

**INDUCTION AND REINDUCTION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN DA RATS: THE EFFECT OF AGE AND ENCEPHALITOGEN**

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*Susceptibility to experimental allergic encephalomyelitis (EAE) was investigated in DA-rats of both sexes, aged 5, 8 or 27 weeks. Guinea pig or Lewis rat spinal cords (GPSC or RSC) emulsified in complete Freund's adjuvant were used for both induction and reinduction of EAE. The results showed that: a) sex has no influence on clinical signs of EAE in young DA rats aged 5 or 8 weeks; b) susceptibility to EAE induction increases with age; c) RSC is a more potent encephalitogen than GPSC; d) GPSC is a more effective antigen for anti-MBP antibody production than RSC; e) anti-MBP antibody levels are not correlated with clinical score of EAE; f) EAE can be reinduced in 27-week-old rats if RSC is used for induction and/or reinduction; and g) anti-MBP antibodies are not related to resistance to EAE reinduction in DA rats.*

*Key words: experimental allergic encephalomyelitis, DA rats, anti-myelin basic protein antibodies*

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is a T cell-mediated inflammatory demyelinating disorder of the central nervous system (CNS) which serves as an animal model for the human disease multiple sclerosis (MS) (Swanborg, 1995). The disease is characterized by opening of the blood-brain barrier, perivascular infiltration of lymphocytes into the CNS, local inflammation and demyelination in the form of plaques. EAE is accompanied by the presence of serum autoantibodies against nervous tissue antigens, particularly myelin basic protein (MBP). The significance of these autoantibodies is not yet understood since both pathological and protective effects have been described (reviewed in Paterson and Halberg, 1980). Neurological symptoms consist of pareses and paralyses, which first affect the tail, and then progress to hindlimbs and forelimbs. Spontaneous recovery from the disease, that appears several days later, is associated with an increase in endogenous corticosteroid serum levels, indicating neuroendocrine regulation of EAE (MacPhee and Mason, 1990). A second milder

attack of the disease may develop 5-10 days following recovery, and thereafter the animals are refractory to reinduction of EAE.

Marked differences in susceptibility to EAE among strains originate from genetic variations in the expression of major histocompatibility products (Fritz et al., 1983; Williams and Moore, 1973) and differential cytokine production after challenge with the encephalitogen (Vukmanovic et al., 1989). The susceptibility of Lewis rats was ascribed to deficient secretion of corticotrophin-releasing hormone in the hypothalamus in response to inflammation (Sternberg, 1995).

EAE can be induced by immunization with spinal cord homogenate, myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), or encephalitogen peptides from these myelin components. The form of EAE, acute, chronic or relapsing, in the susceptible strains partly depends on the antigen and adjuvant utilized for immunization. For instance, MBP and PLP induce relapsing EAE in the H-2b strain of mice, while MOG peptide 35-55 causes chronic neurological impairment without relapses (Mendel et al., 1995). In Lewis and DA rat strains immunization with MBP or spinal cord homogenate in complete Freund's adjuvant (CFA) induces acute EAE. However, it was demonstrated that DA rats develop severe, protracted and relapsing EAE (SPR-EAE) after immunization with syngeneic spinal cord and incomplete Freund's adjuvant (Lorentzen et al., 1995). The induction of SPR-EAE was associated with humoral autoreactivity to MOG and cellular autoreactivity to rat MBP peptides 69-87 and 87-101.

Most of the studies on EAE in rats were performed in the Lewis strain. However, during the last decade several laboratories have demonstrated that DA rats were equally or even more susceptible to the induction of autoimmune diseases in comparison with Lewis rats. The aim of the present study was to investigate the effect of age and encephalitogen on the induction and reinduction of EAE in DA rats. Secondly, the possible correlation between anti-MBP antibodies and the severity of the clinical disease was tested.

