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CLINICAL MANIFESTATIONS OF EXPERIMENTAL AUTOIMMUNE UVEITIS

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SUMMARY
THE CLINICAL SIGNS OF EXPERIMENTAL AUTOIMMUNE UVEITIS

Introduction: Autoimmune uveitis is a sight threatening disease which in many cases fails to respond to conventional immunosuppressive or biological therapy. The research in experimental models of autoimmune uveitis helps to find new therapeutical strategies. The aim of this study is to present the clinical and histological signs of experimental autoimmune uveitis (EAU) in mice.

Methods: EAU was induced in C57BL/6 mice by subcutaneous application of IRBP (interphotoreceptor retinoid binding protein) in complete Freund's adjuvant and intraperitoneal application of pertussis toxin. Clinical evaluation of uveitis was performed in vivo using special imaging system with otoscope. Histological evaluation of uveitis was performed at day 35 post induction of EAU on hematoxylin and eosin stained frozen sections. Clinical and histological grading was used to assess the inflammation intensity of EAU.

Results: The intensity of inflammation is depicted on representative fundus images and histological images of retina at day 35 post induction.

Conclusion: The model of EAU is robust and reproducible and allows us to study the immunopathological mechanisms of inflammation and its regulation. The inflammatory signs in our model are similar to findings of posterior uveitis of autoimmune etiology in humans, thus we may apply our experimental results in human medicine.

Key words: experimental autoimmune uveitis, autoimmunity, mice

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INTRODUCTION

Together with diabetic retinopathy, uveitis is the main cause of blindness in developed countries in people of productive age. Uveitis represents ten percent of the cases of blindness in these countries (20, 9) despite the ever broadening range of therapeutic options.

The etiology of autoimmune uveitis in humans is very difficult to determine. In approximately 25% of patients, autoimmune uveitis is linked with a systemic pathology of the type of sarcoidosis, ankylosing sponditis, multiple sclerosis, systemic lupus erythematosus, Wegener's granulomatosis etc. In almost one half of cases, however, we are unable to determine the etiology.

The fundamental therapeutic modality of autoimmune uveitis is immunosuppression. Research over the last five years has improved the therapeutic options, and basic research into uveitis in experimental models has made a significant contribution to this development.

The first model of uveitis in rats (18) used the application of Freund's adjuvant containing mycobacteria and later also endotoxin (10). Since then a model of autoimmune uveitis has been developed in rats, guinea pigs, horses and in primates, through immunisation by one of the retinal antigens – arrestin or S-antigen in complete Freund's adjuvant (22, 12). Its application to experimental animals generated a pathology with a clinical picture very similar to uveitis in humans. In ordinarily used species of mice, however, S-antigen does not generate inflammatory ocular pathology. It was only after the discovery of interphotoreceptor retinoid binding protein (IRBP) that an experimental model of autoimmune uveitis was developed in mice (4). Since that time we have seen the creation of numerous variants of the basic mouse model, which resides in intraperitoneal or subcutaneous application of antigen (IRBP).

In our experiments EAU was induced with the help of IRBP of human origin. The IRBP protein is found in the interphotoreceptor matrix, which serves for the transport of vitamin A derivatives between the photoreceptors and the retinal pigment epithelium. The structure of the IRBP protein is formed by four evolutionary old domains which are assumed to have been generated by gene duplication (1).

A further option for inducing EAU is the adoptive transmission of T lymphocytes from immunised donors, also the "humanised" form of EAU in transgenic species of mice, EAU induced by injection of dendritic cells which have matured in vitro in the presence of an antigen, and finally a model of spontaneous uveitis in mice lacking an autoimmune regulator (AIRE) gene and in athymic "nude" mice with implanted embryonic rat thymus (6).

The model of experimental uveitis we used is very similar to the image of posterior uveitis or panuveitis in humans –

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with vitritis, choroiditis, retinitis and vasculitis. The use of degrees of uveitis is based on a biomicroscopic evaluation in vivo and a histological evaluation post mortem (7).

The objective of this study is to prevent clinical and histological manifestations of experimental uveitis in mice. According to the degree thereof, a standardised evaluation of the intensity of inflammation is performed, which serves for example for an assessment of the efficacy of the new treatment.

