EXPERIMENTAL AUTOIMMUNE UVEORETINITIS (EAU) IN THE MOUSE

マウスにおける実験的自己免疫性ぶどう膜炎

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KO IWASE[1], MUTSUHIKO MINAMI[2], NOBUO KATO[3], and MANABU MOCHIZUKI[4]

[1]Department of Ophthalmology and [2]Department of Blood Transfusion, Faculty of Medicine University of Tokyo; [3]Department of Bacteriology, Nagoya University School of Medicine; and [4]Department of Ophthalmology, Kurume University, School of Medicine.

INTRODUCTION

Experimental autoimmune uveoretinitis (EAU) is an organ-specific autoimmune disease in the eye that can be induced by immunization with retinal specific antigens, i.e., retinal soluble antigen (S-antigen)(1) and interphotoreceptor retinoid-binding protein (IRBP)(2). The immunopathogenic mechanisms involved in EAU are still controversial, but accumulating data indicate that the disease is primarily mediated by T-lymphocytes (3-5).

EAU can be readily induced by immunization with the retinal antigens emulsified in complete Freund's adjuvant (CFA) in a variety of experimental animals such as the rat(6), the guinea pig(7) and the monkey(8). EAU induced

in these animal species is highly reproducible and useful to study the immunopathological mechanisms of the disease and the immunopharmacological effects of immunosuppressants in uveitis (9-11). However, these models are inadequate to study the genetic control of EAU. It is, therefore, important to establish an EAU model in a genetically and immunologically well-defined species such as the mouse. Currently, Caspi and her colleagues succeeded to induce EAU in the mouse (12,13). The induction of EAU in the mouse reported by Caspi et al. was achieved by intensified immunization protocol, i.e., pretreating animals with cyclophosphamide 2 days before immunization and immunizing animals twice with high doses of retinal antigens in CFA along with Bordetella pertussis (13). Although their method is reproducible to induce EAU in certain strains of mice, treating animals with an immunosuppressant, cyclophosphamide, may affect the immune responses in the mice and the cells infiltrating the eye.

The aim of the present study was to estalish a method to induce EAU in mice without using immunosuppressants, and also to determine the genetic control in the induction of EAU by using B10 congenic mice.

MATERIALS AND METHODS

§ 1 Animals

Two inbred strains of mice, SMA mice (n=134) and C57BL/6 mice (n=114) and five strains of B10 congenic mice, B10.BR (n=46), B10.A (n=10), B10.A(4R) (n=11), B10.MBR (n=7), B10.A(5R) (n=19) were used. Also, F1(B10.BR x C57BL/6) (n=19) and AKR/J (n=12) were used. The H-2 haplotypes of the inbred strains of mice used in the study are summarized in Table 1. B10.BR, B10.A(4R), B10.MBR and B10.A(5R) were obtained from the Jackson Laboratory (Bar Harbor, Maine, U.S.A.). B10.A was obtained from the Japan SLC (Hamamatsu, Shizuoka, Japan). C57BL/6 was purchased from Charles River Japan (Atsugi, Kanagawa, Japan). AKR/J was purchased from the Seiwa Laboratory (Yoshitomi, Fukuoka, Japan). F1 hybrid was produced by selective breeding by ourselves. The breeders of SMA mice were supplied by the Institute for Laboratory Animal Research, Nagoya University School of Medicine. The SMA strain of mice is an inbred strain originally derived from Swiss mice and its homogeneity has been maintained by brother - sister mating at the Institute for many years. Animals aged 6 to 8 weeks on the first day of each experiment were used in this study. In conducting the research described, the investigators adhered to the "Guide of the Care and Use of Laboratory Animals" (14).

§ 2 Antigen

S-antigen was prepared using fresh bovine retinas according to the method of Dorey et al. (15) and of Fujino et al. (16).

§ 3 Adjuvants

Two different adjuvants were used. One was K03 LPS, which was prepared from Klebsiella pneumoniae strain LEN-1 (03: K1-) as described previously (17). The other was CFA used as a control adjuvant for K03 LPS. CFA was made in our laboratory from incomplete Freund's adjuvant (DIFCO, Detroit, MI) by adding Mycobacterium tuberculosis H37Ra (Mitsui Pharmaceutical Co. Ltd., Tokyo) at a concentration of 2.0 mg/ml.

§ 4 Immunization

Two different protocols for immunization were tested in this study. One was an immunization with S-antigen emulsified (1:1 v/v) in CFA. Test animals received either single or 5 repeated immunizations with 100 μ g of S-antigen emulsified in CFA in a hind footpad. The other was an immunization with S-antigen mixed with K03 LPS. The K03 LPS was dissolved in 0.01M phosphate buffered saline (PBS) and mixed with antigen solution (1:1 v/v). The mixture containing 100 μ g of K03 LPS and one of various doses (4, 20 and 100 μ g/time/mouse) of S-antigen was given by a subcutaneous injection at the inguinal region. The injection was repeated at the same place at intervals

as indicated.

§ 5 Evaluation of EAU induction in mice

Animals were examined daily under an operating microscope to evaluate clinical signs of ocular inflammation. Animals were killed 3 weeks after the last immunization and all eyes were examined histologically. Immediately after enucleation, eyes were placed in 2.5% glutaraldehyde 2% formalin and fixed. Then, the eyes were embedded in glycol methacrylate, cut at 3 μm and stained with hematoxylin and eosin. Intensity of histopathological changes was graded from 0 to 3 as described in Table 2. Figures 1 to 3 showed the examples of histopathological grading.