#### MATERIAL AND METHODS

**Animals:** Inbred Dark Agouti (DA) rats from a breeding colony at the Immunology Research Center "Branislav Janković" were used. Sex- and age-matched animals were housed 3-4 per cage and given a standard diet and tap water ad libitum.

**Induction and reinduction of EAE:** EAE was induced in male and female DA rats aged 5 and 8 weeks and in male rats at the age of 27 weeks. Rats were immunized by intradermal injection in the hind footpad of 0.1 ml of an emulsion containing guinea pig spinal cord (GPSC) homogenate or Lewis rat spinal cord (RSC) homogenate (20 mg/rat) in an equal volume of complete Freund's adjuvant (CFA; 0.3 mg *Mycobacterium tuberculosis*/rat). In addition, rats received a subcutaneous injection of 0.3 ml *Bordetella pertussis* vaccine ( $9 \times 10^9$  organisms/rat) in the dorsum of the same foot. Reinduction of EAE was performed 3 weeks after induction of EAE or injection of CFA only. Animals were scored daily for clinical signs of the disease on a scale from 0 to 4 defined as follows: 0, no clinical signs; 1, flaccid tail; 2, weakness of hind limbs; 3, paralysis of hind limbs; and 4, quadriplegia and moribund state.

*Determination of anti-MBP antibodies:* Blood samples were obtained by cardiac puncture under aether anesthesia 20 days after induction and 14 days after reinduction of EAE. Serum levels of antibodies to rat MBP were determined by ELISA as previously described (Djordjevic et al., 1992). Briefly, plates were coated with 1 µg/ml of rat MBP (kindly provided by Dr. George Hashim, St. Luke-Roosevelt Hospital, New York) and saturated with 1% bovine serum albumin solution. Individual serum samples, 1/100 dilution, were tested in triplicate. Peroxidase-conjugated anti-rat IgG in the dilution 1/6000 was used as a secondary antibody. Optical densities were measured in a flow multiscan photometer at 492 nm.

*Data analysis:* All biometric calculations were performed using the statistical package STAT VIEW II. All data were analyzed using three-factorial analyses of variance (ANOVA) with age, sex and encephalitogen as factors, and reanalyzed by means of single factor ANOVA. The Fisher protected least-significant difference test was used for post hoc comparisons between groups. Data are presented as means ± standard errors (S.E.)

## RESULTS

Clinical EAE in DA rats immunized with GPSC or RSC at different ages is presented in Table 1. Since there were no statistically significant differences between the parameters of the disease in male and female DA rats 5 and 8 weeks old, their data were pooled. The results showed that 5-week old DA rats are less

Table 1: Induction of EAE in DA rats 5, 8 or 27 weeks old

Age	Encephalitogen	Incidence (%)	Day of onset (x ±SE)	Clinical score (x ±SE)	Duration in days (x ±SE)
5-week-old	GPSC	12/24 (50)	8.8 ±0.5	0.9 ±0.1*	2.4 ±0.4*
	RSC	8/12 (67)	6.8 ±0.3*	1.0 ±0.1*	2.1 ±0.3*
8-week-old	GPSC	18/19 (95)	8.9 ±0.3	2.7 ±0.3	3.6 ±0.4
	RSC	19/20 (95)	7.1 ±0.2*	2.8 ±0.3	4.5 ±0.3
27-week-old	GPSC	13/17 (76)	9.2 ±0.5	2.2 ±0.4	4.9 ±0.4
	RSC	19/19 (100)	9.5 ±0.5	3.3 ±0.1 <sup>a</sup>	6.6 ±0.6 <sup>a</sup>

\*Statistically significant differences vs. all other groups,  $p < 0.01$ ; and <sup>a</sup>Statistically significant differences vs. 27-week-old rats immunized with GPSC homogenate,  $p < 0.05$ .

susceptible to EAE in comparison with 8- and 27-week old rats (lower incidence, less severe clinical signs and shorter duration of clinical disease). There were no differences in the severity of EAE between 8- and 27-week old rats. In both 5- and 8-week old rats the disease appeared earlier in the RSC immunized group when compared with GPSC immunized rats. Furthermore, in 27-week-old DA rats EAE was more pronounced in the RSC group as revealed by the clinical score and duration of the disease.