METHOD

Mice C57BL/6

Females of the inbred species of mice C57BL/6 at the age of 5 to 8 weeks were supplied by the Centre for Experimental Biomodels (1st Faculty of Medicine, Charles University, Prague). The use of laboratory animals for this project was approved by the academic commission for work with testing animals of the 1st Faculty of Medicine, Charles University in Prague, and the animals were handled in accordance with Act no. 246/1992 Coll., on the protection of animals against mistreatment, on the basis of certification of the Ministry of Agriculture.

Induction of EAU

Application of IRBP, which acts as an antigen, was performed according to the standard protocol (2, 3). 500 µg IRBP 1-20 (interphotoreceptor retinoid binding protein, New England Peptide, Gardner, USA) is applied subcutaneously. DMSO (dimethyl sulfoxide) (Sigma Aldrich, St. Louis, USA) was used for the dissolution of peptide. IRBP was emulsified in a ration of 1:1 with complete Freund's adjuvant (Difco, USA) containing mycobacteria. The reactivity of the immune system was supported by intraperitoneal application of 1.2 µg of pertussis toxin (List Biologicals, Campbell, USA), dissolved in PBS (phosphate buffered saline).

Clinical examination of uveitis

A biomicroscopic examination of animals in vivo was performed with the help of an otoscope (fig. 1). The mice were examined under general intraperitoneal combined anaesthesia with ketamine 80 mg/kg (Narkamon 50 mg/ml, Bioveta, Slovakia) and xylazine 5 mg/kg (Rometar 20 mg/ml, Bioveta, Slovakia). For dilation of the pupil tropicamide (Unitropic 1% oph. gtt., Unimed Pharma, Slovakia) and phenylephrine (Neosynephrin-Pos 10 %, Ursapharm, Czech Republic) were applied locally. An otoscope attached to an external light source and a camera with a front placed lens +4.0 dioptres (19) was placed on the cornea.

Histological processing of material

The animals were killed according to the ethical regulations set by the law of the Czech Republic, by means of cervical dislocation. The eyes were enucleated by cutting from the conjunctival sac immediately post mortem the 35th day following induction of EAU. The eyes were placed in a gel medium (Tissue-Tek® O.C.T. CompoundTm, USA) and frozen in 2-methylbutane (Sigma Aldrich, St. Louis, USA) in an atmosphere of liquid nitrogen. The samples frozen at -70˚C were dissected on a microtome (Leica CM 1850) into 7 µm thick sections. The sections were always cut from the periphery and from the region of the optic nerve of both eyes, and stained using the method of haematoxylin eosin according to the standard protocol. Samples were taken from both eyes with regard to the fact that inflammation can be asymmetrical.

RESULTS

Subcutaneous application of IRBP in complete Freund's adjuvant strengthened with intraperitoneal pertussis toxin generated symptoms of posterior uveitis and in exceptional cases also panuveitis in sensitive species of mice.



Fig 1 Biomicroscopic examination of the retina in mice

An evaluation of the intensity of the inflammation was conducted clinically in vivo or histologically post mortem.

Clinical manifestations of EAU

Clinical manifestations of posterior uveitis are evident on photographs of the retina created with the help of the display system of the otoscope. The symptoms of inflammation in our images identify vasculitis, choroiditis and edema of the optic nerve (fig. 2 - 12).

The clinical system of evaluating the intensity of uveitis is described by Xu et. al. (23). 4 parameters are evaluated on a scale of 1 (minimum inflammation) to 4 (severe inflammation): size and shape of retinal infiltrates, inflammatory changes of optic nerve, degree of encapsulation of capillaries and structural changes of retina (atrophy/scarring).

In our mice the highest intensity of uveitis was observed from the 25th to 28th day after induction, on average of the 3rd degree. At the beginning of observation (day 14) there was isolated identification of an infiltrate or presence of mild edema of the disc of the optic nerve, in the overall evaluation the average degree is 0. In the further development on day 20 to 21, in some eyes there is evident pronounced edema of the disc of the optic nerve, encapsulation of capillaries and whitish retinal linear or granular inflammatory

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Fig. 2 and 3 Physiological fundus in mice