§ 6 Evaluation of immune responses to S-antigen

Immediately after the sacrifice of the mice, the blood and the spleen were obtained. The antibody titers to S-antigen in the serum were measured by the enzyme linked immunosorbent assay (ELISA) as described previously (4) with some modification: a 1:1000 dilution of peroxidase conjugated anti-mouse IgG (Cooper Biochemical Inc., Malvern, PA, USA) was used as the secondary antibody in the present study. The antibody levels were expressed as values of the OD absorbance at 410 nm using a 1:320 dilution of the serum samples. The values of the serum samples of SMA and B10 congenic mice without immunization were less than 0.04.

Proliferative responses of lymphocytes to S-antigen were examined using plastic dish-nonadherent splenocytes as follows: immediately after the sacrifice of the mice, the spleens were removed and teased gently in RPMI-1640 medium with HEPES (GIBCO, Grand Island, NY) supplemented with 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM L-glutamine and 2% fetal calf serum (FCS, Boehringer-Mannheim, West Germany). The cell suspension was washed 3 times in the medium and was deprived of erythrocytes by treatment with Tris-ammonium chloride. The cell suspension was incubated in 100 x 15-mm plastic petri dishes (1 x 10^6 cells/dish, Fisher Scientific Co. Springfield, NJ) at 37°C for 1 hr. Then the nonadherent cells were collected carefully, washed once and resuspended in the medium supplemented with 5% FCS and 5 x 10^{-5} M 2-mercaptoethanol. A mixture of the cells (2 x 10^5cells/ml) and S-antigen (0.2, 2, 5, 20 μ g/ml) was cultured in triplicate in 96-well flat-bottomed plates (Costar, Cambridge, MA), at 37°C with 100% humidity and 5% CO_2 in air for 4 days, and pulsed with 1.5 μ Ci of ^{3}H-thymidine (6.7 Ci/mmol, New England Nuclear, Boston, MA) for the last 16 hr. of culture. The cells were harvested with an automatic cell harvester (Cambridge Technology Inc., Cambridge, MA), and incorporated radioactivity was measured by a liquid scintillation counter (Aloka Model LSC-900, Tokyo, Japan). The results were expressed as Δ cpm (mean cpm in cultures with

S-antigen (2 μ g/ml) minus mean cpm in cultures without S-antigen).

RESULTS

§ 1 Immunization with S-antigen in CFA

None of the SMA or C57BL/6 mice developed EAU after single or 5 repeated immunizations with S-antigen in CFA (n=8 in each group). (data not shown)

§ 2 Immunization with S-antigen in K03 LPS

We immunized SMA and C57BL/6 mice with S-antigen (100 μ g/time/mouse) and K03 LPS various times at 4-week intevals. As shown in Table 3, SMA mice recieved 4-5 immunizations developed EAU changes in all tested animals, although none of the SMA mice (n=20) immunized with K03 LPS alone developed EAU (data not shown). 40% of C57BL/6 mice developed EAU when immunized by the repeated protocol (Table 3). No mice of either strain developed any pathological changes in the eye until after the third immunization. A quarter of the SMA mice immunized 3 times with S-antigen developed mild EAU changes. The more the number of immunizations increased, the more frequently and the more severely SMA mice developed EAU. SMA mice developed strong pathological changes including severe cell infiltration following the fourth immunization. After the fifth immunization, cells

infiltrating the eye disappeared but the most intense change was recorded in the photoreceptor cell layer, which was completely destroyed. C57BL/6 mice also developed EAU, and the incidence of EAU induction depended on the number of immunizations. The incidence and intensity of EAU changes in C57BL/6 mice were less than in SMA mice.

We examined the influence of the following parameters on EAU induction in mice, i.e. (1) day of sacrifice following the first immunization, (2) the dose of antigens and (3) the intervals between immunizations.

The influence of the period after the first immunization on EAU induction was shown in Table 4. SMA mice fully developed EAU after the fourth immunization at 4-week intervals (group C in Table 4). These mice were sacrificed at 15 weeks after the first immunization. However, SMA mice which had been immunized twice at an interval of 4 weeks and sacrificed at 15 weeks after the first immunization did not develop EAU (group B).

The results of a dose-response study using the protocol of 4 immunizations at 4-week intervals are shown in Table 5. In both SMA mice and C57BL/6 mice, the incidence of EAU as well as the intensity of pathological changes depended on the dose of S-antigen. However, SMA mice were more susceptible to EAU than C57BL/6 mice as demonstrated by the higher incidence and the higher grading of the disease intensity at all tested

antigen doses. SMA mice developed mild EAU changes in one-third of the tested animals even with 4 μg of S-antigen, while none of the C57BL/6 mice developed EAU at that dose.

Then, the relationship between EAU induction and the time interval of immunizations was examined. In this experiment, a dose of 20 μg of S-antigen plus K03 LPS was injected 4 times at different intervals (1, 2, 4 weeks). It was found that immunization at 4-week intervals resulted in slightly higher or similar EAU development in the SMA mice and C57BL/6 mice comparing to immunization at 1 or 2-week intervals (data not shown).

