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Experimental posterior uveitis I: A clinical, angiographic, and pathological study

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SUMMARY The clinical, angiographic, and histopathological features of experimental posterior uveitis in the black hooded Lister rat are described. This mild form of experimental allergic uveoretinitis (EAU) is induced by sensitisation with retinal S antigen in Freund's complete adjuvant, and the inflammation produced is confined to the posterior segment of the eye. This allows for the first time precise photographic and angiographic documentation of the evolution of clinical signs, because there is minimal clouding of the vitreous by inflammatory cells. Clinically the disease is characterised by the appearance of disc oedema and periphlebitis, followed by focal infiltrates in the deep retinal layers, with eventual atrophy of the pigment epithelium. Histologically, retinal vasculitis is associated with focal mononuclear cell infiltration and necrosis of the photoreceptor layers. This model closely resembles the clinical features of idiopathic retinal vasculitis seen in man.

Retinal vasculitis is a well recognised syndrome in man that may complicate systemic diseases such as sarcoidosis and Behçet's disease or occur on its own, and it causes a significant visual morbidity in young people.[1] However, its underlying aetiology and pathogenesis are poorly understood, mainly owing to a lack of suitable pathological material. Models developed in primates[2,3] have shown similarities to posterior uveitis in man, while those in the rabbit[4,5] and guinea pig[6] produce an inflammation largely confined to the choroid, because these animals lack a fully developed retinal vasculature. The most widely used laboratory animal is the albino Lewis rat,[7,8] which, because of its inbred nature and susceptibility to disease, has become an appropriate model for the assessment of uveitogenicity of compounds and for studies of therapeutic prevention.[9] Immunological studies strongly support the concept that experimental allergic uveoretinitis (EAU) is a specific antiretinal autoimmune phenomenon predominantly mediated by T lymphocytes.[10] In the Lewis rat, however, certain clinical features such as disc oedema and periphlebitis may be inferred from pathological studies, but direct observation of the evolution of these signs is impossible because of clouding of the ocular media by inflammatory cells. Rapid destruction of the photoreceptor layer prevents the study of early events that may be important in initiating disease, and the regression of disease within one week does not allow time for pharmacological intervention.

In pilot experiments we observed that EAU in the black hooded Lister rat induced by retinal S antigen (S-ag) in Freund's complete adjuvant (FCA) produced a form of EAU that predominantly affected the retina, so that recording the evolution of clinical signs was possible. In this paper we present the clinical, angiographic, and pathological features of this disease in a series of 36 rats. In a subsequent paper the electroretinographic findings will be discussed.

Materials and methods

Animals. In all studies male black hooded Lister rats (Bantin and Kingman) weighing 250–300 g were used.

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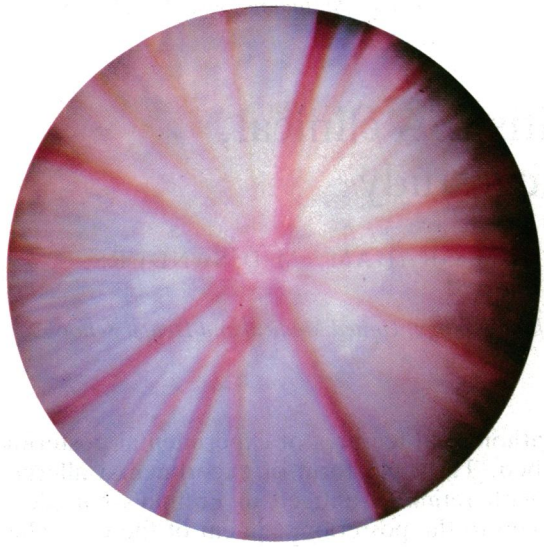