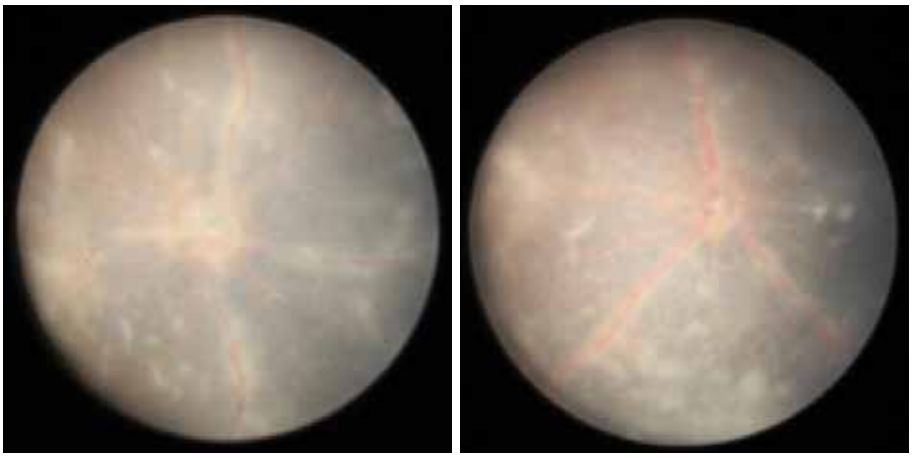


Fig. 4 and 5 Photograph of retina on 20th day after induction of EAU, infiltrates are present in the retina, with encapsulated capillaries and edema of the disc of the optic nerve



Fig. 6 Posterior synechia on photograph of anterior segment present on 25th day after induction of EAU.



Fig. 7 Total serous amotio retinae on 25th day after induction of EAU



Fig. 8 Photograph of fundus on 25th day after induction of EAU, on the retina are evident granular infiltrates, encapsulated capillaries and edema of the disc of the optic nerve

lesions (fig. 4 and 5), the majority of eyes remain without inflammatory changes, the average degree of inflammation is 1. At the peak of inflammation on day 25 to 28, the majority of eyes show pronounced manifestations of inflammation (fig. 6, 7, 8), the average observed degree was 3. From the 35th day atrophic changes are present on the retina (fig. 9 and 10), which intensify over time (fig. 11 and 12).

Histological manifestations of inflammation

The histological symptoms of posterior uveitis include reti-

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Fig. 9 and 10 Photograph of fund on 35th day after induction of EAU, retina with predominant atrophy, granular and linear infiltrates regressing, edema of the disc of the optic nerve persists

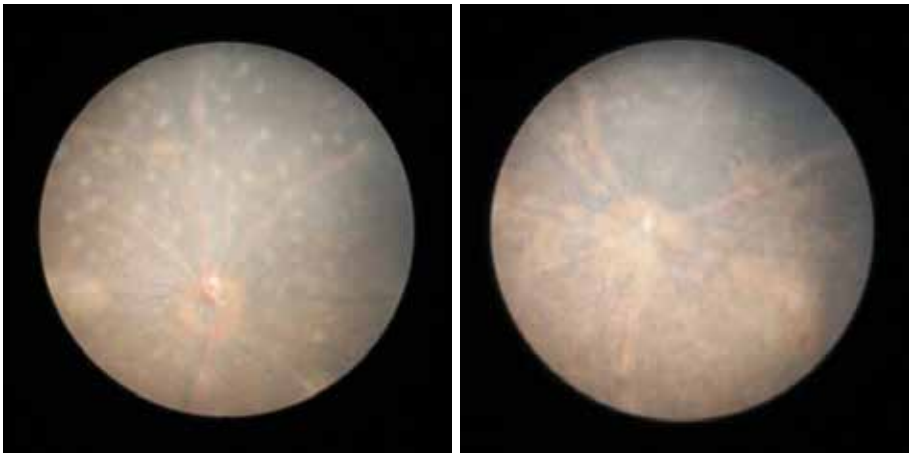


Fig. 11 and 12 Photograph of fundus on 60th day after induction of EAU, with dominant retinal atrophy, infiltrates are more organised, papilla of optic nerve is bordered.

