Vascular Changes in the Posterior Segment in Clinical and Experimental Ocular Inflammatory Disease

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Summary
Posterior segment inflammatory disease can have several different effects on the retinal vasculature, all of which have potentially sight threatening consequences. Blood-retinal barrier breakdown is a common feature of the disease process with resulting retinal and macula oedema. Very little is known about the mechanism of this breakdown and in particular whether it is at the endothelial cell membrane or at the tight junctions between the endothelial cells—a matter of importance if better therapeutic regimes are to be devised in the future. This paper looks at this question in two animal models of posterior uveitis using different techniques.

Perivasculitis, with its clinical accompaniment of vascular sheathing, is a classic component of posterior segment inflammatory disease. This can result in breakdown of the normally tight blood-retinal barrier with consequent oedema of the retina and macula (Fig. 1) which has devastating effects on visual acuity. In addition, in some types of posterior segment inflammation e.g. in Eales' disease (Fig. 2), Behçet's disease (Fig. 3), closure of the retinal vessels can occur, resulting in areas of ischaemic retina. As in other situations where this occurs, such as diabetes, neovascularisation can result, often with sight threatening sequelae.

By the time closure of the blood vessels has occurred many pathogenetic mechanisms are likely to be playing a role. In the early stages of the inflammatory process we know that T-cells of the CD4+ subtype are found surrounding the blood vessels in both human[1] and experimental models of posterior segment ocular inflammatory disease.[2] Blood-retinal barrier (BRB) breakdown also occurs at this time and is related to the presence of activated T-cells.[3] What is not known is exactly what the mechanism is by which these activated T-cells damage the BRB and where the damage occurs. The former is thought to occur by lymphokine secretion[4] but which type(s), are not known. This paper looks at two animal models of disease and examines the evidence obtained as to where the damage to the BRB occurs—is it at the vascular endothelial cell membrane level or the tight junctions in between these cells?

Two approaches have been tried: (1) carrying out angiography with a large molecular weight fluorescein-labelled dextran in a primate model of uveitis. In addition, electron microscopy was used to examine the vascular interendothelial tight junctions in areas leaking the large molecular weight dextran and those only leaking sodium fluorescein. (2) Horse-radish peroxidase was infused in a rat model of posterior uveitis and the tight junctions between the retinal vascular endothelial cells examined by electron microscopy.

Methods
Animal models: (1) *Primate*—Four Rhesus monkeys were immunised subcutaneously

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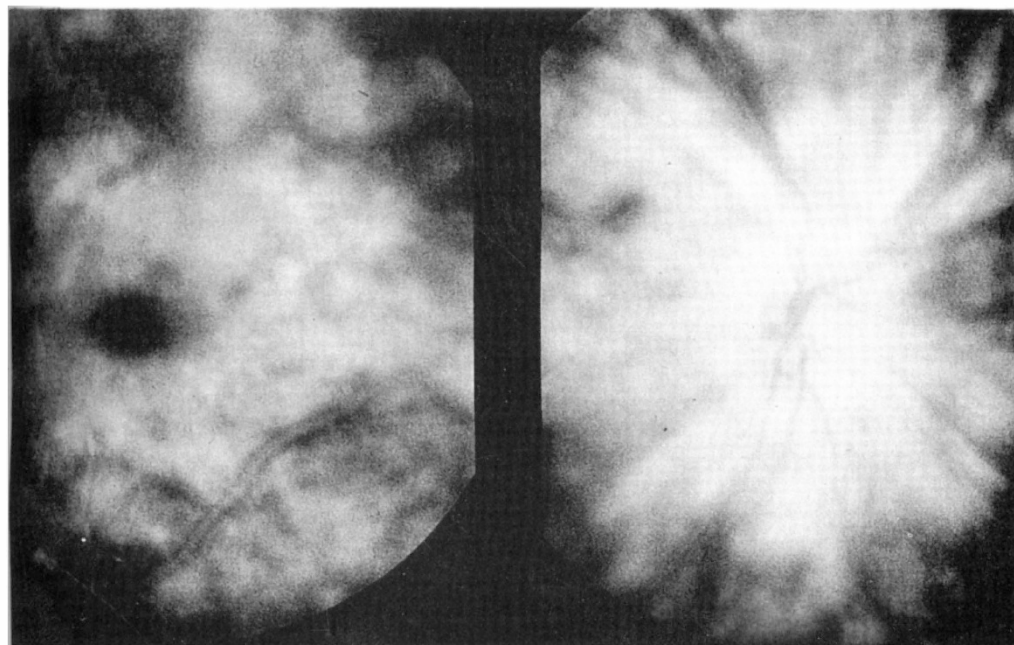
Fig. 1 *Fluorescein angiogram from a patient with posterior uveitis demonstrating massive disc and macular oedema.*

with bovine IRBP (inter-photoreceptor binding protein; 15 µg/kg body weight) emulsified with complete Freund's adjuvant and Bordetella Pertussis given intravenously as an additional adjuvant. The pupils were kept dilated with G. Atropine 1% twice weekly from two weeks post immunisation. (2) *Rat*—Female Lewis rats were immunised in the foot pad with 50 µg retinal S-antigen emulsified in complete Freund's adjuvant together with Bordetella Pertussis bacteria given intraperitonealy.