Fig. 1a

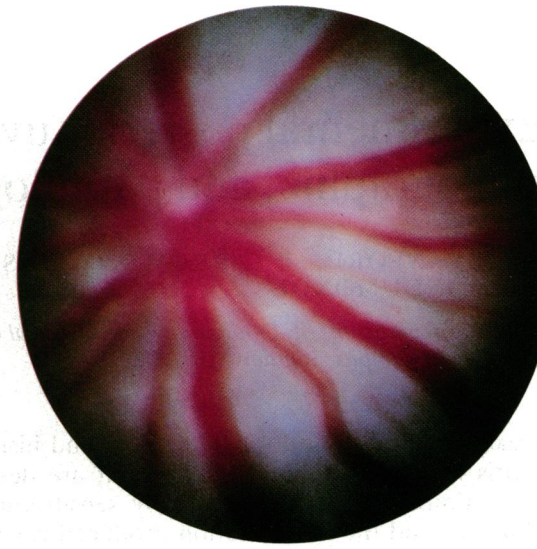

Fig. 1b

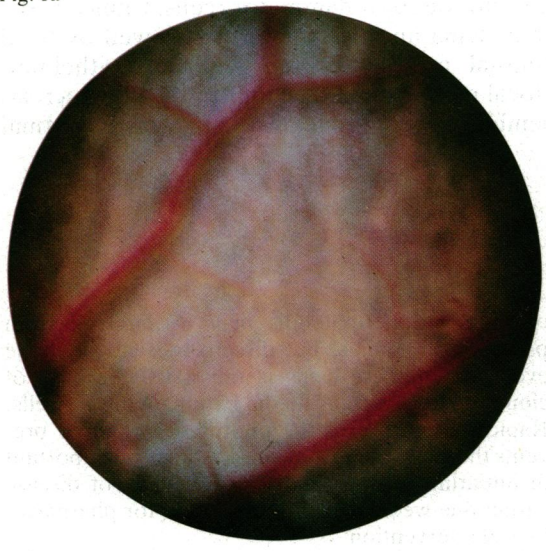

Fig. 1c

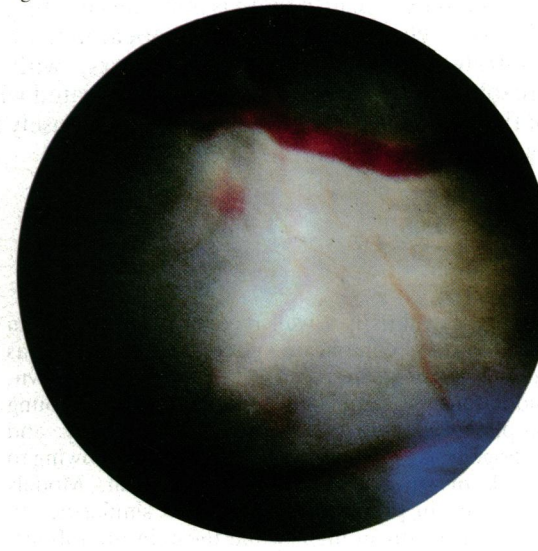

Fig. 1d

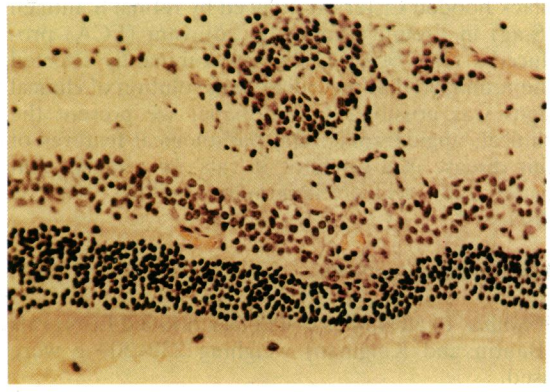

Fig. 1e

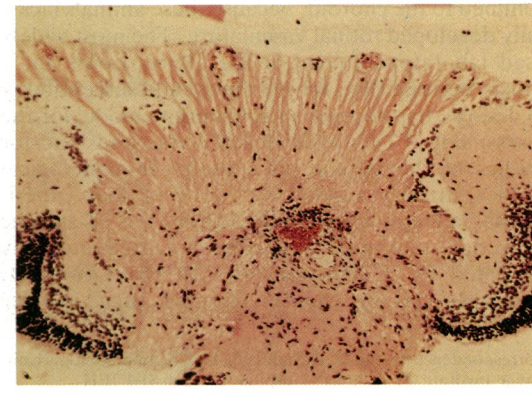

Fig. 1f

Inoculation protocol. In all experiments animals were injected with purified porcine retinal S antigen. Details of the purification procedure are published elsewhere,[11] but in brief this was carried out by extraction from fresh retinas in low ionic strength buffer, ammonium sulphate precipitation, and then purification by size fractionation on Ultrogel AcA34 followed by hydrophobic absorption chromatography on phenyl-sepharose (Pharmacia). The final purity of the injected antigen was 80% as determined by sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) electrophoresis and scanning densitometry. The S antigen in phosphate buffered saline (PBS) was emulsified in Freund's complete adjuvant enriched with *Mycobacterium tuberculosis* 3 mg/ml (Ministry of Agriculture and Fisheries, Weybridge, England). Each animal received 0·1 ml per hind footpad to a final dose of 50 μg S antigen per animal. Control animals were injected in the same manner with PBS/FCA.

Clinical assessment. Each animal was observed on a daily basis from day 10 after inoculation. The pupil was dilated with a drop of tropicamide hydrochloride 1%, and disease activity was assessed by slit-lamp biomicroscopy and fundus photography (Zeiss fundus camera). Where detailed fundus examination was required, the animals were anaesthetised with a single intraperitoneal injection of ketamine hydrochloride (Ketalar) 120–180 mg/kg, which provided a suitable level of anaesthesia for 20 minutes. Serial photographs were taken to record the evolution of clinical signs. Where particular features were noted, or at the termination of the experiment, animals were anaesthetised with intraperitoneal urethane (1·5 g/kg), and fluorescein angiography was carried out.