Fig. 2 to 12 The photographs of the retina in healthy mice and in mice at various intervals after induction of EAU illustrate the dynamic of the mani**festations of inflammation**

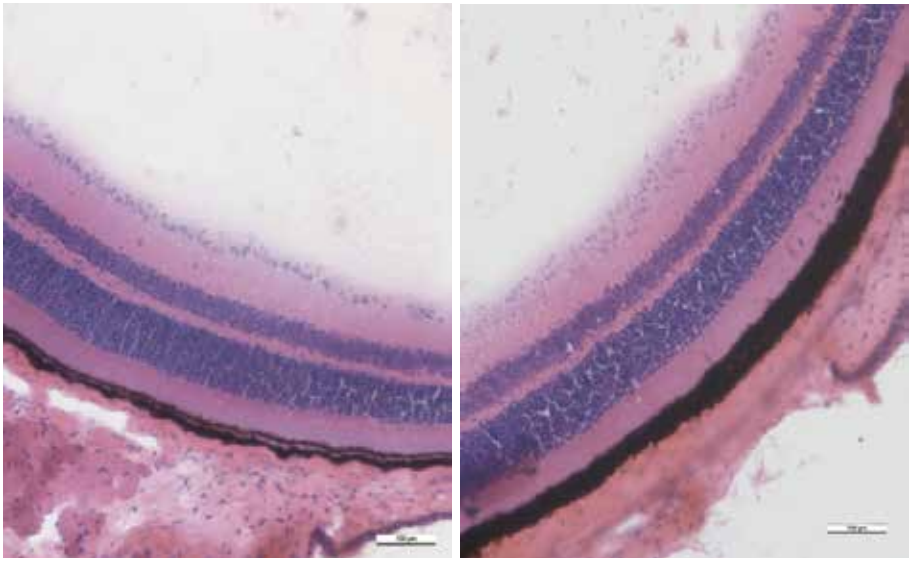


Fig. 13 and 14 Histological section of retina of healthy mouse with regularly arranged layers, enlarged 20x

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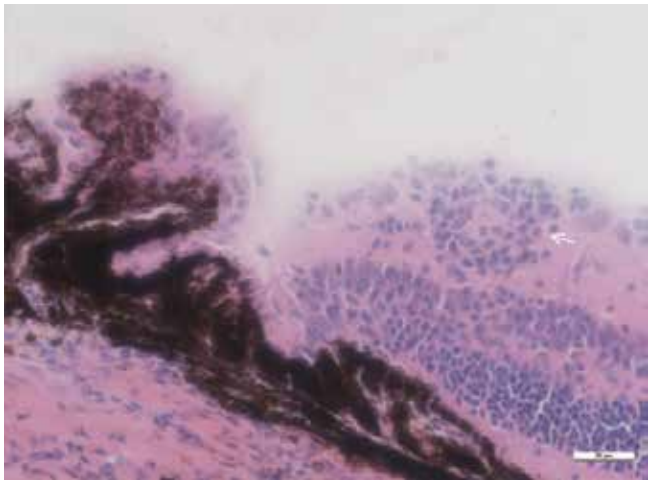


Fig. 15 Inflammatory deposit in internal layer of retina in proximity of ciliary body (arrow), enlarged 40x

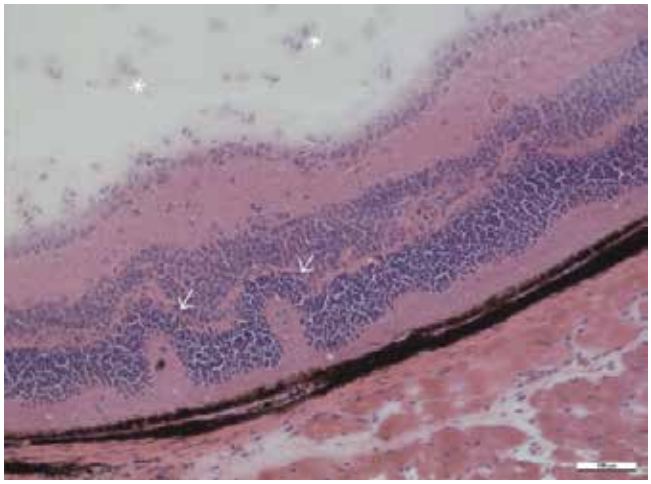


Fig. 16 Retinal buckling in outer layers of retina (arrows), inflammatory cells are present in vitreous body (stars), enlarged 20x

