Clin. exp. Immunol. (1982) **50**, 311–317.

Resistance to the induction of EAE in AO rats: its prevention by the pre-treatment with cyclophosphamide or low dose of irradiation

MARIJA MOSTARICA-STOJKOVIĆ, MILICA PETROVIĆ & M.L. LUKIĆ *Institute of Microbiology and Immunology, School of Medicine, Institute of Physiology, School of Pharmacy, University of Belgrade and Laboratory for Cellular Immunology, Institute for Biological Research, Belgrade, Yugoslavia*

(Accepted for publication 18 June 1982)

SUMMARY

Susceptibility to the induction of EAE was compared in AO, DA and Lewis strain of rats. As evaluated by clinical and histological criteria, AO rats exhibited significantly lower susceptibility to EAE induced with guinea-pig spinal cord (GPSC) tissue and complete resistance to the encephalitogenic challenge with rat myelin basic protein (BP) irrespective of antigen dose and adjuvant used. AO rats pre-treated with BP + Freund's incomplete adjuvant became completely unresponsive to the induction of EAE with GPSC + Freund's complete adjuvant (FCA) indicating that they do possess cells sensitive to some antigenic determinants of rat BP. In order to test whether the resistance to EAE is due to an active suppression, low dose of irradiation (300 rad) and cyclophosphamide (20 mg/kg) was applied prior to the induction of EAE. Selective depletion of radiosensitive cells facilitated the induction of EAE. Similarly, cyclophosphamide given 2 days prior to BP + FCA completly abrogated the resistance to EAE induction. Thus, it appears that the inability of BP + FCA to produce EAE in AO rats is due to the disproportionate activation of suppressor cells.

INTRODUCTION

The existence of genetic differences in the ability of animals to produce a specific immune response is well established. It is also well known that inbred strains of experimental animals differ in susceptibility to the induction of experimental autoimmune diseases. Most of the mouse strains (Levine & Sowinski, 1973), Brown-Norway (BN) rats (Kornblum, 1968) and Strain 2 guinea-pigs (Stone, Lerner & Goode, 1969) are relatively resistant to the induction of experimental allergic encephalomyelitis (EAE).

In an attempt to delineate the cellular basis of the susceptibility to the induction of EAE in rats, we studied comparatively the antigenic requirements for the induction of EAE in Lewis, Dark August (DA) and Albino Oxford (AO) rats. By using various regimens of immunization, we have demonstrated that AO rats exhibited lower susceptibility to the induction of EAE with complete spinal cord tissue and unresponsiveness to the induction of EAE with rat myelin basic protein (BP). Here we will submit the evidence indicating that AO rats do possess lymphocytes sensitive to the antigenic determinants of rat BP and that the resistance to the induction of EAE could be partially overcome by the low dose of irradiation or by pre-treatment with cyclophosphamide. These results

Correspondence: Dr Miodrag L. Lukić, Institute of Microbiology and Immunology, School of Medicine, University of Belgrade, st. dr. Subotića 1, 11000 Belgrade, Yugoslavia.

0009-9104/82/1100-0311$02.00

indicate that the inability of BP to induce EAE in AO rats is not due to its failure to induce effector T cells but to the disproportionate induction of suppressive mechanisms. Suppressor cell activity was found responsible for the unresponsiveness induced by multiple injections of BP in Freund's incomplete adjuvant (FIA) (Swierkosz & Swanborg, 1975, 1977). Active suppressor mechanisms were also implicated in the recovery from disease and acquired resistance to the re-induction of EAE in Lewis rats (Willenborg, 1979). We here present the evidence suggesting that the hyporesponsiveness to guinea-pig spinal cord homogenate (GPSC) emulsified in Freund's complete adjuvant (FCA) and inability to develop EAE after immunization with BP + FCA, observed in AO rats, may also be due to an active suppression.

MATERIALS AND METHODS

Animals. AO, DA and Lewis rats, 12–16 weeks old, obtained from the animal colony maintained at the Institute for Biological Research, Belgrade, were used in the experiments. Rats were provided access to food and water without restriction, and were hand watered during periods of paralysis.