Fluorescein angiography. The tail vein of the anaesthetised animal was cannulated with a 25 gauge butterfly needle (Abbott Ireland Ltd) that had been heparinised to ensure patency. In some cases the hypotensive effect of the anaesthetic was such that the tail vein could not be cannulated, and in these animals a cannula was introduced into the deep femoral vein via a flank incision. 0·1 ml of 5% sodium fluorescein was injected and a fluorescein angiogram recorded with the Zeiss fundus camera and Ilford FP4 film using the appropriate filters. For peripheral views of the retina the head of the animal was rotated manually. The angiogram was terminated at five minutes. The animals were then killed after cardiac puncture and the eyes removed for histological processing.

Histology. Fresh eyes removed from the animals were placed in 4% glutaraldehyde (EM grade, Emscope Laboratories) for 10 minutes. The globe was then incised with a razor blade to allow access of the fixative to both anterior and posterior chambers and remained in glutaraldehyde for a further 12 hours before being trimmed and placed in 10% neutral buffered formalin. Elimination of this step resulted in a high incidence of artefactual retinal detachment. The eyes were dehydrated in graded alcohols, cleared in toluene, and embedded in paraffin for sectioning. Haematoxylin and eosin staining was used throughout.

Antibody studies. All animals used in this study were bled weekly under ether anaesthesia. Antibodies against S antigen (S-ag) were detected by means of a modified radiometric plate binding assay as described elsewhere.[12] Briefly, polystyrene microtitre plates (Dynatek) were coated with porcine S-ag 5 μg/ml in PBS for two hours at 37°C. The plates were washed in PBS and then incubated with 0·4% bovine serum albumin (BSA) in PBS for two hours at room temperature. Sera from the animals were diluted (1/4 to 1/256) in PBS/BSA and incubated on the plate (1 hour 37°C, 1 hour 4°C) and washed. Fixed antibodies were detected with ^{125}I protein A (Pharmacia) diluted to approximately 1:100 in radioimmunoassay diluent (0·4% BSA in PBS and 0·05% Tween (polysorbate) depending on the specific activity. The plates were then incubated (1 hour 37°C, 1 hour 4°C), washed, and counted in a gamma counter (Compugamma). The antibody titre was expressed as a reciprocal of the dilution that gave half the maximum counts.