§ 3 Histopathological features of EAU

Fig. 4 shows the histology of the retina of a naive SMA mouse. The histopathological changes of EAU in SMA mice were characterized by infiltration of inflammatory cells at the retinal blood vessels as well as in the retina and the choroid, and destruction of the photoreceptor cell layer. The early EAU changes following 3 immunizations were moderate cell infiltration in and around retinal blood vessels as well as in the retina and in the choroid but minimal destruction of the photoreceptor cell layer (Fig. 5). The infiltrating cells in those tissues were predominantly mononuclear cells, while infiltration of polymorphonuclear leukocytes (PMNs) was minimal.

The cell infiltration was most remarkable at retinal blood vessels. Following 4 immunizations, inflammation became more severe and partial destruction of the photoreceptor cell layer was observed (Fig. 6). When a fifth immunization was given, the photoreceptor cell layer was completely destroyed, the retina became atrophic, and the cells infiltrating into the retina and the choroid disappeared, as shown in Fig. 7. In contrast to the clear histological changes in the posterior segment of the eye, no significant changes were detected in the anterior segment of the eye (Fig. 8). The histopathological changes of EAU in C57BL/6 mice were essentially same as those in SMA mice. However, intensity of cell infiltration observed in C57BL/6 mice was much less than in SMA mice.

It should be noted that naive mice of any strains used in this study had shown no pathological changes in the eye (the eyes of 5 naive animals of each strain were histologically examined at the age of 12 weeks).

§ 4 Immune responses to S-antigen in SMA mice and C57BL/6 mice

Figures 9 and 10 demonstrate the immune responses to S-antigen in SMA mice and C57BL/6 mice with regard to the number of immunizations. When immunized with 100 μ g of S-antigen plus K03 LPS at intervals of 4 weeks, antibody response to S-antigen in SMA mice increased following the secondary

immunization and the antibody levels were gradually elevated thereafter, while that in C57BL/6 mice slightly increased after the tertiary immunization. The antibody levels in SMA mice were significantly higher than those in C57BL/6 mice (Fig. 9). The proliferative responses to S-antigen in SMA mice were also elevated following the secondary immunization, and the response increased gradually thereafter as the number of immunizations increased. On the other hand, the proliferative responses to S-antigen in C57BL/6 mice slightly increased after the tertiary immunization, and were markedly elevated only after the fourth immunization (Fig. 10).

As shown in Table 6, the antibody levels and the proliferative responses to S-antigen in both strains of mice depended on the dose of S-antigen used for the immunization. In addition, it was found that the lowest dose of S-antigen (4 μ g/mouse), which was an insufficient dose to induce EAU in C57BL/6 mice, did not cause any significant elevation of antibody titers and proliferative responses to S-antigen in this strain of mice. However, in SMA mice the lowest dose of S-antigen, which induced EAU in one-third of this strain of mice, caused moderate elevation of antibody titers and proliferative responses to S-antigen.

§ 5 EAU susceptibility in various strains of mice

Table 8 summarizes the susceptibility to EAU in various strains of mice. Mice were immunized with the mixture containing 100 μ g of K03 LPS and 20 μ g of S-antigen 4 times at 4-week intervals, since a dose of 20 μ g of S-antigen was the submaximum dose to induce EAU in B10.BR (Table 7). As shown in Table 8, among the five strains of B10 congenic mice, B10.BR, B10.A, B10.A(4R) and B10.MBR were highly susceptible to EAU, whereas B10.A(5R) was not susceptible. F1(B10.BR x C57BL/6) was also highly susceptible. C57BL/6 and AKR/J showed low to intermediate susceptibility to EAU.

Table 9 summarizes the antibody levels and the proliferative responses to S-antigen of each strain of mouse used in this experiment. Both antibody titer levels and the proliferative responses to S-antigen were comparable to susceptibility to EAU.

DISCUSSION

The present data showed an establishment of a new method for the induction of EAU in the mouse, which has been considered to be a species resistant to EAU. Also, these data suggested that I-A subregion genes regulated EAU susceptibility in the mice.

Induction of EAU in SMA mice and C57BL/6 mice was achieved by immunization with retinal uveitogenic antigen mixed with K03 LPS. The

immunization was repeated 3-5 times at intervals of 1-4 weeks. The successful induction of EAU in mice can be attributed to the following two factors, (1) the adjuvant used and (2) the strains of mice used in the study.

We could induce EAU in either SMA or C57BL/6 by immunization with S-antigen in K03 LPS, although both strains did not develop EAU by immunization with antigen emulsified in CFA. K03 LPS has been reported to have very powerful adjuvant activities in augmenting immune responses (18,19,20), the delayed type hypersensitivity (21-23) and susceptibility to various autoimmune diseases, such as thyroiditis (24,25), pancreatitis (26), hepatitis (27), hemolytic anemia (28) and orchitis (29) in SMA mice or other strains of mice. K03 LPS has also been shown to have several biological activities such as enhancing interleukin-1 production by activated macrophages (30,31), induction of PMN infiltration in the lymph node (32), and promoting antigen retention in the lymph node (33). In addition, successful induction of other experimental autoimmune diseases in mice has been reported by repeated immunization with pathogenic antigens mixed with K03 LPS (18,19,24-29). EAU in the mouse was also readily induced by the repeated immunization protocol with S-antigen mixed in K03 LPS. Although the precise immunologic mechanisms by which K03 LPS augments the immune responses is still unknown, it was reported that much more pronounced antibody response

was induced after the secondary and tertiary immunizations with the protein antigen mixed with K03 LPS than after the primary immunization (20). This is in accord with the present results: no mice developed EAU and antibody response and proliferative response to S-antigen in those animals were very low after the primary immunization with S-antigen in K03 LPS. However, the disease induction and high immune responses to S-antigen were achieved only after the tertiary immunization or thereafter (Table 3 & Figs. 9, 10).

