Myelination Inhibition Factor: Dissociation from Induction of Experimental Allergic Encephalomyelitis

Abstract. *Sensitization of guinea pigs with purified myelin basic protein induces experimental allergic encephalomyelitis (EAE) but does not induce a serum factor which inhibits myelin formation in vitro. This factor, induced by some unidentified constituent of whole central nervous system tissue, should not be characterized as a component of "EAE serum."*

Sensitization of animals with whole central nervous system (CNS) tissue and mycobacteria induces experimental allergic encephalomyelitis (EAE), a disease that has been extensively investigated because of its possible relation to multiple sclerosis (MS) (*1*). Serums from humans with acute MS as well as serums from animals in which EAE had been induced with whole CNS tissue have been shown to demyelinate CNS tissue cultures (*2*). This in vitro phenomenon has been interpreted as evidence for a pathogenic role of circulating antibody in EAE. However, EAE is induced by a single component of CNS tissue, myelin basic protein (*3*); and Seil *et al.* (*4*) showed that serums from guinea pigs sensitized with myelin basic protein and mycobacteria failed to demyelinate CNS tissue cultures, results consistent with those reported earlier by Lumsden (*5*) that sensitization with an encephalitogenic peptide did not induce the demyelinating factor.

In contrast, Yonezawa *et al.* (*6*) reported that demyelination occurred when serums from guinea pigs or rabbits sensitized with myelin basic protein were added to CNS cultures. This variance could possibly be related to differences in criteria used to assess demyelination in vitro. A more sensitive technique that can be evaluated more objectively has been described (*7*). This method is based on the capacity of unmyelinated cultures to myelinate when they are continuously exposed to test serum from the time of explantation. In the present study, the myelination inhibition activity of serums from animals sensitized with basic protein was compared with that of serums from animals sensitized with whole CNS tissue.

In our earlier study (*4*), production of demyelinating factor was dissociated from induction of EAE by use of two types of sensitization: (i) 50 mg (wet weight) of whole homologous spinal cord plus 2.5 mg of killed *Mycobacterium tuberculosis* (H37Rv) and (ii) 10 to 100 μg of homologous myelin basic protein plus 0.1 mg of mycobacteria. In the present study the amounts in the latter mixture were increased to 0.5 mg of myelin basic protein and 2.5 mg of mycobacteria in order to achieve equivalent sensitization in both groups.

Twenty randomly bred adult NIH guinea pigs weighing between 445 and 530 g were divided into four groups of five animals each. Each guinea pig was injected with 0.5 ml of an emulsion consisting of equal parts of physiologic saline and a mixture of mineral oil : Arlacel (85 : 15). Each guinea pig in two of the groups received 50 mg (wet weight) of homologous spinal cord plus 2.5 mg (dry weight) of heat-killed *M. tuberculosis* (H37Rv). Each guinea pig in the remaining two groups received the same emulsion except that 0.5 mg of bovine myelin basic protein replaced the 50 mg of whole cord. Injections were made intracutaneously in five separate shaved sites on the upper back and over the sternum. All animals were killed 11 days after sensitization.

Tissue culture techniques were similar to those described previously (*4, 8*). Parasagittal sections of newborn Swiss-Webster mouse cerebellum were explanted onto collagen-coated cover slips with a drop of nutrient medium, sealed in Maximow double cover-slip assemblies, and incubated at 35.5° to 36.0°C. Each newborn mouse cerebellum yielded 7 to 8 explants, and 28 explants were usually prepared in one sitting. The nutrient medium for control cultures consisted of 2 parts of low-zinc insulin (3 unit/ml), 1 part of 20 percent dextrose, 4 parts of bovine serum ultrafiltrate, 4 parts of Eagle minimum essential medium with Hanks base and added L-glutamine, 7 parts of Simms X-7 balanced salt solution, and 12 parts of human placental serum; 90 of 114 cultures grown in this medium became myelinated. Test cultures were exposed to medium containing 3 parts of pooled normal guinea pig serum as a complement source and 7.7 parts of test serum in place of 10 parts of human placental serum; that is, the concentration of test serum was 25 percent. A given test medium was applied to six or seven explants. The test or control media were renewed twice weekly. Test and control cultures were observed for 15 to 16 days for evidence of myelin formation, a period considered sufficiently long for evaluating myelination because the usual time for appearance of myelin in cerebellar cultures derived from newborn Swiss-Webster mice is between 9 and 12 days (*9*). Cultures were generally first evaluated for myelination at a time after myelin was expected to have begun to appear in control cultures, and they were last evaluated on day 15 or 16, after which they were fixed in formalin.