Results

Immunisation of black hooded Lister rats with retinal S antigen produced a retinal vasculitis in 90% of the animals observed. The onset of disease, defined by the appearance of vitreous cells and tortuous retinal vessels, varied from 14 to 57 days. The duration of disease, defined as the time from onset until the disappearance of vitreous cells and regression of disc

Fig.1 *Colour photographs of the rat fundus.* (a) *Normal rat optic disc.* (b) *Diseased disc at day 18 after inoculation showing massive swelling, thickening, and tortuosity of retinal vessels and loss of the normal nerve fibre layer reflex. There is a slight haze due to vitreous cells.* (c) *Normal rat retinal periphery.* (d) *Disease in the periphery showing retinal exudates surrounding the capillaries, vascular engorgement with some sludging of blood, and intraretinal haemorrhages.* (e) *Photomicrograph of diseased rat retina showing a perivasculitis composed mainly of lymphocytes and a small mononuclear cell infiltrate in the photoreceptor layer (haematoxylin and eosin).* (f) *Photomicrograph of diseased rat optic disc in oblique section showing swelling, vasculitis of central vessels, predominantly the vein, and some peripapillary retinal folds (H and E).*
Figs. 1a-d, ×44. Figs. 1e,f, ×200.

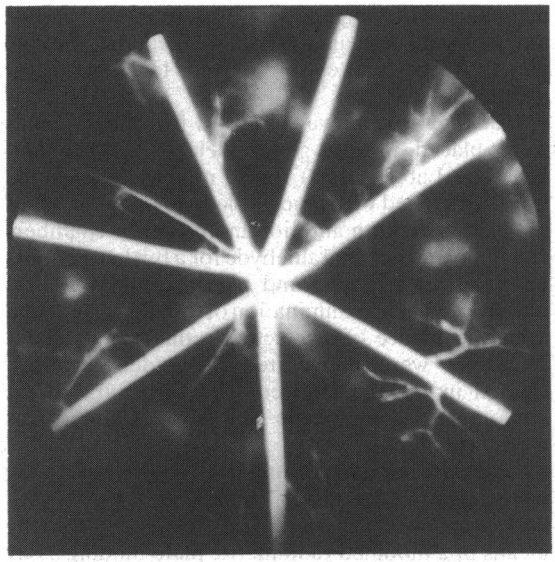

Fig. 2a

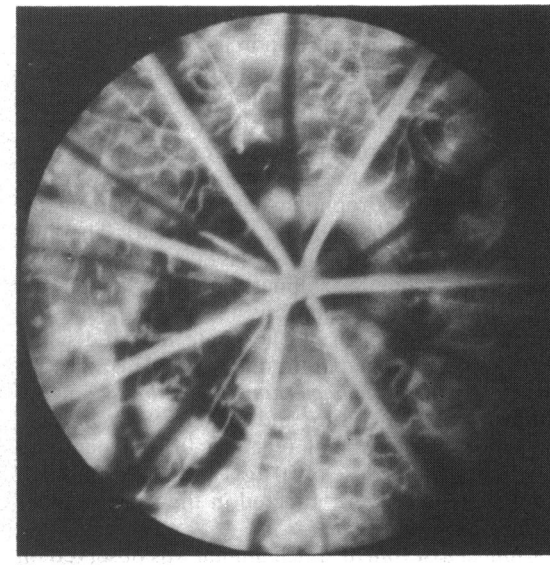

Fig. 2b

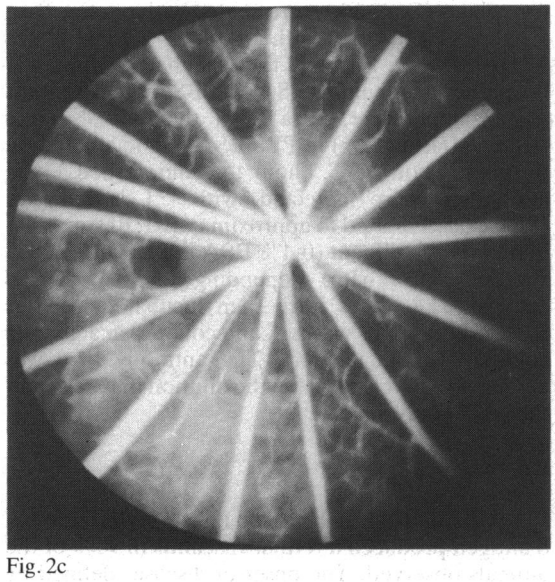

Fig. 2c

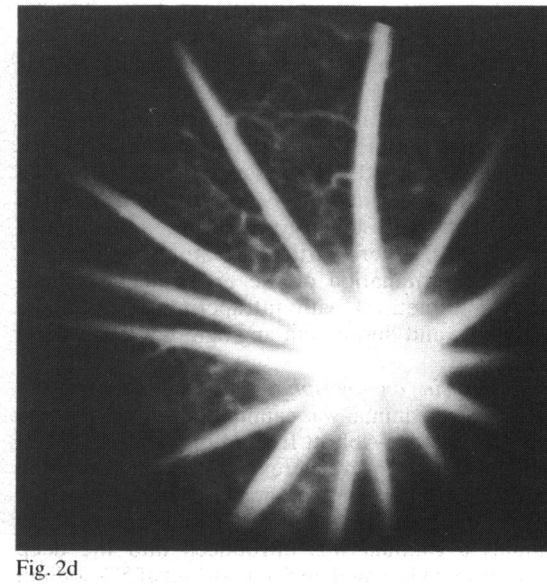

Fig. 2d

Fig. 2 *Fluorescein angiogram of a normal rat retina showing* (a) *early arterial,* (b) *early venous* (c) *late venous, and* (d) *late stages of the angiogram.*

oedema and tortuous vessels, ranged from 5 to 23 days (Table 1). The disease did not recur in any animal for at least three months as judged by these criteria.

CLINICAL COURSE

The disease could be divided into early, intermediate, and late stages. The appearances of a normal rat retina are shown in Figs. 1a and 1c. There is a small hyaloid remnant on the disc which is common to most rat eyes. The retinal vessels are generally straight and there is a well defined nerve fibre layer.

The early stage of the disease was defined by dilation and tortuosity of the retinal vessels (veins more than arteries) and the appearance of a few cells in the vitreous. One to two days after this the optic disc became swollen, the definition of the nerve fibre