Although the present data (Table 3) suggest that at least 4 injections with S-antigen are needed to achieve maximal EAU, one could argue that mice injected fewer than 4 times were killed before the immunopathogenic process reached its maximal level, and therefore total days after the S-antigen immunization could be an essential factor for EAU development rather than the number of S-antigen injections. This argument, however, was ruled out by the findings in SMA mice (Table 4): the 4 repeated injections with S-antigen (100 μ g/time) at 4-week intervals achieved a 100% incidence of EAU (group C in Table 4) and yet the 2 repeated injections with S-antigen did not cause EAU even in mice sacrificd 11 weeks after the second immunization (group B).

It is noteworthy that the pathological features of EAU in mice were different from those in rats. As reported in many studies (6,34), EAU in rats

is acute in nature and characterized by severe PMN infiltration throughout the eye, particularly in the anterior segment of the eye. Although T lymphocytes play the essential role in EAU induction in rats (3-5), the pathological features suggest significant involvement of Arthus-type reaction in the rat EAU (4). In contrast, EAU in the mouse was chronic in nature and characterized by mononuclear cell infiltration in the retina and the choroid, particularly around the retinal blood vessels and in the photoreceptor cell layer. However, inflammation of the anterior segment of the eye was minimal. Also, infiltration of PMNs was minimal throughout the eye at any stage of the disease. It is, therefore, likely that Arthus reaction is less involved and that cellular immunity may be more essential factor in the mouse EAU model than in the rat model. It is not clear why these two species of rodents exhibit different pathological changes of EAU.

It has been reported that genes within the major histocompatibility complex (MHC) influence the susceptibility to certain experimental autoimmune diseases in mice, e.g., thyroiditis (35), encephalomyelitis (36), myasthenia gravis (37), hemolytic anaemia (38) and diabetes (39). The data described here demonstrated that genes within H-2 have a significant role in controlling the susceptibility to EAU induction in the mouse. As demonstrated in Table 8, among the five strains of B10 congenics, all the

highly susceptible strains, B10.BR, B10.A, B10.A(4R), B10.MBR, have I-A^k allele, whereas C57BL/6, a low-susceptible strain, and B10.A(5R), a non-susceptible strain, have I-A^b allele. It was, therefore, suggested that EAU susceptibility in the mouse is controlled by genes in the I-A subregion. Also, F1(B10.BR x C57BL/6) is a strain highly susceptible to EAU. This suggested that the high responder genes within H-2 are dominant. Further, B10.A(5R) (I-A^b) was non-responder although C57BL/6 (I-A^b) was low-responder. Therefore, I-E^k or D^d region genes may play an inhibitory role on induction of EAU in mice of I-A^b, since several authors reported that I-E region genes play a suppressive effect on an immune response (40-42). The H-2 haplotype of the AKR/J mouse is the same as that of B10.BR. And yet, the AKR/J mouse was a intermediate responder for the EAU development while B10.BR mouse was a high responder (Table 8). Therefore, the non H-2 genetic background may play a certain role in influencing susceptibility to EAU.

Serum antibody titers and the proliferative responses to S-antigen were correlated to the disease susceptibility among the tested strains of mice, including the AKR/J mouse. (Table 9). Thus, intensity of immune responses to S-antigen in the present experiments may determine EAU susceptibility.

In conclusion, the mouse EAU model offers important advantages over other animal models of EAU. The extensive knowledge of the immunologic

factors of the mouse and the availability of genetically defined strains should be of great value in studies of cellular and molecular mechanisms and immunogenetics of ocular autoimmune disease.

Recently, immunophathogenic mechanisms of experimental autoimmune encephalomyelitis (EAE) have been extensively studied. In EAE murine model, the encephalitogenic T cell lines were established (43) and encephalitogenic epitopes on myelin basic protein (MBP) or myelin proteolipid apoprotein (PLP) were determined (44). Further, MBP-binding site of MHC class II molecules were analyzed (45), and the usages of V β of T cell receptors on MBP-specific encephalitogenic T cell clones were analyzed in murine and rat models (46). Also, some new therapies for EAE have been developed. Vaccination with attenuated T cell lines successfully prevented the induction of EAE(47). Administration of antibodies directed to MHC class II molecules (46) or monoclonal antibodies to some TCR V β (48) were also effective for prevention of the induction of EAE. Further, administration of competitor peptides, which effectively compete with an encephalitogenic peptide for binding to MHC class II molecules, was reported to prevent the induction of EAE (49).

In future, we will further analyze immunopathogenic mechanisms involved in EAU using the established EAU model. It is possible in murine model to

establish T cell lines and clones capable of adoptively transferring uveitis, to analyze the structure of T cell receptors of these uveitogenic T cell clones, and to dissect the relative contribution of the humaral and cellular immunity to EAU induction. Also, we will develop effective therapeutic protocols for EAU. These trials will provide important clues to overcoming of human autoimmune uveoretinitis.