EAE induction. BP isolated from rat spinal cord according to the method of Martenson, Deibler & Kies (1970), or GPSC were used for the induction of EAE. Antigens were emulsified in FCA and injected in one hind footpad. Each animal received 0·1 ml of encephalitogenic emulsion containing either 50 mg GPSC (wet weight) or 0·3–0·6 mg of BP in 0·05 ml saline plus 0·05 ml of FCA (DIFCO Laboratories, Detroit, USA) enriched with 250 μg of *Mycobacterium tuberculosis* H37Ra (kindly provided by Professor M. Jovanović of Institute of Microbiology and Immunology, Belgrade).

EAE prevention. Rats were given a course of eight intradermal injections of BP, 1 mg each, emulsified in FIA, at 2 day intervals. Induction of EAE was performed 2 days after finishing this regimen.

Evaluation of EAE. Rats were observed daily for clinical signs of neurological dysfunction which were scored as follows: 0–no clinical signs of disease; 1–flaccid tail; 2–hind limb paresis; 3–hind limb paralysis; 4–death. Surviving animals were sacrificed on day 21 post-immunization and their brains and spinal cords were fixed in 10% neutral formalin, embedded in paraffin, sectioned and stained with haematoxilin & eosin. Sections of the brain at the level of the thalamus, mesencephalon and cerebellum–pons as well as sections representing cervical, thoracic and lumbosacral spinal cord were examined for focal perivascular mononuclear infiltrates characteristic of EAE. Histopathological changes were scored as follows: 0–absence of histological lesions; 1—slight mononuclear infiltrates only in submeningeal areas; 2—1–4 perivascular infiltrates on a slide; 3—5–10 infiltrates on a slide; 4—more than 10 infiltrates on a slide. The coded slides were read independently and blindly by two investigators. A score of 4 was arbitrarily given for animals found dead before day 21, because post mortem artifacts rendered it histologically unreadable.

Treatment with cyclophosphamide. Solutions of cyclophosphamide (Endoxan, Bosnalijek, Sarajevo) were freshly prepared by dissolving in distilled water. Cyclophosphamide was injected i.v. 2 days before EAE induction in doses of 20 mg/kg body weight.

Irradiation. Whole body 300 rad X-irradiation was delivered by a Phillips therapeutical X-ray machine which was run at 200 kV and 20 mA, through additional filtration of 0·5 mm Cu filter and at the target distance of 54·5 cm. The dose rate was 74 rad/min. Animals were irradiated 21, 14 and 3 days before or at the time of immunization.

RESULTS

Strain differences in susceptibility to the induction of EAE

As shown in Table 1, the induction of EAE with GPSC + FCA produced disease in all three strains of rats, though the severity of disease and speed of onset of EAE signs were significantly lower in AO rats. Encephalitogenic capacity of partially purified BP was tested in DA rats in doses ranging from 0·01 to 1·2 mg of BP (data not shown). By both, clinical and histological criteria doses of 0·3–0·6 mg were found to be most effective in EAE induction. On the contrary, BP in doses of 0·3–0·6 mg (Table

Table 1. Susceptibility to EAE induction in three rat strains

Encephalitogen	Strain	Clinical signs* Onset†	Incidence‡	Grade§	Histological EAE Incidence‡	Grade§
GPSC+FCA	AO	13·3	11/15	1·7	11/13	1·9
	DA	9·5	9/9	3·1	4/4	3·2
	Lewis	8·4	9/9	2·6	9/9	3·5
BP+FCA (0·3 mg)	AO	—	0/15	0	0/15	0
	DA	12·7	4/4	1·5	4/4	1·7
	Lewis	13·8	5/5	3	5/5	3·2
BP+FCA (0·6 mg)	AO	—	0/15	0	0/15	0
	DA	8·8	9/9	2·4	9/9	2·8
	Lewis	13·1	14/14	2·7	12/12	2·5

* Combined results of three experiments.

† Average day after immunization on which signs were first observed; rats without signs did not enter into calculation.

‡ Numerator = number of rats with signs or lesions; denominator = total number of rats.

§ Average of grades 0 to 4; all rats entered into calculation.

1) as well as higher and lower doses of BP (non-optimal for DA rats) were completely ineffective in AO rats in which neither clinical signs nor histological lesions developed. Pertussis vaccine given as an additional adjuvant with BP+FCA enhanced the disease in Lewis and DA rats but could not overcome resistance to the induction of EAE in AO rats (data not shown).

