Brief Definitive Report

MYELIN BASIC PROTEIN SERUM FACTOR

An Endogenous Neuroantigen Influencing Development of Experimental Allergic Encephalomyelitis in Lewis Rats*

By ROBERT S. FUJINAMI, PHILIP Y. PATERSON, EUGENE D. DAY, AND VINCENT A. VARITEK

From the Department of Microbiology-Immunology, The Medical and Dental Schools, Northwestern University, Chicago, Illinois 60611, and the Departments of Microbiology-Immunology and Surgery, Duke University Medical Center, Durham, North Carolina 27710

Studies in our laboratory have uncovered important distinctions concerning the relative capacity of suckling as opposed to adult Lewis rats to develop experimental allergic encephalomyelitis (EAE) as well as age-related differences in histopathologic features of disease (1, 2). For example, EAE could not be induced in 10-day-old suckling rats sensitized to rat myelin basic protein (RMBP) in complete Freund's adjuvant (CFA) whereas sensitization of adult animals to RMBP-CFA induced typical disease (1). Whereas sensitization of suckling rats with guinea pig (GP) myelin basic protein (MBP) or spinal cord did induce clinical neurological signs of EAE of a transient nature which were accompanied by focal central nervous system (CNS) lesions, these perivascular cellular infiltrates contained disproportionately large numbers of segmented neutrophils and occurred more often in white matter than was the case in similarly sensitized adult animals (1, 2). Furthermore, appropriately sensitized suckling rats were as effective as adult animals with respect to serving as donors of lymph node cells for transfer of EAE to adult recipients (3). This observation, in particular, suggested that the milieu of the immature rat diminishes expression of effector cell activity.

It occurred to us that the reduced incidence and severity of EAE in suckling Lewis rats might be due to a circulating MBP or MBP-like moiety of endogenous origin capable of interacting with sensitized effector cells so as to reduce their capacity to bind to CNS target MBP antigen and initiate injury. In support of this hypothesis, we were able show that sera of normal suckling Lewis rats contained a factor which additively inhibited primary binding of radiolabeled RMBP with syngeneic RMBP reagent antibodies (4, 5). We concluded that the MBP serum factor, designated MBP-SF, was immunochemically indistinguishable from native RMBP with respect to immunodeterminants specific for reagent RMBP antibody (4). It was of obvious importance to determine the relationship between mean MBP-SF values and occur-

* Supported by U. S. Public Health Service grants NS 06262 and NS 10237; National Institute General Medical Sciences Training grant 3T01-GM 00724-15; the James C. Hemphill grant 811-A-1; and research grant 833-D-4 from the National Multiple Sclerosis Society.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/78/1201-1716$1.00
Volume 148 December 1978 1716–1721

rence of EAE in Lewis rats as functions of age. Our initial observations summarized here suggest that MBP-SF has an important immunoregulatory influence on development of EAE in rats sensitized to RMBP-CFA.

Materials and Methods

Animals. Adult male Lewis rats, 8–12-wk old, were obtained from Microbiological Associates (Walkersville, Md.), Simonsen Laboratories, Inc. (Gilroy, Calif.) and Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). Suckling Lewis rats, by definition <28 days old, were born and reared at Northwestern University or Duke University.

Antigen and Sensitization of Animals. RMBP obtained from Dr. Marian Kies, National Institutes of Health, Bethesda, Md., was used for a single experiment. In all other experiments, RMBP as well as GPMBP was prepared in our laboratory from syngeneic adult Lewis rats or from random bred guinea pig spinal cord (purchased from Pel-Freeze Biologicals Inc., Rogers, Ark.), using the method of Deibler et al. (6). Rats were injected with 50 μg RMBP or GPMBP emulsified in CFA, distributed between each hindleg footpad as previously described (1).

Serum and Plasma Specimens. Blood was collected by cardiac puncture from etherized rats. Serum and plasma from several litters of sucklings (usually 8–12 animals per litter) and individual adult rats were obtained as previously described (4). These frozen samples were shipped over dry ice to Duke University and assayed for MBP-SF content. During the initial phases of our work, these specimens were coded and ages of serum or plasma donor animals divulged only after final bioassay results were recorded. Confirmatory data also were generated by bioassay of specimens collected from Lewis suckling or adult rats at Duke University.