Table 1 *Time of onset and duration of disease*

	Experimental animals	*Controls*
No. developing disease	32/36 (90%)	0/15
Disease onset	14 to 57 days (25·1±12·4)	0
Disease duration	5 to 23 days (12±5·6)	0

Figures in brackets are the mean±SD.

layer was lost, and small intraretinal infiltrates appeared.

The intermediate stage showed the maximum number of clinical signs. Figs. 1b and 1d show a retina at this stage 18 days after inoculation. This stage lasted from seven to 10 days.

In the late stage of disease the optic disc and peripapillary area returned to normal, the vessels regained their normal calibre, and vitreous cells disappeared. In the periphery the exudates faded but pigment epithelial atrophy remained in these areas. Significant anterior uveitus, cataract formation, and the development of posterior synechiae did not develop in any animal. Control animals showed no evidence of vitreous cells, and the fundus remained normal throughout.

FLUORESCEIN ANGIOGRAPHY

Fluorescein angiograms were performed to characterise the early, intermediate, and late stages of disease and at the end of observation. A normal rat fluorescein angiogram is shown in Figs. 2a, b, c, d. In the late phase of the angiogram there was a small amount of leakage of dye from the disc in control animals.

In the early stage of disease leakage of dye was evident at the disc and in peripheral areas associated with intraretinal infiltrates. In the intermediate stage (Figs. 3a, b, c) marked thickening and tortuosity of vessels at the disc was evident in the early phase, with extensive leakage of dye in the late phase. In the periphery there was early leakage of dye, which persisted into the late films. Even when the disc had returned to normal clinically, there was persistent leakage of dye, though this became less with time. Areas of peripheral pigment epithelial atrophy showed staining but no leakage at four months.

HISTOLOGY

Anterior segment (all stages of disease). A mild mononuclear cell infiltrate was observed in the anterior chamber in some animals at the height of the intermediate stage. Infiltration of the vitreous round the ciliary body was seen but was always small and reflected changes occurring in the vitreous as a whole. Histological evidence of posterior synechiae were not seen.