Serums from one group of five guinea pigs that had been sensitized with whole CNS tissue (the A group) were identified as being positive for myelination inhibition before evaluation to provide a positive control. The remaining three groups were coded and evaluated as unknowns. Usually, serums from three test groups were applied to a group of simultaneously prepared explants, and the remaining explants were set up as controls in normal nutrient media. Some serums had to be retested after Millipore filtration because of bacterial contamination. In accordance with Bornstein and Raine's criteria (*7*), a culture was considered negative for myelination inhibition if only a single fiber became myelinated. A serum was judged to be negative (that is, lacking in myelination inhibition factor) if half or more of the cultures to which it was applied became myelinated.

Antibody to myelin basic protein was determined by coprecipitation of antigen-antibody complexes (containing ^{125}I-labeled antigen) with rabbit antiserum against guinea pig globulin (*10*). Serums were incubated with ^{125}I-labeled myelin basic protein from guinea pig for 1 hour at 37°C, and globulins were precipitated with a commercial antiserum against guinea pig globulin (Hyland). After 18 to 20 hours at 4°C, the precipitated globulins were separated by centrifugation, washed, and dissolved in 1*M* NaOH. The radioactivity of the latter solution was used to calculate the percentage of total radioactivity specifically bound to globulins.

All ten guinea pigs sensitized with whole CNS tissue had clinical signs of EAE when serums were collected on day 11. Each guinea pig had lost 50 to 135 g in the prior 3 days. All had

Table 1. Serum factors induced by sensitization of guinea pigs with CNS antigens. The test serum comprised 25 percent of the medium in myelination studies. The fraction of cultures myelinated is the number of positive cultures divided by the number of cultures tested. Specific antibody is expressed as the percentage of radioactivity bound by 1 ml of undiluted serum after incubation with 3 μg of ^{125}I-labeled myelin basic protein; NT, not tested.

Whole CNS tissue				Myelin basic protein			
Guinea pig	EAE	Cultures myelinated (fraction)	Specific antibody	Guinea pig	EAE	Cultures myelinated (fraction)	Specific antibody
1A	+	1/7	NT	1C	+	3/5*	NT
2A	+	0/6*	9	2C	+	4/5	NT
3A	+	1/12	12	3C	+	3/6*	55
4A	+	0/6	22	4C	+	3/6	47
5A	+	1/6†	NT	5C	−	2/5	60
1B	+	3/6	16	1D	+	2/7	63
2B	+	0/7*	NT	2D	+	4/5	39
3B	+	0/7	12	3D	+	4/6*	60
4B	+	0/6*	NT	4D	+	5/6*	NT
5B	+	0/6	15	5D	+	3/5*	NT
Totals	100%	9%‡	14.3 ± 1.8§		90%	59%‡	54 ± 3.8§

* Serum tested at 10 percent concentration gave the same result. † Serum tested at 10 percent concentration: 6/6 myelinated. ‡ Normal cultures: 90/114 (79 percent) myelinated. § Normal serums: 6.9 ± 0.4 (mean ± standard error).

histologic signs of lesions of EAE: four were rated +, five were rated ± to +, and one was rated ±. Of the ten guinea pigs sensitized with 0.5 mg of bovine myelin basic protein, two had lesions rated +, five were rated ± to +, two were rated ±, and one was rated −. All but the latter animal showed the same clinical signs as the ten guinea pigs sensitized with whole CNS tissue, with weight losses ranging from 40 to 95 g.

Serums from nine of the ten guinea pigs sensitized with whole CNS antigen inhibited myelin formation in cerebellar cultures, whereas serums from only two of the ten animals sensitized with myelin basic protein inhibited the formation of myelin (Table 1).