Radioimmunoassay (RIA) of Samples for MBP-SF. The quantitative binding inhibition procedure used throughout was as previously described (4) and employed ^{125}I-RMBP as reagent antigen and anti-RMBP raised in rabbits as reagent antibody. This procedure could detect ≥0.6 ng of MBP eq/μl.

Results

Mean values of MBP-SF in > 45 different serum and plasma pools and individual serum specimens collected from normal Lewis rats of widely varying ages are shown in Fig. 1. Elevated levels of MBP-SF expressed as MBP-equivalents ranged from 5 to 8 ng/μl during the first 10 days after birth to peak concentrations usually exceeding 10 ng/μl during the period 11–20 days old. Thereafter, MBP-SF levels gradually fell to the barely detectable or undetectable levels found in rats 7 or more wk old, viz., ≤ 6 ng/μl.

A striking inverse relationship between MBP-SF levels and susceptibility to EAE, in terms of incidence of histopathological changes with or without clinical signs of disease after sensitization with RMBP-CFA, was observed among Lewis rats of differing ages. This relationship is apparent in Fig. 2. With increasing age, mean MBP-SF levels fell (as previously depicted in Fig. 1) whereas occurrence of EAE increased to close to 100% incidence by 41–50 days of age. It should be stressed that this inverse relationship (Fig. 2) holds only for rats 11 days or older. Rats much < 11 days old were found to be insusceptible to EAE due to limiting amounts of encephalitogenic antigen in their maturing CNS target tissue (1).

The relationship between MBP-SF levels and occurrence of EAE among suckling and adult Lewis rats sensitized to a xenogeneic MBP, viz., GPMBP, is shown in Fig. 3. Because we had already noted the capacity of a moderate proportion of suckling and virtually all adult Lewis rats to develop disease when sensitized to GPMBP-CFA (1), it was not surprising to find no evident inverse relationship between MBP-SF levels and occurrence of EAE in these animals.

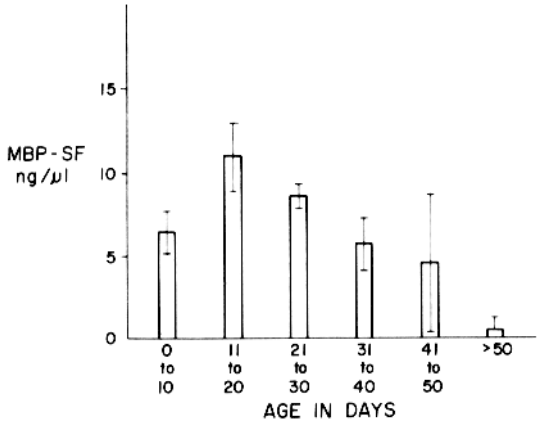

FIG. 1. MBP-SF levels in normal Lewis rats ranging in age from <1 day to 14 wk old, expressed as means (± SEM) for age ranges indicated. Individual values ranged from < 0.6 ng/µl (the sensitivity of the RIA-inhibition procedure) to 21.3 ng/µl. □, MBP-SF.

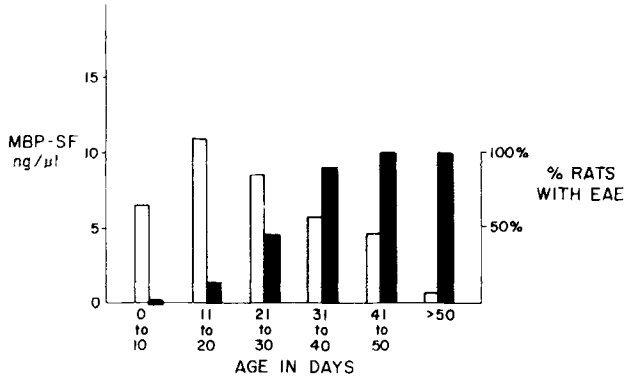

FIG. 2. Mean MBP-SF levels of normal Lewis rats (as in Fig. 1) in relationship to occurrence of EAE among animals sensitized to RMBP-CFA, for age ranges shown. □, MBP-SF; ■, percent rats with EAE.