SUMMARY

We have established a new method for the induction of Experimental autoimmune uveoretinitis(EAU) in the mouse, which has been considered to be a species resistant to EAU. EAU was induced in two strains of mice by repeated-immunization protocol. SMA mice and C57BL/6 mice were immunized repeatedly with S-antigen mixed with Klebsiella 03 lipopolysaccharide (K03 LPS) at intervals of 1 to 4 weeks. Following the tertiary immunization, the mice exhibited histopathological changes of EAU as well as significant immune responses to the antigen. The histopathology of EAU was characterized by retinal vasculitis, infiltration of mononuclear cells in the retina and the choroid, and destruction of the photoreceptor cell layer.

We also analyzed the genetic control for the induction of EAU using the established mouse EAU model. Five strains of B10 congenic mice, F1(B10.BR X

C57BL/6), C57BL/6 and AKR/J were used. The susceptibility is regulated by genes in the I-A subregion of the H-2 complex. Also, the non H-2 genetic background may play a certain role in influencing susceptibility to EAU.

Since the mouse is a genetically and immunologically well-defined species, this model is useful for study of the immunopathogenic mechanisms of EAU.

KEY WORDS

Experimental autoimmune uveoretinitis(EAU), mice, S-antigen, Klebsiella 03 lipopolysaccharide(K03 LPS), H-2 restriction of EAU induction, I-A subregion of H-2 complex

Table 1: H-2 haplotypes of mice used in this study

Strain	H-2 haplotype			
	K	A	E	D
B10. BR	k	k	k	k
B10. A	k	k	k	d
B10. A (4R)	k	k	—	b
B10. MBR	b	k	k	q
B10. A (5R)	b	b	k	d
F1 (B10. BR X C57BL/6)	k/b	k/b	k/b	k/b
C57BL/6	b	b	—	b
AKR/J	k	k	k	k
SMA	?	?	?	?

Table 2: Histopathological grading for EAU

Grading	Pathological changes		
	Cell infiltration at retinal vessels	Cell infiltration in the retina & choroid	Destruction of photoreceptor cells
0	No infiltrating cells	No infiltrating cells	No destruction
1	Mild cell infiltration at some retinal vessels	Mild cell infiltration	Partial destruction
2	Moderate cell infiltration at many retinal vessels	Moderate cell infiltration	Moderate destruction
3	Severe cell infiltration at most retinal vessels	Severe cell infiltration	Complete destruction and disappearance of photoreceptor cells

Table 3: Effects of the number of immunizations with S-antigen mixed with KO3 LPS on EAU development

Strain	Number of immunization*	Incidence of EAU	Histopathological grade of EAU			
			Cell infiltration at retinal vessels	Cell infiltration in the retina & choroid	Damage of photoreceptor cells	Irido-cyclitis
SMA	1	0/6	0	0	0	0
	2	0/8	0	0	0	0
	3	2/8	1.0±1.0	1.0±1.0	0.8±0.8	0
	4	10/10	2.8±0.4	2.8±0.4	2.3±0.4	0
	5	12/12	0	0	3.0±0.0	0
C57BL/6	1	0/8	0	0	0	0
	2	0/8	0	0	0	0
	3	2/8	0.5±0.5	0.3±0.4	0.3±0.4	0
	4	4/10	0.4±0.5	0.6±0.8	0.4±0.5	0
	5	4/10	0.6±0.8	0.6±0.8	0.6±0.8	0

* Immunization was performed with S-antigen (100μg/mouse) at 4-week intervals.

Table 4: Influence of the day of sacrifice on EAU development in SMA mice.

Group	Protocol of immunization*	Incidence of EAU
A	SAg ↓ 4W SAg ↓ 3W †	0/10
B	SAg ↓ 4W SAg ↓ 11W †	0/10
C	SAg ↓ 4W SAg ↓ 4W SAg ↓ 4W SAg ↓ 3W †	8/8

* Immunization was performed with S-antigen (100μg/mouse) at 4-week intervals.

Table 5: Dose-dependent effects of S-antigen on EAU development*

Strain	Dose of S-antigen (μg/mouse)	Incidence of EAU	Histopathological grade of EAU			
			Cell infiltration at retinal vessels	Cell infiltration in the retina & choroid	Damage of photoreceptor cells	Irido-cyclitis
SMA	4	2/6	1.0±1.4	1.0±1.4	1.0±1.4	0
	20	5/12	1.0±1.2	1.0±1.2	0.8±0.2	0
	100	12/12	2.8±0.4	2.8±0.4	2.3±0.4	0
C57BL/6	4	0/8	0	0	0	0
	20	2/8	0.5±0.9	0.5±0.9	0.5±0.9	0
	100	4/10	0.5±0.5	0.6±0.8	0.4±0.5	0

* Immunization with S-antigen was repeated 4 times at 4-week intervals.

Table 6: Dose-dependent effects of S-antigen on the immune responses*

Strain	Dose of S-antigen (μg/mouse)	Immune responses to S-antigen	
		Antibody level**	Proliferative responses of lymphocytes***
SMA	4	0.79±0.01	9118±353
	20	1.26±0.08	16556±954
	100	1.60±0.05	15147±1327
C57BL/6	4	0.05±0.02	2223±112
	20	0.88±0.15	9873±254
	100	1.10±0.12	13538±1127

* Immunization with S-antigen was repeated 4 times at 4-week intervals.
** The values indicate OD values at 410 nm of a serum dilution at 1:320 by ELISA assay (mean±SE).
*** ΔCPM (mean±SE). Background were from 3318 to 9127 cpm.

