed to 5 out of 62 (8%) guinea pigs sacrificed earlier than 19 days. This difference is also highly significant ($p<0.001$). The 5 guinea pigs that had no clinical disease had no antibody; 3 of these had mild histologic lesions.

Antibody incidence was not related to severity of histologic lesions (Table II). The highest incidence of anti-BP antibody was found in animals with mild lesions; if lesions were minimal or absent, no antibody was detected. When the lesions were severe, the antibody incidence was low. (The same relationship obtained if disease index instead of histologic severity was used to rank the experimental groups.)

All 11 of the sera in which antibody to homologous BP was detected bound myelin BP from CNS of other species. This organ-

TABLE II. Severity of EAE and Incidence of Anti-BP Antibody.

Histologic severity[a]	No. of animals	Antibody incidence (%)	Disease index	No. of animals	Antibody incidence (%)
Absent–minimal (0 or 0–±)	5	0	0–3	7	0
Mild (± or ±–+)	26	20	4–6	19	26
Severe (+ or ++)	56	12	≧7	61	13

[a] Histology graded as described in Alvord and Kies (24).

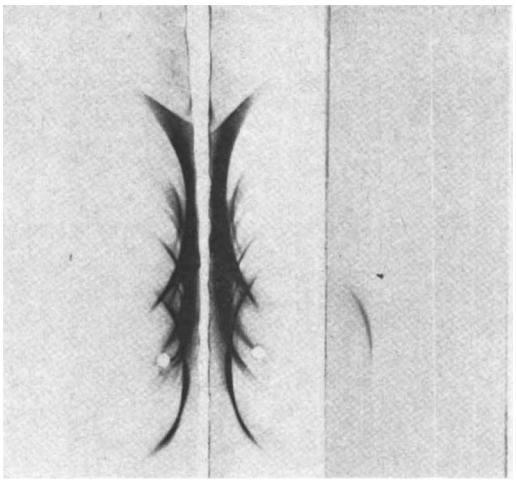

FIG. 2. Radioimmunoelectrophoresis of guinea pig serum: (left and right as in Fig. 1) (left well) hyperimmune guinea pig serum + rat-BP-^{125}I; (right well) normal guinea pig serum + rat-BP-^{125}I; anode at top of figure.

specific species-cross-reactivity was also seen in hyperimmune sera (Fig. 2).

Discussion. As noted in many reports from several laboratories, myelin BP is highly effective in inducing EAE (4–7). The present study has utilized a sensitive method which is specific for antibody to encephalitogenic BP and which does not depend upon any secondary activity of the antibody for its detection (28). Although both antibody and disease (EAE) are presumably induced by the same protein, this does not prove that the antigenic and encephalitogenic determinants of the protein molecule are identical or even related. It should be emphasized also that the results have no direct bearing on whether the antibody is cytotoxic, protective, or possibly unrelated to the pathogenesis of EAE.

Even though guinea pigs with mild clinical and histologic reactions develop a higher incidence of anti-BP antibodies than those with more severe signs of disease, it does not necessarily follow that the antibodies are protective. Over 60% of guinea pigs with mild disease have no demonstrable antibody. A more likely explanation is that the animals with a milder and more chronic course merely survive long enough to permit antibody production to reach detectable levels. Even when guinea pigs are protected against subsequent encephalitogenic challenge by hyperimmunization, it was shown that the high levels of antibody which develop in protected animals can be almost completely suppressed by concomitant treatment with an immunosuppressant drug (Methotrexate) without restoration of the animals' susceptibility to subsequent encephalitogenic challenge (30). In this situation, circulating antibody does not appear to be an essential factor in protection.

The infrequent occurrence of anti-BP antibodies as detected by the sensitive method employed in the present study suggests that this antibody is not responsible for the *in vitro* demyelinating effect of EAE serum demonstrable in CNS tissue culture (15). The high incidence of *in vitro* demyelinating activity in sera from guinea pigs sensitized with whole CNS has been confirmed (31–33), whereas evidence is accumulating that similar demyelinating activity is not present in sera from guinea pigs sensitized with purified encephalitogens (31, 32, 34).

Lumsden (31) suggested that failure to detect demyelinating activity in sera from guinea pigs sensitized with bovine encephalitogenic peptide (BEP) was related to lack of antigenicity of BEP. However, Seil *et al.* (32) showed that sensitization with BP in Freund's adjuvant which induces EAE may result in formation of antibody to BP but does not induce the serum factor responsible for *in vitro* demyelination of CNS in tissue culture. It was reported by Yonezawa *et al.* (33) that *in vitro* demyelinating factor could be detected after BP sensitization but sufficient details were not given to account for the difference between their results and those of Seil *et al.* (32).

Summary. Radioimmunoelectrophoresis was used to detect antibodies to myelin basic protein (BP) in sera from guinea pigs given a single encephalitogenic challenge containing homologous BP or CNS. Antibodies which were found in only 13% of sensitized guinea pigs were detected late in the course of disease and were found only in the 7S-γ_2 class of immunoglobulins. With such a low incidence, it is unlikely that this antibody has

any pathogenetic significance. Although the incidence of antibodies is higher in guinea pigs with mild clinical and histologic evidence of allergic encephalomyelitis (EAE) than in the entire group, the increased incidence is not sufficient to suggest a protective function for the antibody.

The authors wish to thank Mr. Jack Lieberman, National Heart Institute, Biometrics Section, for assistance in performing the statistical analysis and Mr. John Stream for technical assistance with the experimental animals and sera.

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Received Sept. 20, 1968. P.S.E.B.M., 1969, Vol. 130.