Angiography with fluorescein-labelled dextrans:

The technique is fully described elsewhere.[5] Angiography was carried out weekly from two weeks post induction of disease. In brief, anaesthetised monkeys had an intravenous line inserted and angiography with a fluorescein-labelled dextran of 70,000 molecular weight was carried out followed by an angiogram with the standard fluorescein sodium. Nine weeks after induction of disease, and after angiography, an eye was enucleated for examination by electron microscopy.

Infusion with horse-radish peroxidase:
Rats, (three animals per time point) at various stages of ocular inflammation induced by retinal S-antigen, were anaesthetised and injected intravenously with 50 µg horse-radish peroxidase, having been pretreated with an anti-histamine (diphenhydramine). This was allowed to circulate for 15 minutes. The animals were killed, the eyes enucleated, and fixed, cut and incubated with 3'-3' diaminobenzadine (Sigma) prior to cutting 70 nm sections and examination by electron microscopy.[6]

Results
All monkeys remained healthy, apart from their intraocular inflammation. The sequential fluorescein angiography was tolerated well. The disease pursued an identical course to that described by Hirose *et al.*[7] and was first noticed clinically at about five weeks after immunisation. Ophthalmoscopic examination showed a swollen disc with marked sheathing of the retinal vessels, deep retinal lesions and associated diffuse pigmentary mottling and chorioretinal atrophy throughout the fundus. Anterior segment inflammation was minimal in the monkeys given 15 µg/kg of IRBP, and there was very little vitritis.
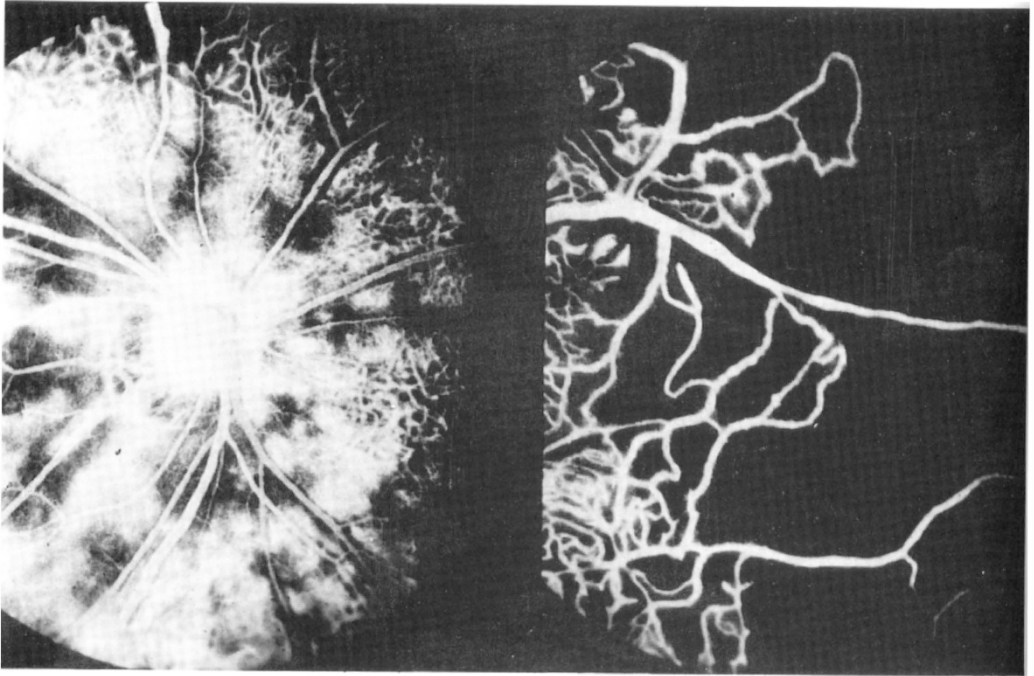
Fig. 2   *Fluorescein angiogram of patient with Eales' Disease showing peripheral vascular closure.*

Angiographic Findings

Diffuse sheathing was apparent on all the vessels and there was no difference clinically between areas that leaked fluorescein and those that did not. No leakage of fluorescein dye was seen prior to the clinical onset of disease. By seven weeks after disease induction spot leaks were seen with dextran 70,000 in the retinal vessels but many more lesions were seen in the peripheral retinal vessels that leaked fluorescein sodium alone (Fig. 4). An area not leaking fluorescein sodium during week six was seen to be leaking dextran 70 during week seven. During week eight, a large area that had not leaked before now leaked fluorescein sodium but none of the dextrans. New lesions that leaked dextran 70 were appearing in areas with no previous leakage.