Posterior segment (early stage). The earliest histological feature noted was leakage of inflammatory cells from the disc accompanying a mild vasculitis of central disc vessels. This was followed by a panvasculitis affecting the superficial retinal vessels (Fig. 4b), the veins being more affected than the arteries. At this stage mononuclear cells containing pigment were observed singly in areas of the photoreceptor layer (Fig. 1e). The appearance of these cells was associated with pigment epithelial thinning, but whether these cells were derived from blood borne monocytes that had acquired pigment during their passage through the RPE, or whether they were transformed pigment epithelial cells could not be ascertained.

Posterior segment (intermediate stage). The intermediate stage was characterised by swelling of the optic disc due to oedema (Fig. 1f) with increasing numbers of inflammatory cells in the vitreous and a panvasculitis (Figs. 4a, b). In the retina itself mononuclear cell infiltrates were observed, which formed clusters in the photoreceptor layer causing retinal folds, and in all cases were associated with focal photoreceptor necrosis. The retinal pigment epithelium (RPE) always remained intact, and the choroid was thickened in only the severest cases.

Posterior segment (late stage). In this stage the optic disc returned to normal. The retinal vessels appeared normal but in the photoreceptor layers areas of focal necrosis were superseded by fibrous replacement with concomitant loss of the outer nuclear and plexiform layers. In severe cases there was also reduction in the thickness of the inner nuclear layer, probably due to transsynaptic degeneration.

Antiretinal S antigen antibody titres. The antibody levels against S antigen as determined by the plate binding assay are shown in Fig. 5. The titres were highest in the third week and remained raised for some time afterwards. Correlation between antibody titres and clinical or pathological disease was not evident in individual animals.

Discussion

Since the original work of Elschnig[12] and Woods,[13] which implied that some forms of uveitis were autoreactive, considerable advances have been made in refining animal models of human uveitis. There are no models that exactly mirror the clinical variety of disease in man, but all have increased our knowledge by correlation of clinical signs with underlying pathological events. The production of EAU, which has been produced by the injection of whole retinas, retinal extracts, and rod outer segments,[14,15] may now be elicited by a single minute dose of highly purified