AO rats do possess cells sensitive to the antigenic determinants of BP

In order to establish whether the resistance to EAE in AO rats is the consequence of the inability of their lymphocytes to recognize BP, we attempted to induce unresponsiveness to BP+FCA or GPSC+FCA by pre-treatment of animals with BP+FIA. It was previously shown that pre-treatment of rats with multiple injections of BP+FIA induces the unresponsiveness to the consecutive challenge with BP+FCA (Swierkosz & Swanborg, 1975). AO and Lewis rats received eight injections of 1 mg BP over a 2 week period. Two days after the last injection rats were immunized with BP+FCA or with GPSC+FCA. The results are summarized in Table 2. Tolerogenic pre-treatment rendered Lewis rats unresponsive to the consecutive induction of EAE with BP+FCA. Though tolerant to the induction of EAE with BP+FCA, Lewis rats pre-treated with BP+FIA developed disease when challenged with GPSC+FCA. On contrary, AO rats, after pre-treatment with BP+FIA, became unresponsive to the induction of EAE with GPSC+FCA. Pre-treatment of AO rats with FIA did not alter their capacity to develop EAE after GPSC+FCA. These results suggested that AO rats possess the cells sensitive to the encephalitogenic determinants present in BP.

Enhancing effect of low dose irradiation on the development of EAE in AO rats

AO and DA rats were given 300 rad of whole body X-irradiation at 10 weeks of age and were immunized with GPSC+FCA or with BP+FCA on the same day or 3, 14 or 21 days thereafter. Irradiation at the time of or 3 and 14 days prior to immunization did not enhance the clinical signs of EAE in AO rats challenged with GPSC+FCA and did not alter the resistance of AO rats to the induction of EAE with BP+FCA (data not shown). However, when AO rats were irradiated with 300 rad 21 days prior to the encephalitogenic challenge EAE was produced in all rats treated with GPSC+FCA or BP+FCA (Table 3). In non-irradiated controls only two out of five AO rats treated with GPSC+FCA developed a mild form of the disease while control AO rats treated with

Table 2. Suppression of EAE in Lewis and AO rats by pre-treatment with multiple injections of BP+FIA

Strain	BP+FIA	Encephalitogen	Clinical signs			Histological EAE	
			Onset*	Incidence†	Grade‡	Incidence†	Grade‡
Lewis	−	BP+FCA	9·2	4/4	4	4/4	3·2
	+	BP+FCA	—	0/5	0	1/5	0·4
Lewis	−	GPSC+FCA	8·0	4/4	3·4	4/4	4·0
	+	GPSC+FCA	11·4	5/5	3·5	5/5	3·2
AO	−	GPSC+FCA	10·0	5/5	2·2	3/3	2·3
	+	GPSC+FCA	—	0/5	0	1/5	0·4

* Average day after immunization on which signs were first observed; rats without signs did not enter into calculation.

† Numerator = number of rats with signs or lesions; denominator = total number of rats.

‡ Average of grades 0 to 4. All rats entered into calculation.

Table 3. Effect of the low dose of irradiation on susceptibility to the induction of EAE

Strain	Encephalitogen	Irradiation (300 rad day −21)	Clinical signs			Histological EAE	
			Onset*	Incidence†	Grade‡	Incidence†	Grade‡
AO	GPSC+FCA	−	13·5	2/5	0·4	3/5	1·4
AO	GPSC+FCA	+	15·4	5/5	1·2	4/4	2·0
DA	GPSC+FCA	−	9·2	5/5	3·4	4/4	3·2
DA	GPSC+FCA	+	9·4	5/5	3·0	4/4	2·7
AO	BP+FCA	−	—	0/5	0	0/5	0
AO	BP+FCA	+	16·3	3/5	1·0	4/4	1·7§
DA	BP+FCA	−	10·2	5/5	1·8	3/3	2·0
DA	BP+FCA	+	10·0	5/5	1·0	3/3	2·0

* Average day after immunization on which signs were first observed. Rats without signs did not enter into calculation.

† Numerator = number of rats with signs or lesions; denominator = total number of rats.

‡ Average of grades 0 to 4. All rats entered into calculation.

§ Significantly different ($P_{(2)} < 0{\cdot}05$) from non-irradiated control group by Wilcoxon's rank sum test.

BP+FCA exhibited the resistance to the induction of EAE. In DA rats, low dose of irradiation applied at the time of or 3 and 14 days prior to the induction of EAE significantly suppressed clinical signs of the disease (data not shown). Irradiation applied in DA rats 21 days prior to the encephalitogen did not not affect or tended to suppress the development of EAE (Table 3). These results seem to indicate that the depletion of radiosensitive cells could facilitate EAE induction in resistant AO strain but would not enhance the disease in susceptible DA rats.

