Brief Definitive Report

MYELIN BASIC PROTEIN SERUM FACTOR
An Endogenous Neuroantigen Influencing Development
of Experimental Allergic
Encephalomyelitis in Lewis Rats *

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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 num-
bers 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-

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herence 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 125I-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.
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 125I-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.

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