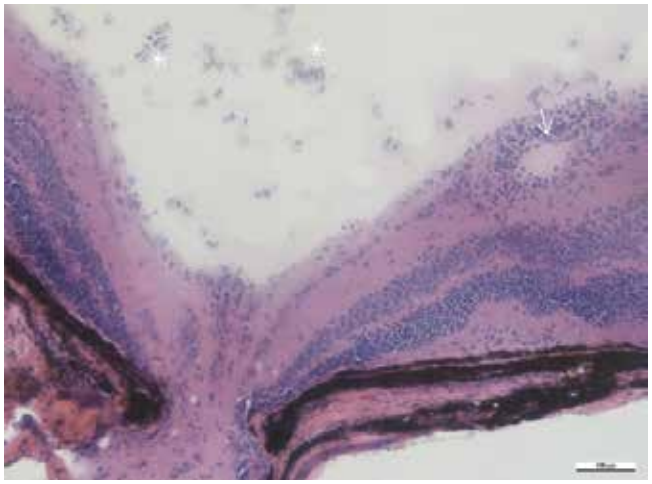


Fig. 17 Inflammatory infiltrations in area of retinal capillary close to disc of optic nerve (arrow), inflammatory cells are present in vitreous body (stars), enlarged 20x

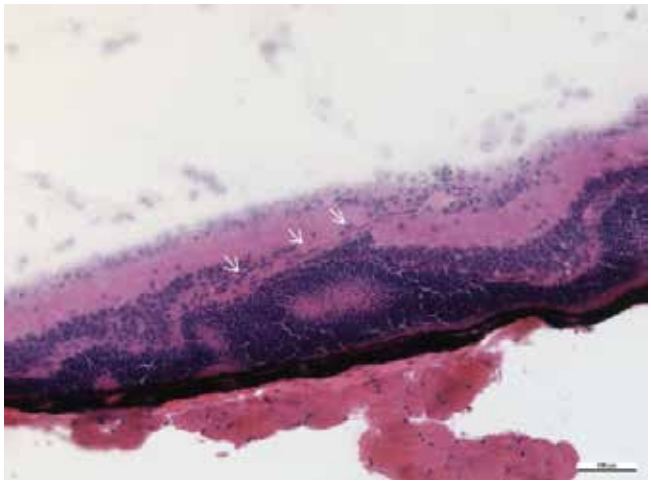


Fig. 18 Newly formed capillary passes through layers of retina (arrows), enlarged 20x

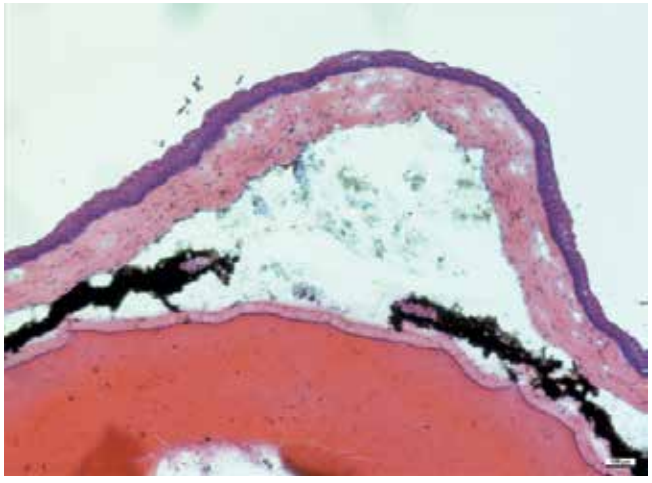


Fig. 19 Histological section of anterior part of eye, chamber fluid is turbid, iris congested, anterior and posterior synechae are present. Behind the iris is a lens which physiologically occupies a large part of the eye. Enlarged 10x.

Fig. 13-19 Histological sections of the eye in normal mice and in mice after induction of EAU 35. dyed with hematoxylin and eosin. The scale is shown in the images, line corresponds to the size of 100 microns.

nal buckling, inflammatory deposits in the retina, frequently localised close to the ciliary body, vasculitis, vitritis, neovascularisation of the retina or loss of photoreceptors (fig. 15 – 18). In healthy mice the retina has perceptibly separated layers of the retina, without irregularities (fig. 13 and 14).

Microscopic evaluation of the intensity of uveitis in sections stained by the haematoxylin-eosin method is conducted according to the established system of evaluation from degree 0 (no inflammation) to degree 4 (severe inflammation) according to Caspi (4, 21, 8).

Histologically the maximum inflammatory changes in our observation are present on the 35th day after induction. The intensity of inflammation assessed histologically in our mice is 1.0 to 2.0.