Effect of cyclophosphamide on the induction of EAE

We tested whether effect similar to that demonstrated for low dose of irradiation could also be achieved using low dose of cyclophosphamide. Lando, Teitelbaum & Arnon (1980) have shown that in BALB/c mice cyclophosphamide had a marked effect on EAE which is dose-dependent. At doses of 20 mg/kg, it drastically affected the resistance to EAE while lower and higher doses were less

Table 4. Effect of the pre-treatment with cyclophosphamide (20 mg/kg) on the induction of EAE with BP + FCA

Strain	BP + FCA	Cyclophosphamide (day −2)	Clinical signs			Histological EAE	
			Onset*	Incidence†	Grade‡	Incidence†	Grade‡
Lewis	0·3 mg	−	11·2	5/5	2·4	5/5	2·2
		+	11·4	5/5	3·2	3/3	2·3
AO		−	—	0/5	0	0/5	0
		+	11·3	3/5	0·8	4/5	1·6
Lewis	0·6 mg	−	12·0	4/4	2·8	3/3	2·3
		+	11·2	5/5	2·8	5/5	2·2
AO		−	—	0/5	0	0/5	0
		+	13·0	5/5	2·0§	4/4	2·2§

§ Average day after immunization on which signs were first observed. Rats without signs did not enter into calculation.

† Numerator = number of rats with signs or lesions; denominator = total number of rats.

‡ Average of grades 0 to 4. All rats entered into calculation.

§ Significantly different ($P_{(2)} < 0{\cdot}05$) from corresponding control group non-treated with cyclophosphamide by Wilcoxon's rank sum test.

effective. Similarly, Kayashima, Koga & Onoue (1978) found that the administration of 25–50 mg/kg of cyclophosphamide given 2 days before wax D resulted in significant enhancement of the adjuvant arthritis in rats of low susceptibility whereas larger doses of cyclophosphamide were less effective. We found that cyclophosphamide in a dose of 50 mg/kg administered i.v. 2 days prior to EAE induction led to the mild suppression of the disease in susceptible rat strains. Therefore we used the dose of 20 mg/kg in order to establish whether the resistance to the induction of EAE could be due to the preferential activation of cyclophosphamide sensitive suppressor T cell precursors in AO rats. Table 4 shows that pre-treatment with cyclophosphamide partially abolished this resistance in some rats challenged with 0·3 mg BP in FCA and in all rats challenged with 0·6 mg of BP in FCA. In Lewis rats, however, cyclophosphamide slightly increased the response to the lower but not to the higher dose of encephalitogen.

DISCUSSION

AO rats exhibited lower susceptibility to the induction of EAE. They showed a milder form of EAE when immunized with guinea-pig nervous tissue and complete lack of disease when rat BP was used as encephalitogen. The BN strain of rats has been previously described as 'resistant' to EAE. However, Levine & Sowinski (1975) and Lennon *et al.* (1976) demonstrated that appropriate choice of antigen, adjuvant and routes of inoculation made it possible to produce EAE in BN rats. These results suggested that designation of particular strain as resistant to EAE must be restricted and qualified according to the specific details of immunization. In AO rats, however, the addition of *B. pertussis* or carbonyl iron as additional adjuvants could not overcome the resistance to the induction of EAE with BP + FCA.

It was claimed that Lewis rats recognize more than one site on certain myelin basic proteins as encephalitogenic (Martenson, Levine & Sowinski, 1975). The unresponsiveness to BP suggested the possibility that AO rats lack lymphocytes capable of recognizing the encephalitogenic fragment present in our BP preparation. However, this possibility seemed unlikely since AO rats pre-treated with BP + FIA became tolerant to the induction of EAE with GPSC + FCA, indicating that AO rats do have lymphocytes sensitive to antigenic determinants of BP.