Table 7: Dose-dependent effects of S-antigen on EAU development in B10.BR mice*

Strain	Dose of S-antigen (μg/mouse)	Incidence of EAU	Histopathological grade of EAU			
			Cell infiltration at retinal vessels	Cell infiltration in the retina & choroid	Damage of photoreceptor cells	Irido-cyclitis
B10.BR	4	0/12	0	0	0	0
	20	10/12	2.5±0.4	2.4±0.5	1.9±0.6	0
	100	10/10	2.7±0.4	2.6±0.4	2.2±0.3	0

* Immunization with S-antigen was repeated 4 times at 4-week intervals.

Table 8: H-2 restriction of EAU in the mouse

Strain	H-2 haplotype				Induction rate of EAU
	K	A	E	D	
B10.BR	k	k	k	k	10/12 (83.3%)
B10.A	k	k	k	d	7/10 (70.0%)
B10.A (4R)	k	k	—	b	7/11 (63.6%)
B10.MBR	b	k	k	q	4/7 (57.1%)
B10.A (5R)	b	b	k	d	0/19 (0.0%)
F1 (B10.BR x C57BL/6)	k/b	k/b	k/b	k/b	13/19 (68.4%)
C57BL/6	b	b	—	b	2/8 (25.0%)
AKR/J	k	k	k	k	4/12 (33.3%)

Immunization with S-antigen (20µg/mouse) was repeated 4 times at 4-weeks intervals.

Table 9: Immune responses to S-antigen in various strains of mice.

Strain	Antibody level*	Proliferative responses of lymphocytes**
B10.BR	1.59±0.14	18675±4126
B10.A	1.39±0.10	18095±1215
B10.A(4R)	1.61±0.07	17883±2753
B10.MBR	1.22±0.33	15669±1732
B10.A(5R)	0.57±0.37	2754±956
F1(B10.BR x C57BL/6)	1.22±0.22	15904±1854
C57BL/6	0.88±0.15	10512±352
AKR/J	1.18±0.13	8631±1963

* The values indicate OD values at 410 nm of a serum dilution at 1:320 ELISA assay (mean±SE)

** ΔCPM (mean±SE). Backgrounds were from 7321 to 9829 cpm.

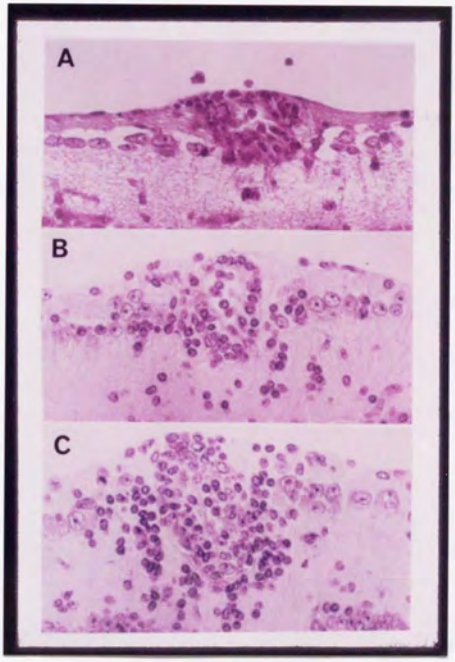

Fig. 1 Histopathological grading of cell infiltration at retinal blood vessels. A,B and C indicate grade 1,2 and 3, respectively.

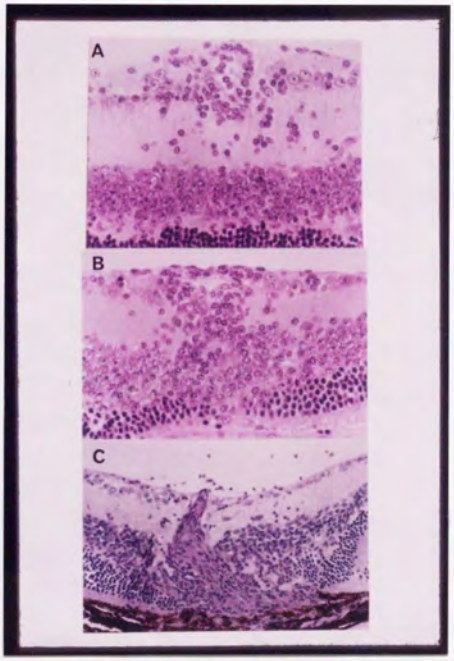

Fig. 2 Histopathological grading of cell infiltration in the retina and choroid. A,B and C indicate grade 1,2 and 3, respectively.

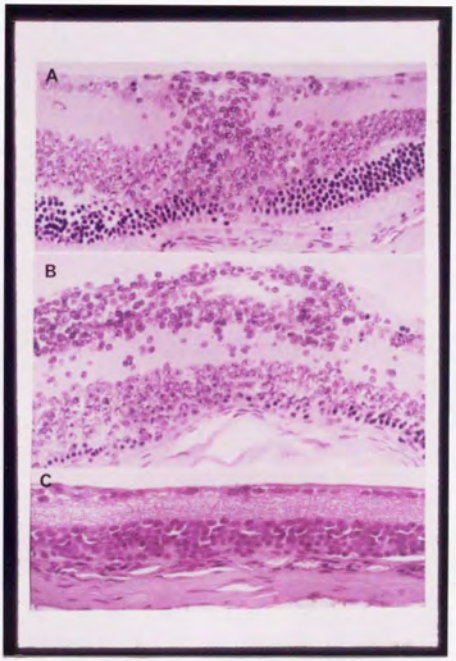

Fig. 3 Histopathological grading of photoreceptor cells destruction. A,B and C indicate grade 1,2 and 3, respectively.