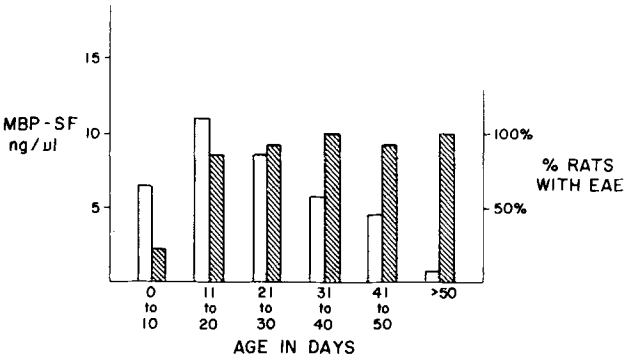

FIG. 3. Mean MBP-SF levels of normal Lewis rats (as shown in Fig. 1) in relationship to occurrence of EAE among animals sensitized to GPMBP-CFA, for age ranges shown. □, MBP-SF; ▨, percent rats with EAE.

Discussion

MBP-SF levels decrease progressively among suckling and weanling Lewis rats as they mature (Fig. 1), whereas the occurrence of EAE, after sensitization with RMBP-CFA, increases with age (Fig. 2). This striking inverse relationship, we believe, implicates MBP-SF as an endogenous immunoinhibitory neuroantigen acting to suppress development of EAE among Lewis rats sensitized to syngeneic MBP. MBP-SF may bear immunodeterminants other than those detected in our binding-inhibition RIA (4, 5) specific for surface receptors on circulating effector lymphoid cells activated by RMBP-CFA. By interacting with MBP-SF, such effector cells otherwise destined to interact with autologous MBP in the host's CNS target may be desensitized and rendered less injurious for MBP antigen in the CNS compartment. As a result of diminished interaction of effector cells with target antigen, tissue injury translating to EAE would be reduced or inhibited. Efforts are under way to demonstrate that injections of pooled suckling rat serum containing high levels of MBP-SF have the capacity to suppress EAE in adults sensitized to RMBP-CFA. We already have reported that injections of pooled immune serum collected from adult rats after recovery from EAE will suppress or completely inhibit disease in rats actively sensitized to neuroantigen (7).

The lack of relationship between MBP-SF levels and occurrence of EAE in rats sensitized to a xenogeneic neuroantigen, i.e., GPMBP (Fig. 3), deserves comment in view of the fact that most syngeneic antibodies raised against RMBP cross-react extensively with MBP of guinea pig origin as well as MBP derived from many other mammalian species (8). Because the only MBP immunodeterminants detected by RIA are those which elicit and/or bind to MBP antibody, and because other laboratories (9–11) have provided evidence for other immunodeterminants specifically engendering EAE activity and cell-mediated immunity, there are good reasons to believe that endogenous RMBP-SF would not interact with EAE-receptor sites on lymphocytes sensitized to GPMBP to a degree anticipated with lymphocytes sensitized to syngeneic RMBP. Indeed, this could be one explanation for the well known greater encephalitogenic activity of GPMBP, compared to RMBP, in both suckling and adult Lewis rats (1, 2).

Only trace amounts of MBP have been reported in fetal or postnatal maturing rat CNS tissues until ≅ 2 wk after birth, when substantial amounts of this myelinated nerve protein begin to accumulate in nerve fibers together with cerebrosides and other major components of myelin (12–14). It is our premise that MBP probably is synthesized within the CNS of very immature rats but its intercalation into maturing myelin requires cerebrosides and other lipoproteins, which are not synthesized until ≅ 2 wk old. Soluble MBP of relatively low molecular weight, if not incorporated into myelin, might well enter the systemic circulation. Shedding of endogenous neuroantigen into the vascular compartment with distribution to peripheral lymphoid tissues might be a means for early induction of immunologic tolerance to autologous MBP, at least at the level of T cells, in agreement with the tenets of self tolerance developed by Weigle and his associates (15). There is every reason to believe that MBP-SF is representative of the type of serum factor described by Cohen and his associates (16–18) preventing sensitization of cultured lymphoid cells to syngeneic nervous tissue and other autoantigens.

Summary

Age-related concentrations of myelin basic protein serum factor (MBP-SF), an endogenous neuroantigen detected and quantitated by inhibition of binding of rat myelin basic protein (RMBP) antibody with ^{125}I-RMBP reagent antigen and immunochemically indistinguishable from native RMBP in this respect, reach peak levels as high as 21 ng/μl among 2–3-wk-old normal suckling Lewis rats. Levels then progressively decline to low, usually undetectable levels of ≤ 0.6 ng/μl MBP-equivalents in adult animals by 7 wk of age. MBP-SF levels are inversely related to the age-related increasing capacity of maturing Lewis rats to develop experimental allergic encephalomyelitis (EAE) after sensitization to MBP of syngeneic, but not xenogeneic, origin. MBP-SF appears to be an endogenous neuroimmunoregulatory product of potential importance for immunologic tolerance to autologous RMBP in Lewis rats.