In an attempt to explain the results discussed so far we were left with three different possibilities:

(i) AO rats recognize tolerogenic but not encephalitogenic determinants of BP (Swanborg, 1975); (ii) BP + FCA fails to induce effector cells in AO rats; (iii) failure to develop EAE after the injection of BP + FCA is not due to a primary inability to respond to the encephalitogenic determinants but to an active suppression. In order to test the third possibility we applied to AO rats, prior to the induction of EAE, two types of treatment which, in other experimental models of cell-mediated immunity, were shown to affect suppressor cell activity. Both of those approaches, low dose irradiation 21 days prior to the induction of EAE and pre-treatment with low dose of cyclophosphamide 2 days prior to the induction of EAE indicated that active suppressive mechanisms are involved in the resistance of AO rats to the encephalitogenic effect of BP + FCA (Tables 3 & 4).

Several workers interpreted the increase of delayed hypersensitivity following treatment with low doses of cyclophosphamide as evidence for the elimination of suppressor cell activity (Turk & Parker, 1973; Sy, Miller & Claman, 1977). Cyclophosphamide administered 2 days before the encephalitogenic challenge was also found to abrogate the unresponsiveness to EAE induced by tolerogenic pre-treatment of (SJL × BALB/c)F_1 mice (Lando, Teitelbaum & Arnon, 1979) as well as natural resistance to EAE in BALB/c mice (Lando, Teitelbaum & Arnon, 1980). To this we now add the evidence that pre-treatment with cyclophosphamide may abrogate the resistance to the induction of EAE with BP + FCA in AO rats.

Rats exposed to sublethal whole body X-irradiation up to 14 days prior to immunization show a consistent decrease in their capacity to develop EAE (Vitale, Allegretti & Matošić, 1966; Paterson, 1976). Similarly, DA rats irradiated with 300 rad up to 14 days prior to the induction of EAE exhibited a milder form of the disease, when compared to non-irradiated controls. At 3 week intervals, however, the irradiation did not significantly affect the development of EAE in DA rats (Table 3). In contrast, low dose of irradiation applied 21 days prior to the induction of EAE in AO rats resulted in subsequent development of the disease in some of the rats treated with BP + FCA and the enhancement of EAE in animals treated with GPSC + FCA (Table 3). It is of interest to note that Lando *et al.* (1980) observed that when BALB/c mice were irradiated with 350 rad only 2 days before the encephalitogenic challenge 50% of the otherwise resistant mice developed EAE. This apparent species difference is not completely understood. However, our results are in agreement with previous findings in rats, showing that: (a) 600 rad irradiation reduced the incidence of EAE if applied up to 10–14 days prior to immunization (Vitale *et al.*, 1966); (b) a 3 week interval was needed between the irradiation with 200 rad and wax D application in order to demonstrate the elimination of radiosensitive precursors of suppressor T cells responsible for the resistance of WKA strain to adjuvant arthritis (Kayashima, Koga & Onoue, 1976; Kayashima *et al.*, 1978). It appears, therefore, that cyclophosphamide sensitive and radiation sensitive cells, the properties of which agree with those known for suppressor T lymphocyte, may exert a regulatory role in strain dependent resistance to EAE induction.

Relevant are also findings that severe adjuvant arthritis was produced in the rat strains of low susceptibility after adult thymectomy and/or irradiation. However, potentiating effect of elimination of suppressor cells by such procedures was not apparent in PVG/c strain of rats which was highly susceptible to adjuvant arthritis (Kayashima *et al.*, 1976). Similarly, low dose cyclophosphamide pre-treatment and low dose irradiation, which enhanced EAE in AO rats, did not significantly affect the development of EAE in highly susceptible Lewis and DA strains.

In conclusion, it appears that the susceptibility to develop experimentally induced autoimmunity could be dependent on the quantitative differences in suppressor cell activity. It is, however, possible that genetically influenced differences in the activity of antigen presenting cells, shown in other system in mice (Lukić & Leskowitz, 1974; Lukić, Wortis & Leskowitz, 1975) may be responsible for preferential induction of suppressive cell activity in rat strain of lower susceptibility to EAE.

We wish to thank Professor M.M. Simić for his interest and helpful discussions. We thank Ms Vesna Nikolić for her excellent technical assistance. Support was provided by grants from Republic Scientific Fund and VMA Fund, Belgrade, Yugoslavia.

REFERENCES

KAYASHIMA, K., KOGA, T. & ONOUE, K. (1976) Role of T lymphocytes in adjuvant arthritis. I. Evidence for the regulatory function of thymus-derived cells in the induction of the disease. *J. Immunol.* **117,** 1878.