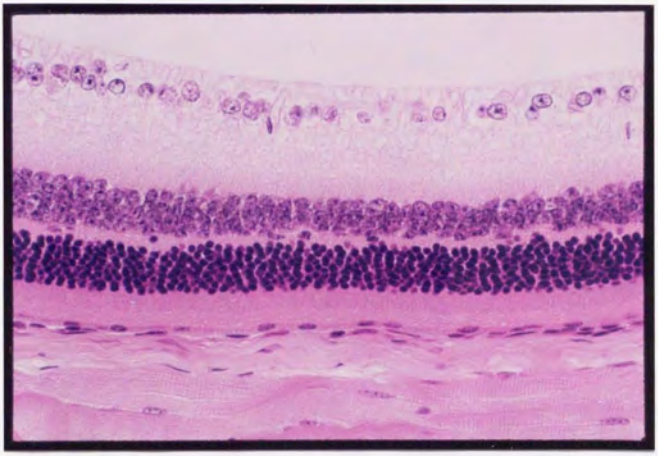

Fig. 4 Histology of the reina of a normal SAM mouse (H&E staining, X400).

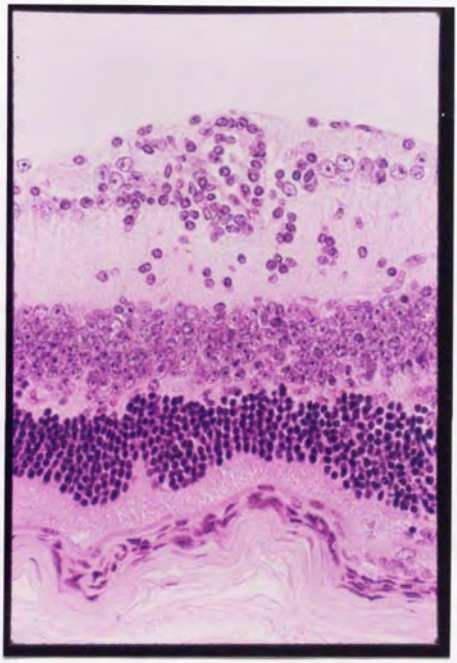

Fig. 5 Histopathology in a SMA mouse following 3 immunizations with S-antigen (100 μ g) mixed with K03 LPS showing moderate mononuclear cell infiltration in and around the retinal blood vessels, in the photoreceptor cell layer and the choroid (H&E staining, X400).

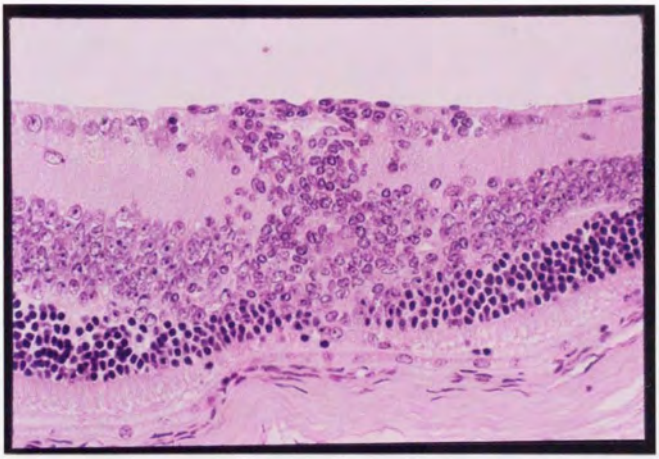

Fig. 6 Histopathology in a SMA mouse following 4 immunizations with S-antigen (100 μg) mixed with K03 LPS showing advanced retinal vasculitis and partial destruction of the photoreceptor cell layer. (H&E staining, X400).

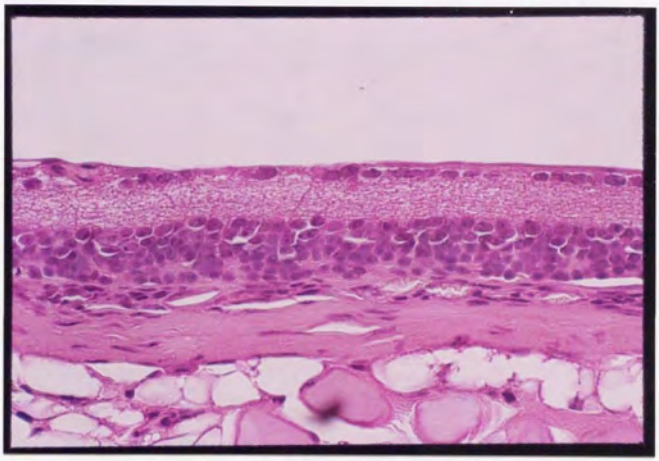

Fig. 7 Histopathologic changes in the retina of SMA mouse following 5 immunizations with S-antigen (100 μg) mixed with K03 LPS. Inflammatory cells in the retina and the choroid were not observed but the photoreceptor cell layer was totally destroyed. (H&E staining, X400).