Reinduction of EAE in DA rats aged 5 and 8 weeks was performed with the same antigen that was used for the induction of the disease. However, none of the rats developed clinical disease. Rats preimmunized with CFA were completely resistant to EAE after injection of encephalitogen. Conversely, 27-week old rats were susceptible to EAE reinduction only when RSC was used for induction and/or reinduction of the disease (Table 2). The incidence and the severity of clinical signs were lower in this second episode of the disease in comparison with acute EAE.

Table 2: Reinduction of EAE in DA rats at the age of 27 weeks

Encephalitogen for induction/ reinduction		Incidence (%)	Day of onset (x ± SE)	Clinical score (x ± SE)	Duration in days (x ± SE)
GPSC/	GPSC	0/8 (0)			
RSC/	GPSC	3/7 (43)	6.0 ± 0.6	1.3 ± 0.4	3.3 ± 0.2
CFA/	GPSC	0/5 (0)			
GPSC/	RSC	3/9 (33)	7.0 ± 0.3	0.8 ± 0.4	3.0 ± 0.6
RSC/	RSC	3/7 (43)	7.7 ± 0.6	1.0 ± 0.4	2.7 ± 0.6
CFA/	RSC	0/5 (0)			

The level of anti-MBP antibodies was lower in rats immunized with GPSC or RSC for the induction of EAE at age of 5 weeks in comparison with 8-week old rats (Figure 1). After reinduction of EAE there were no differences in the anti-MBP antibody levels between 5- and 8-week old rats. In both age groups, higher anti-MBP antibody secretion was observed in GPSC immunized animals. The level of anti-MBP antibodies was decreased in the CFA/GPSC and CFA/RSC groups in comparison with GPSC/GPSC, but this was not statistically significant in comparison with the RSC/RSC group.

Similarly to 5- and 8-week-old DA rats, increased anti-MBP antibody levels were observed in 27-week-old rats after induction of EAE with GPSC compared to the RSC group (Figure 2). As for the reinduction of EAE, higher anti-MBP antibody levels were found in the GPSC/GPSC, GPSC/RSC and RSC/GPSC groups in comparison with the RSC/RSC, CFA/GPSC and CFA/RSC groups.

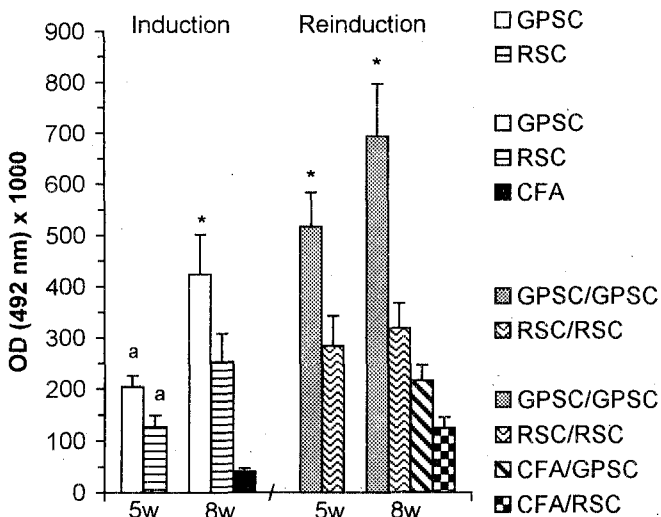


Figure 1. Figure 1: Anti-MBP antibodies in sera of 5- and 8-week-old DA rats after induction and reinduction of EAE with GPSC or RSC homogenate in CFA. Statistically significant differences: \* GPSC vs. RSC and CFA, and GPSC/GPSC vs. RSC/RSC, CFA/GPSC and CFA/RSC,  $p < 0.01$ ; and <sup>a</sup> 5-week vs. 8-week,  $p < 0.05$ .