KAYASHIMA, K., KOGA, T. & ONOUE, K. (1978) Role of T lymphocytes in adjuvant arthritis. II. Different subpopulations of T lymphocytes functioning in the development of the disease. *J. Immunol.* **120,** 1127.

KORNBLUM, J. (1968) The application of the irradiated hamster test to the study of experimental allergic encephalomyelitis. *J. Immunol.* **101,** 702.

LANDO, Z., TEITELBAUM, D. & ARNON, R. (1979) Effect of cyclophosphamide on suppressor cell activity in mice unresponsive to EAE. *J. Immunol.* **123,** 2156.

LANDO, Z., TEITELBAUM, D. & ARNON, R. (1980) Induction of experimental allergic encephalomyelitis in genetically resistant strains of mice. *Nature,* **287,** 551.

LENNON, V.A., WESTALL, F.C., THOMPSON, M. & WARD, E. (1976) Antigen, host and adjuvant requirements for the induction of hyperacute experimental autoimmune encephalomyelitis. *Eur. J. Immunol.* **6,** 805.

LEVINE, S. & SOWINSKI, R. (1973) Experimental allergic encephalomyelitis in inbred and outbred mice. *J. Immunol.* **110,** 139.

LEVINE, S. & SOWINSKI, R. (1975) Allergic encephalomyelitis in the reputedly resistant Brown Norway strain of rats. *J. Immunol.* **114,** 597.

LUKIĆ, M.L. & LESKOWITZ, S. (1974) Tolerance induction with bovine gamma globulin in mouse radiation chimeras depends on macrophages. *Nature,* **252,** 605.

LUKIĆ, M.L., WORTIS, H.H. & LESKOWITZ, S. (1975) A gene locus affecting tolerance to BCG in mice. *Cell. Immunol.* **15,** 457.

MARTENSON, R.E., DEIBLER, G.E. & KIES, M.W. (1970) Myelin basic proteins of the rat central nervous system. Purification, encephalitogenic properties, and amino acid composition. *Biochim. Biophys. Acta,* **200,** 353.

MARTENSON, R.E., LEVINE, S. & SOWINSKI, R. (1975) The location of regions in guinea pig and bovine myelin basic proteins which induce experimental allergic encephalomyelitis in Lewis rats. *J. Immunol.* **114,** 592.

PATERSON, P.Y. (1976) Experimental autoimmune (allergic) encephalomyelitis: induction, pathogenesis and suppression. In *Textbook of Immunopathology, Vol. I.* (ed. by P.A. Miescher & H.J. Müller-Eberhard). pp. 179. Grune and Stratton, New York.

STONE, S.H., LERNER, E.M. & GOODE, J.H. (1969) Acute and chronic autoimmune encephalomyelitis: age, strain, and sex dependency. The importance of source of antigen. *Proc. Soc. exp. Biol. Med.* **132,** 341.

SY, M.S., MILLER, S.D. & CLAMAN, H. (1977) Immune suppression with supra-optimal doses of antigen in contact sensitivity. I. Demonstration of suppressor cells and their sensitivity to cyclophosphamide. *J. Immunol.* **119,** 240.

SWANBORG, R.H. (1975) Antigen-induced inhibition of EAE. III. Localization of an inhibitory site distinct from the major encephalitogenic determinant of myelin basic protein. *J. Immunol.* **114,** 191.

SWIERKOSZ, J.E. & SWANBORG, R.H. (1975) Suppressor cell control of unresponsiveness to experimental allergic encephalomyelitis. *J. Immunol.* **115,** 631.

SWIERKOSZ, J.E. & SWANBORG, R.H. (1977) Immunoregulation of experimental allergic encephalomyelitis: conditions for induction of suppressor cells and analysis of mechanisms. *J. Immunol.* **119,** 1501.

TURK, J.L. & PARKER, D. (1973) Further studies on B lymphocyte suppression in delayed hypersensitivity, indicating a possible mechanism for Jones–Motte hypersensitivity. *Immunology,* **24,** 751.

VITALE, B., ALLEGRETTI, N. & MATOŠIĆ, M. (1966) Influence of X-irradiation on experimental allergic encephalomyelitis in rats. *Rad. Res.* **28,** 727.

WILLENBORG, D.O. (1979) Experimental allergic encephalomyelitis in the Lewis rat: studies on the mechanism of recovery from disease and acquired resistance to reinduction. *J. Immunol.* **123,** 1145.