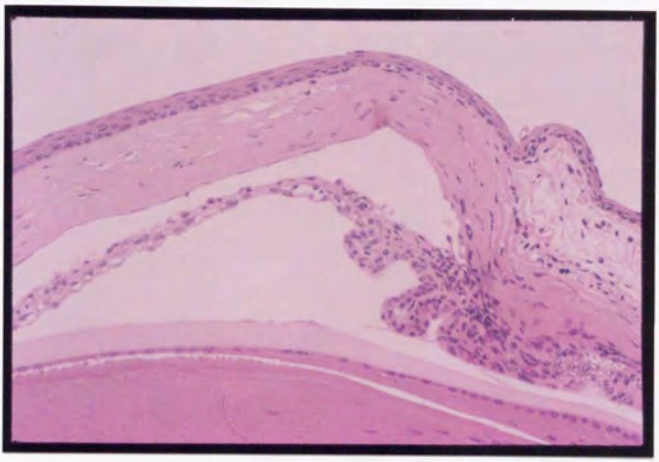

Fig. 8 Histopathology of the anterior segment of the eye in a SMA mouse following 4 immunizations with S-antigen (100 μg) mixed with K03 LPS showing no inflammatory changes in the anterior segment of the eye. (H&E staining, X200).

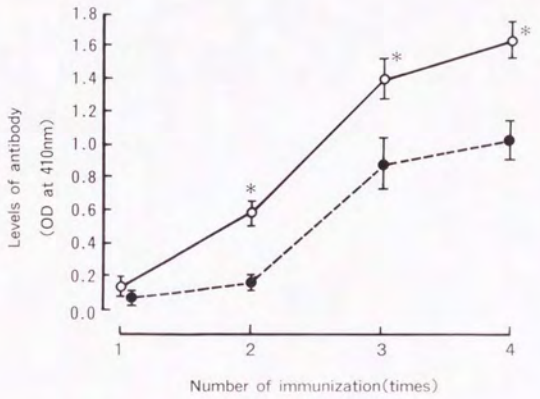

Fig. 9 The levels of antibody to S-antigen in SMA mice (○) and C57BL/6 mice (●) immunized with S-antigen mixed with K03 LPS. More than 5 animals were examined in each group. * indicates significant difference ($p<0.01$) between SMA mice and C57BL/6 mice.

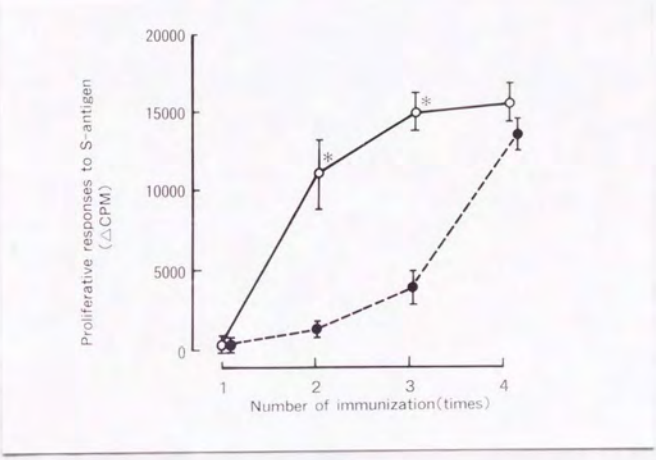

Fig. 10 Proliferative responses of lymphocytes to S-antigen in SMA mice (○) and C57BL/6 (●) immunized with S-antigen mixed with K03 LPS. More than 5 animals were examined in each group. * indicates significant difference ($p<0.01$) between SMA mice and C57BL/6 mice.

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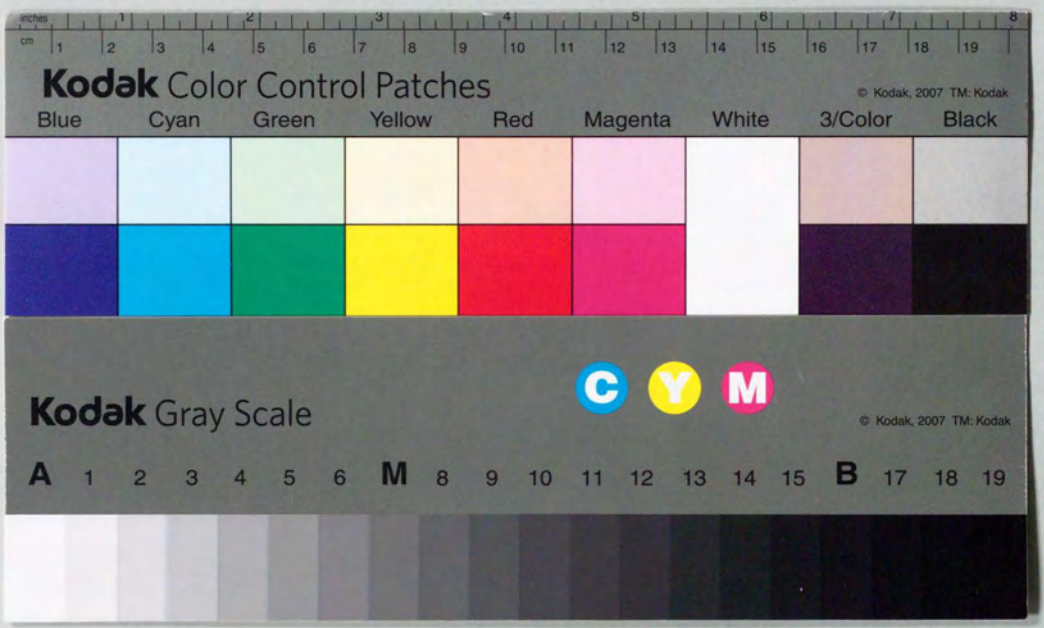
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