The authors thank Mrs. Carrie Clark and Mrs. Cecelia Walker for their assistance in tissue and slide preparation. Mrs. Geane Kraus provided valuable secretarial assistance with the preparation of the manuscript and typescript.

Received for publication 22 August 1978.

References

1. Fujinami, R. S., and P. Y. Paterson. 1977. Induction of experimental allergic encephalomyelitis in suckling Lewis rats: role of age and type of sensitizing neuroantigen. *J. Immunol.* **119:**1634.
2. Dal Canto, M. C., R. S. Fujinami, and P. Y. Paterson. 1977. Experimental allergic encephalomyelitis in suckling Lewis rats. Comparison with the disease in adult animals. *Lab. Invest.* **37:**395.
3. Fujinami, R. S., and P. Y. Paterson. 1976. Contrasting patterns of experimental allergic encephalomyelitis (EAE) in adult versus immature Lewis rats. *Fed. Proc.* **35:**436. (Abstr.)
4. Day, E. D., V. A. Varitek, R. S. Fujinami, and P. Y. Paterson. 1978. MBP-SF, a prominent serum factor in suckling Lewis rats, that additively inhibits the primary binding of myelin basic protein (MBP) to syngeneic anti-MBP antibodies. *Immunochemistry.* **15:**1.
5. Day, E. D., V. A. Varitek, and P. Y. Paterson. 1978. Myelin basic protein serum factor (MBP-SF) in adult Lewis rats: a method for detection and evidence that MBP-SF influences the appearance of antibody to MBP in animals developing experimental allergic encephalomyelitis. *Immunochemistry.* **15:**437.
6. Deibler, G. E., R. E. Martenson, and M. W. Kies. 1972. Large scale preparation of myelin basic protein from central nervous tissue of several mammalian species. *Prep. Biochem.* **2:** 139.
7. Paterson, P. Y., and S. M. Harwin. 1963. Suppression of allergic encephalomyelitis in rats by means of anti-brain serum. *J. Exp. Med.* **117:**755.
8. Pitts, O. M., V. A. Varitek, and E. D. Day. 1976. The extensive cross-reaction of several syngeneic rat-anti-BP antiserums with myelin basic protein (BP) of other species. *Immunochemistry.* **13:**307.
9. Driscoll, B. F., A. J. Kramer, and M. W. Kies. 1974. Myelin basic protein: Location of multiple independent antigenic regions. *Science (Wash. D. C.).* **184:**73.
10. Bergstrand, H. 1973. Localization of antigenic determinants on bovine encepalitogenic protein. Further studies with the macrophage migration inhibition assay in guinea pigs. *Immunochemistry.* **16:**611.
11. Spitler, L. E., C. M. von Muller, H. H. Fudenberg, and E. H. Eylar. 1972. Experimental allergic encephalitis. Dissociation of cellular immunity to brain protein and disease production. *J. Exp. Med.* **136:**156.

12. Norton, W. T. 1975. Myelin: structure and biochemistry. *In* The Nervous System. D. B. Tower, editor. Raven Press, New York. 1:467.
13. Benjamins, J. A., and M. E. Smith. 1977. Metabolism of myelin. *In* Myelin. P. Morell, editor, Plenum Publishing Corporation, New York. 233.
14. Norton, W. T. 1977. Chemical pathology of diseases involving myelin. *In* Myelin. P. Morell, editor. Plenum Publishing Corportion, New York. 383.
15. Weigle, W. O. 1973. Immunological unresponsiveness. *Adv. Immunol.* **16:**61.
16. Cohen, I. R., and H. Wekerle. 1973. Regulation of autosensitization. The immune activation and specific inhibition of self-recognizing thymus-derived lymphocytes. *J. Exp. Med.* **137:**224.
17. Orgad, S., and I. R. Cohen. 1974. Autoimmune encephalomyelitis: Activation of thymus lymphocytes against syngeneic brain antigens in vitro. *Science* (*Wash. D. C.*). **183:**1083.
18. Steinman, L., I. R. Cohen, D. Teitelbaum, and R. Arnon. 1977. Regulation of autosensitization to encephalitogenic myelin basic protein by macrophage-associated and soluble antigen. *Nature* (*Lond.*). **265:**173.