Twelve of the 20 serums were analyzed for specific antibody to myelin basic protein. In contrast to our earlier finding that very few guinea pigs sensitized with myelin basic protein and small amounts of mycobacteria make antibody (*10*), we have observed that most guinea pigs sensitized with myelin basic protein produce antibody if the amount of mycobacteria in the emulsion is increased to 2.5 mg. When whole spinal cord is used in place of myelin basic protein for sensitization, the amount of antibody is much less than when myelin basic protein is used (Table 1). Either the mixture of other antigens present in whole CNS tissue (presumably responsible for the induction of demyelinating factor or myelination inhibition factor) suppresses the induction of antibodies to myelin basic protein, or the basic protein is less effective as an antigen when it is combined with lipids in intact myelin. Indeed, in our earlier study (*4*) antibody to myelin basic protein was not detected at all in serums of guinea pigs sensitzed with whole CNS tissue. The quantitative coprecipitation technique used currently is apparently more sensitive than the qualitative radioimmunoelectrophoretic assay used earlier.

We confirmed Bornstein and Raine's findings that unmyelinated cerebellar explants exposed to serums from animals sensitized with whole CNS tissue fail to myelinate (*7*), and we demonstrated that most serums from guinea pigs sensitized with myelin basic protein do not contain the factor that inhibits myelin formation. Inasmuch as all guinea pigs were sensitized with sufficient amounts of whole CNS or myelin basic protein to induce acute EAE in 90 percent or more of the animals, failure to induce the serum factor with myelin basic protein cannot be attributed to a weaker encephalitogenic challenge with the protein. These results are similar to those we reported earlier (*4*) in that sensitization with whole CNS tissue induced both EAE and a demyelinating factor, whereas sensitization with myelin basic protein induced EAE but failed to induce demyelinating factor.

In two respects, serums from animals in which EAE is induced with whole tissue differ significantly from those in which EAE is induced with purified myelin basic protein: (i) in the capacity to cause demyelination or inhibit myelination, and (ii) in the concentration of specific antibody to myelin basic protein. It behooves investigators, therefore, not to refer simply to "EAE serum," as has been done frequently (*7*, *11*). No identification has been made of the antigen or antigens in whole CNS tissue responsible for induction of factors involved in demyelination and myelination inhibition. Because EAE can be induced without simultaneous induction of these factors, it is concluded that they do not play a significant role in the pathogenesis of EAE.

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References and Notes

1. M. W. Kies and E. C. Alvord, Jr., Eds., *"Allergic" Encephalomyelitis* (Thomas, Springfield, Ill., 1959).
2. M. B. Bornstein, *Nat. Cancer Inst. Monogr.* **11**, 197 (19(→ S. H. Appel and M. B. Bornstein, *J. Exp. Med.* **119**, 303 (1964).
→ R. H. Laatsch, M. W. Kies, S. Gordon, E. C. Alvord, Jr., *J. Exp. Med.* **115**, 777 (1962).
→ F. J. Seil, G. A. Falk, M. W. Kies, E. C. Alvord, Jr., *Exp. Neurol.* **22**, 545 (1968).
5. C. E. Lumsden, *Proc. Int. Congr. Neuropathol. 5th, Zurich, 1965* (1966), p. 231.
6. T. Yonezawa, Y. Ishihara, Y. Sato, *J. Neuropathol. Exp. Neurol.* **28**, 180 (1969).
7. M. B. Bornstein and C. S. Raine, *Lab. Invest.* **23**, 536 (1970).
→ M. B. Bornstein and M. R. Murray, *J. Biophys. Biochem. Cytol.* **4**, 499 (19: → F. J. Seil, *Brain Res.* **42**, 33 (1972).
9. F. J. Seil, unpublished observations based on experience with several thousand cerebellar cultures.
10. R. P. Lisak, R. G. Heinze, M. W. Kies, *Int. Arch. Allergy Appl. Immunol.* **37**, 621 (1970); ———, E. C. Alvord, Jr., *Proc. Soc. Exp. Biol. Med.* **130**, 814 (1969).
11. C. S. Raine and M. B. Bornstein, *J. Neuropathol. Exp. Neurol.* **29**, 177 (1970); *ibid.*, p. 554; M. B. Bornstein and H. Iwanami, *ibid.* **30**, 240 (197 → J. M. Fry, G. M. Lehrer, M. B. Bornstein, *Science* **175**, 192 (1972).
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