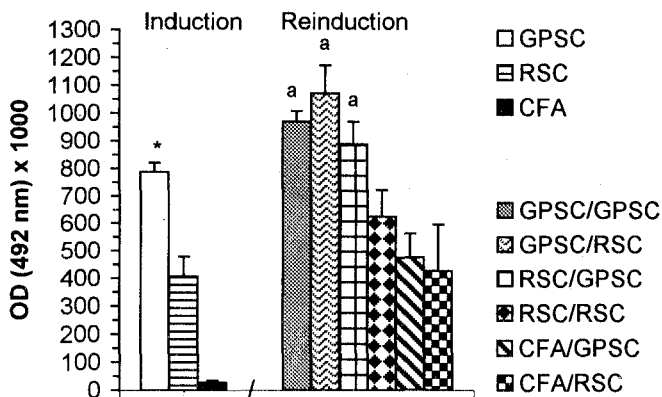


Figure 2. Figure 2: Anti-MBP antibodies in sera of 27-week old DA rats after induction and reinduction of EAE with GPSC or RSC homogenate in CFA. Statistically significant differences: \* GPSC vs. RSC,  $p < 0.001$ ; and <sup>a</sup> GPSC/GPSC, GPSC/RSC and RSC/GPSC vs. RSC/RSC, CFA/GPSC and CFA/RSC,  $p < 0.01$ .

#### DISCUSSION

The results of the present experiments showed that susceptibility to EAE induction in DA rats increased with age. The high susceptibility to EAE of DA rats aged 8 or 27 weeks is in accordance with the reported sensitivity of this rat strain to autoimmune inflammatory diseases, particularly those mediated by CD4+

lymphocytes (Lorentzen et al., 1995a,b). The sensitivity of DA rats to EAE induction was related to both MHC and non-MHC gene products (Lorentzen et al., 1997) and to the profound secretion of proinflammatory cytokines in response to the encephalitogen (Vukmanović et al., 1989; Diab et al., 1997). In contrast, we have found that 5-week-old DA rats were relatively resistant to EAE induction, as revealed by lower incidence and less severe clinical signs in comparison with 8 weeks old rats. Since the adrenocortical system has fully developed by the age of 8 weeks, it can be speculated that the difference in hypothalamo-pituitary axes (HPA) activity between 5- and 8-week-old rats is responsible for the observed distinction in the incidence of EAE (Sapolski and Meaney, 1986). In addition, the age-related susceptibility to the development of EAE in rats is in agreement with the age-related sensitivity to MS.

The finding that RSC homogenate is a more potent encephalitogen than GPSC homogenate for the induction of EAE in DA rats indicated that more than one clone of CD4+ T lymphocytes is involved in the disease. These cell clones may recognize different epitopes on MBP originating from rat or guinea pig spinal cord, or epitopes on other nervous tissue proteins, such as MOG. An investigation of T cell epitopes of guinea pig MBP that induce EAE in DA rats, using synthetic peptides that correspond to regions of the guinea pig MBP molecule that are homologous to rat MBP, disclosed four peptides encephalitogenic when tested in DA rats (Stepaniak et al., 1997). T cells from DA rats immunized with intact MBP proliferated in response to the whole protein and to MBP79-99, but were not stimulated to a significant extent by the other encephalitogenic peptides, suggesting that these may represent cryptic or subdominant epitopes. Our finding that EAE can be reinduced in DA rats at the age of 27 weeks only if RSC was used either for induction or reinduction of the disease suggests that subdominant epitopes could activate T cells after second challenge with the encephalitogen.