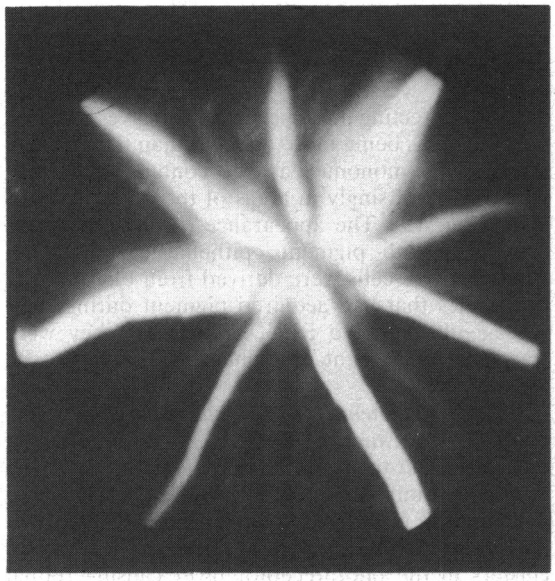

Fig. 3a

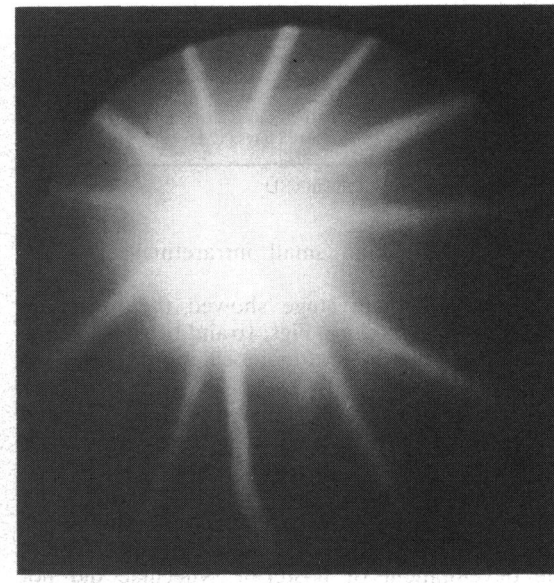

Fig. 3b

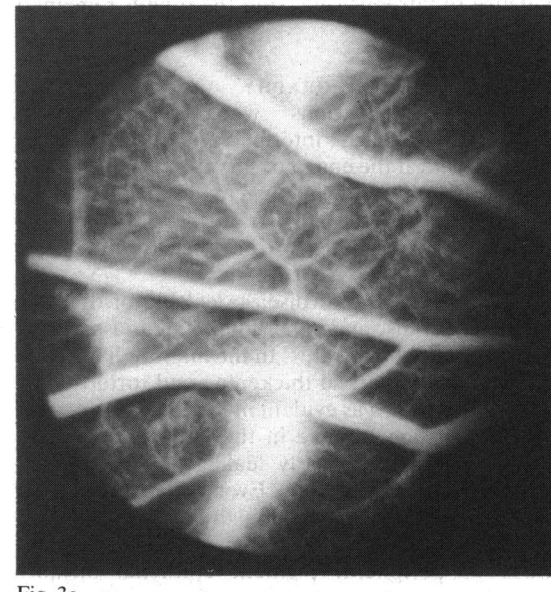

Fig. 3c

Fig. 3 *Fluorescein angiogram of a rat with disease 16 days after inoculation:* (a) *early arterial phase shows marked thickening and tortuosity of vessels,* (b) *late phase of disc shows marked leakage of dye, and* (c) *peripheral view shows leakage of dye from inflamed retinal vessels.*

material from the retina, one of which is retinal S antigen. Other purified antigens, notably rhodopsin and interphotoreceptor binding protein (IBRP), have also been shown to produce disease.[16 17] The nature of the inflammation produced and the extent to which it affects various parts of the eye are dependent on the amount of antigen given,[18] the adjuvants given with it,[7] the species from which the antigen is derived—whether homologous or heterologous—and the species and strain of the experimental model,[19 20] the latter depending largely on the animal's vascular architecture. Correlation between disease and the purity of the administered antigen has not yet been reported. The disease in the rat may thus vary from an acute necrotising panophthalmitis, with involvement of the anterior segment in genetically susceptible Lewis rats, to a mild focal uveoretinitis in genetically resistant pigmented animals.[19 20]