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DISCUSSION

Autoimmune uveitis incorporates a whole range of units which differ in their clinical picture and course of the pathology. With regard to the fact that this furthermore represents a rare pathology, in human medicine it is very difficult to study autoimmune uveitis. A number of experimental models have been developed which enable a more detailed study of uveitis (4, 23, 6). Over the last 40 years these models have helped clarify the genetic dispositions in the course of intraocular inflammation, examine the fundamental mechanisms of the pathogenesis of uveitis and test new strategies of immunological treatment (11, 17).

Experimental autoimmune uveitis in mice represents a reproducible model which opens up further possibilities in the field of research into posterior uveitis of autoimmune etiology. Uveitis induced in mice of the species C57BL/6 is mild and chronic, in a pattern highly similar to autoimmune uveitis in humans (5). In this it differs from the older model of acute uveitis in the mice species B10.RIII, in which severe short-term panuveitis is generated. In this model the peak of inflammation is from day 12 to 15, and it is not possible to examine the fundus biomicroscopically due to turbid optical media (13).

The sensitivity of the ordinarily used species of mice C57BL/6 to retinal peptitis (IRBP) is relatively variable. The success of induction is influenced by multiple factors, for example the sex and age of the mice or conditions of breeding. In our sample we have mice aged 5 to 8 weeks old, whereas some authors prefer mice aged 8 to 12 weeks old (23). Female mice are usually selected due to the higher prevalence of autoimmune pathologies in women. To date no study has been published comparing the intensity of EAU inflammation between the sexes in mice. The unsuccessful induction of uveitis may be the consequence of chronic stress (higher levels of corticosteroids suppress inflammation) or acute pathology (increased circulating interferons) (1).

The intensity of the generated inflammation evaluated histologically in the mice species C57BL/6 fluctuates between the individual laboratories depending on the protocol of induction of uveitis. On average authors state a degree of inflammation of 1 to 2: Kim et al. (15) 2.13, Xu et al. (15) 1 to 2, Keino et al. (14) 1.2, Kitamei et al. (16) 1.63. In our experiments the average degree of inflammation in the histological evaluation was 1 to 2.

It is noteworthy that the intensity of inflammation on a scale of 1 to 4 determined in a clinical examination with an otoscope is higher than upon the histological evaluation (23), which is also confirmed by our observation. This discrepancy could be explained for example by the different range of the evaluated retina. In the clinical examination the entire retina is surveyed. In the histological evaluation 8 to 10 sections of one eye with a thickness of 7 µm are assessed, with the result that not all the lesions may be identified.

The advantage of the model of chronic EAU in C57BL/6 mice is the relatively long period of activity of the inflammation, persisting for approximately 3 to 4 weeks. In our mice the highest intensity of inflammation biomicroscopically was from day 25 to 28 after induction. Histologically the maximum inflammatory changes were demonstrated on the 35th day after induction. Our observations correlate with the results of other others: Xu et al. (23) recorded the peak of inflammation around the 25th day after induction, Caspi (4) describes active inflammation from the 3rd to 7th week after induction.

The progressive development of clinical and histological manifestations of inflammation in our observation corresponds with the description in the study by the collective of authors Xu et al. (23). In the initial stages (14th day after induction) an edema of the disc of the optic nerve is clinically present, on the histological section the disc of the optic nerve is without visible structural changes. At the peak of inflammation (25th day after induction) there is clinically visible pronounced edema of the disc of the optic nerve, encapsulation of capillaries and whitish retinal linear lesions. On the histological sections these changes correspond to infiltration of the optic nerve with inflammatory cells, vasculitis and retinal buckling. In the late stage (80th day after induction) the clinical picture is dominated by retinal scars, whilst histologically gliosis and loss of the outer segments of the photoreceptors was demonstrated.

The minimal or zero affliction of the anterior segment in our model enables better clarify and easy biomicroscopic examination of the retina even upon high activity of inflammation.

CONCLUSION

Clinical and histological evaluation of the intensity of inflammation in the EAU model is of fundamental significance for the use of this model in basic research. The introduction of a stable and reproducible model of EAU enables detailed study of the immunopathological mechanisms of inflammation and their targeted regulation, which may contribute to effective treatment of intraocular inflammations in human medicine.

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