Complete resistance to the reinduction of EAE in rats treated with CFA before immunization with the encephalitogen could be a consequence of a CFA induced shift in Th1/Th2 balance towards Th2 response. It was reported that a peptide of the mycobacterial heat shock protein, HSP65, and the myelin protein 2',3' cyclic nucleotide 3' phosphodiesterase (CNP) share sequence similarity and exhibit immunologic cross-reactivity (Birnbaum et al., 1996). Moreover, this hsp-CNP peptide that itself is non-encephalitogenic protects against EAE and largely stimulates peptide-specific antibody production (i.e. Th2 response). However, anti-MBP antibody levels in CFA pretreated rats (CFA/RSC and CFA/GPSC groups) did not differ from the RSC/RSC group. Taking into account that CFA contains mycobacterium tuberculosis, and that immune responses to either hsp or myelin proteins cross-reactive with hsp may influence the development of EAE, it can be hypothesized that antibodies, other than those specific for MBP, could be responsible for CFA-induced refractoriness to EAE.

We have found that serum anti-MBP antibody levels were significantly higher in GPSC immunized rats in comparison with RSC immunized rats three weeks after induction of EAE. Nevertheless, there was no correlation between antibody level and clinical score of EAE. Moreover, the production of anti-MBP antibodies was the lowest in the 5-week-old DA rats that exhibited only moderate sensitivity to EAE. Our results suggest that anti-MBP antibodies did not have a protective effect in the induction and reinduction of EAE in DA rats. On the other hand, it was reported that infusion of MBP primes the immune response such that subsequent challenge with an encephalitogenic inoculum pushes the response down to a

non-destructive Th2 autoimmune pathway (Staykova et al., 1997). Determination of antibody isotype following challenge revealed a change in the ratio of IgG1 to IgG2a with a significant increase in the amount of IgG1 produced. A higher level of IgG1, but not total IgG, was found in Wistar rats tolerized by intraperitoneal infusion of MBP (Rivero et al., 1997). With respect to the above, it would be interestingly to test if specific anti-MBP antibodies of IgG1 isotype are negatively related to the severity of clinical EAE in DA rats.