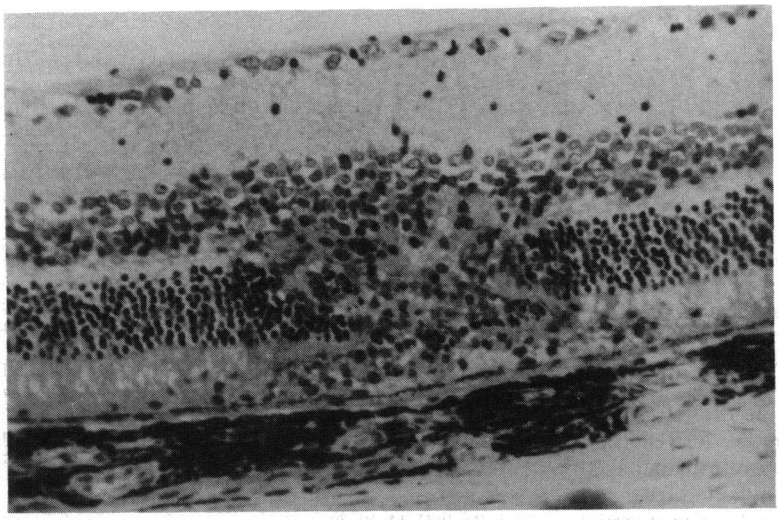

Fig. 4a

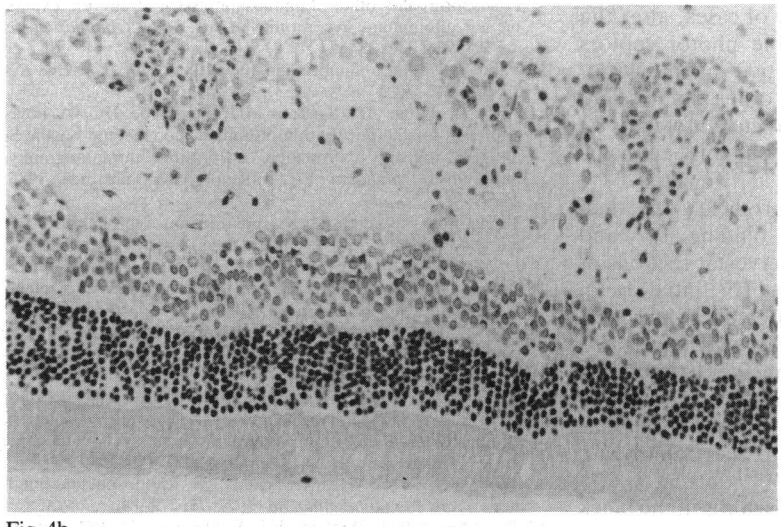

Fig. 4b

Fig. 4 (a) *Photomicrograph of diseased rat retina showing a focal mononuclear cell infiltrate in the photoreceptor, outer nuclear, and plexiform layers. The pigment epithelium appears intact and there is no choroidal involvement (H and E).* (b) *Photomicrograph of diseased retina in the early stage of disease showing perivasculitis of the retinal vessels as an early sign. The photoreceptor layer appears normal. The retina is artefactually detached (H and E).* × *300.*

In this study we have documented the clinical, angiographic, and histological features of EAU in the black hooded Lister rat. The disease in this animal is variable in both its time of onset and duration, and some animals did not develop disease over a period of four months' continuous observation. However, this inconsistency has several practical advantages. The slowness of onset of disease, in some cases taking more than one week from the initial changes to full development of clinical signs, allows early histopathological events to be studied in greater depth, particularly with regard to the anatomical site of early lesions. The duration of disease, though again variable, is more prolonged than in albino animals, and this is relevant when considering the design of regimens for therapeutic intervention. The clinical features such as disc swelling and retinal infiltrates around blood vessels, with accompanying fluorescein leakage, are strikingly similar to those found in man, though we did not find any evidence of occlusive vasculitis as seen in Behçet's disease.

Pathologically, inflammation in this animal is almost entirely confined to the retina, with only minimal focal involvement of the choroid in animals with very severe disease. This is in contrast to practically all other animal models. Actual destruction of the photoreceptors was associated with a mononuclear cell infiltrate, which has also been

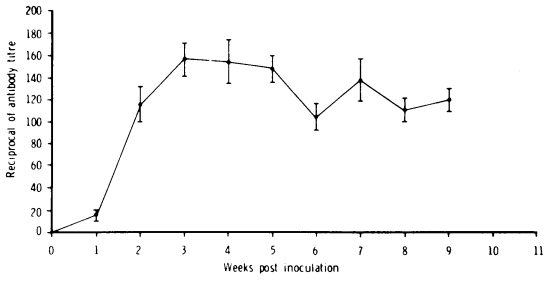

Fig. 5 *Graph showing the relationship between antiretinal S-antigen antibody titres and time after inoculation. Controls are not shown as no rise in titre was observed.*

observed by other authors,[6] but the origin of these cells remain uncertain. They may be derived from monocytes in the blood gaining access from damaged retinal vessels or through an inflamed pigment epithelium, or possibly they may be transformed retinal pigment epithelial cells, though the latter seems less likely. In the majority of cases, after the disease has run its course, viable photoreceptors remain in the absence of any active inflammation. This has been observed in few other models and raises the possibility of inducing recurrent uveitis by further immunological attacks on this antigenic target.

These experimental animals produce antiretinal antibodies as shown by the plate binding assay and indirect immunofluorescence on cryostat sections of rat retinal tissue (data not shown). The initial rise in antibody titre is presumably directed against the immunising antigen, with the levels being maintained by the depot effect of the adjuvant. We could find no evidence of a correlation between disease activity and antibody levels, a finding that confirms the work of other authors and is similar to that found with uveitis in man.[21]

The present model of retinal vasculitis in the black hooded Lister rat is a useful addition to the other models of retinal S antigen induced disease. Direct observation of the clinical and angiographic features of this slowly evolving disease will promote our understanding of the mechanisms underlying posterior uveitis in animals, with important clinical and therapeutic implications for the study of this disease in man.

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