## REFERENCES

1. Birnbaum G, Kotilinek L, Schlievert P, Clark HB, Trotter J, Horvath E et al. 1996. Heat shock proteins and experimental autoimmune encephalomyelitis (EAE): I. Immunization with a peptide of the myelin protein 23,23 cyclic nucleotide 3' phosphodiesterase that is cross-reactive with a heat shock protein alters the course of EAE. *J Neurosci Res* 44: 381-396.
2. Diab A, Zhu J, Xiao BG, Mustafa M, Link H. 1997. High IL-6 and low IL-10 in the central nervous system are associated with protracted relapsing EAE in DA rats. *J Neuropathol Exp Neurol*. 56: 641-650.
3. Djordjević S, Bukilica M, Dimitrijević M, Marković BM, Janković BD. 1992. Anti-myelin basic protein autoantibodies in rats stressed by overcrowding. *Ann NY Acad Sci*. 540: 729-731.
4. Fritz CB, Chou CH, McFarlin DE 1983. Induction of experimental allergic encephalomyelitis in PL/J and (SJL/J X PL/J)F1 mice by myelin basic protein and its peptides. Localization of a second encephalitogenic determinant. *J Immunol*. 130: 191-194.
5. Lorentzen JC, Andersson M, Issazadeh S, Dahlman I, Luthman H, Weissert R et al. 1997. Genetic analysis of inflammation, cytokine mRNA expression and disease course of relapsing experimental autoimmune encephalomyelitis in DA rats. *J Neuroimmunol* 80: 31-37.
6. Lorentzen JC, Issazadeh S, Storch M, Mustafa M, Lassman H, Linington et al. 1995a. Protracted, relapsing and demyelinating experimental autoimmune encephalomyelitis in DA rats immunized with syngeneic spinal cord and incomplete Freund's adjuvant. *J Neuroimmunol*. 63: 193-205.
7. Lorentzen J C, Olsson T, Klareskog L 1995b. Susceptibility to oil-induced arthritis in the DA rats is determined by MHC and non-MHC genes. *Transplant. Proc.* 27: 1532-1534.
8. MacPhee IAM, Mason DW. 1990. Studies on the refractoriness to reinduction of experimental allergic encephalomyelitis in Lewis rats that have recovered from one episode of the disease. *J Immunol*. 27: 9-19.
9. Mendel I, Kerlero de Rosbo N, Ben-Nun A. 1995. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor V beta expression of encephalitogenic T cells. *Eur J Immunol*. 25: 1951-1959.
10. Paterson PY, Halberg MK. 1989. Antineural antibodies in experimental allergic encephalomyelitis. In (E. J. Goetzl, N. H. Spector eds.) *Neuroimmune Networks: Physiology and Diseases (251-257)* New York: Alan R. Liss, Inc.
11. Rivero VE, Maccioni M, Bucher AE, Roth GA, Riera C.M. 1997. Suppression of experimental autoimmune encephalomyelitis (EAE) by intraperitoneal administration of soluble myelin antigens in Wistar rats. *J. Neuroimmunol*. 72: 3-10.
12. Sapolsky RM, Meaney MJ 1986. Maturation of adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Res Rev* 11: 65-76.
13. Staykova MA, Simmons RD, Willenborg DO. 1997. Infusion of soluble myelin basic protein protects long-term against induction of experimental autoimmune encephalomyelitis. *Immunol. Cell. Biol.* 75: 54-64.
14. Stepaniak JA, Wolf NA, Sun D, Swanborg R.H. 1997. Interstrain variability of autoimmune encephalomyelitis in rats: multiple encephalitogenic myelin basic protein epitopes for DA rats. *J. Neuroimmunol*. 78: 79-85
15. Sternberg EM. 1995. Neuroendocrine factors in susceptibility to inflammatory disease: focus on the hypothalamic-pituitary-adrenal axis. *Hormone Res*. 43: 159-61.
16. Swanborg RH. 1995. Experimental autoimmune encephalomyelitis in rodents as a model for human demyelinating disease. *Clin. Immunol. Immunopathol*. 77: 4-13.
17. Vukmanović S, Mostarica-Stojković M, Lukić M L. 1989. Experimental autoimmune encephalomyelitis in low and high interleukin 2 producer rats. I. Cellular basis of induction. *Cell. Immunol*. 121: 237-264.

18. Williams RM, Moore MJ. 1973. Linkage of susceptibility to experimental allergic encephalomyelitis to the major histocompatibility complex in the rat. *J. Exp. Med.* 138: 775-783.

#### **INDUKCIJA I REINDUKCIJA EKSPERIMENTALNOG ALERGIJSKOG ENCEFALOMIJELITISA U DA PACOVA: UTICAJ STAROSTI I ENCEFALITOGENA**

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#### **SADRŽAJ**

U radu su prikazani rezultati ispitivanja osetljivosti na indukciju i reindukciju eksperimentalnog alergijskog encefalomyelitisa (EAE) u DA pacova, oba pola, starosti 5, 8 i 27 nedelja. Za imunizaciju su korišćeni homogenati kičmene moždine zamorca (KMZ) ili kičmene moždine Lewis pacova (KMP) u kompletnom Freund-ovom adjuvansu. Rezultati su pokazali da: a) pol ne utiče na razvoj EAE-a u 5 i 8 nedelja starih DA pacova; b) osetljivost na EAE raste sa starošću; c) KMP je jači encefalitogen od KMZ; d) produkcija anti-mijelin bazni protein (MBP) antitela je veća nakon imunizacije sa KMZ nego sa KMP; e) nivo anti-MBP antitela nije u korelaciji sa kliničkim znakom EAE-a; f) reindukcija EAE-a je moguća u pacova starih 27 nedelja ako se za indukciju i/ili reindukciju koristi KMP; i g) anti-MBP antitela nisu odgovorna za rezistenciju na reindukciju EAE-a u DA pacova.