Light and electron microscopic findings

At nine weeks post induction of disease, angiography prior to enucleation demonstrated areas that leaked dextran 70 and others that leaked fluorescein sodium alone. The light microscopic appearances were similar to those previously described and

showed extensive venous and mild periarterial infiltration with mononuclear cells and polymorphonuclear leukocytes.

Sections for electron microscopy were taken from areas leaking either fluorescein alone or the 70,000 dextrans, so that damage to the intercellular junctions could be assessed. In an area leaking dextran 70, the intercellular junctions were seen to be grossly abnormal (Fig. 5) and were completely open in places. No abnormal junctions could be detected in areas leaking only fluorescein sodium.

Rat model: All animals given retinal S-antigen developed severe retinal inflammatory disease. The earliest signs of histological retinal disease was at 10 days when early lymphocyte infiltration was seen. Horseradish peroxidase (HRP) was infused IV at several time points from day eight to day 14 post immunisation, and also in uninjected control animals. In retinae from animals on day 13 and 14 post immunisation, there was so much destruction and anatomical distortion that it was not possible to comment on the vessels at the EM level. Obvious retinal oedema was apparent at days 11 and 12 but in no animal
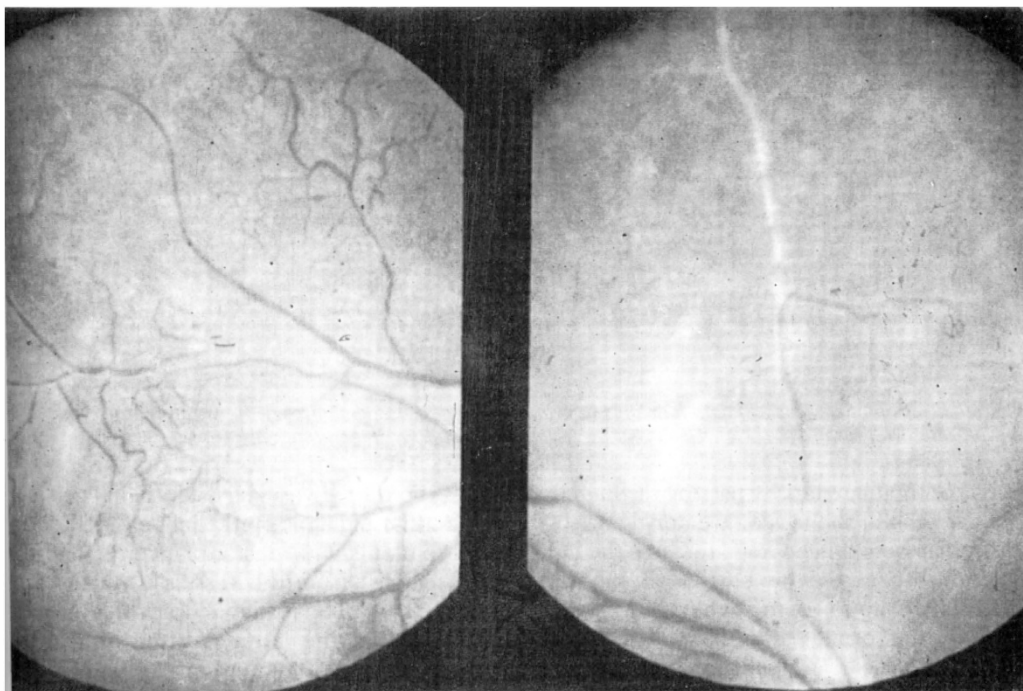
Fig. 3 *Fundus photograph from a patient with Behçet's Disease showing vascular closure.*

was HRP seen outside the retinal vessels and examination of the tight junctions showed them to be normal (Fig. 6).

Discussion

In this study, we have compared two experimental models of posterior segment ocular inflammation and looked at BRB breakdown in each. In the primate model, large areas of leakage occurred in the retinal vessels to the small molecular weight sodium fluorescein. Small transient spot leaks to the high molecular weight dextran were seen. In the rat model, oedema was seen in the retina at a time when no leakage of horse-radish peroxidase was evident.

In both models, breakdown of the blood-retinal barrier had undoubtedly occurred. Solutes of molecular weight less than 1,000 molecular weight, which includes water,
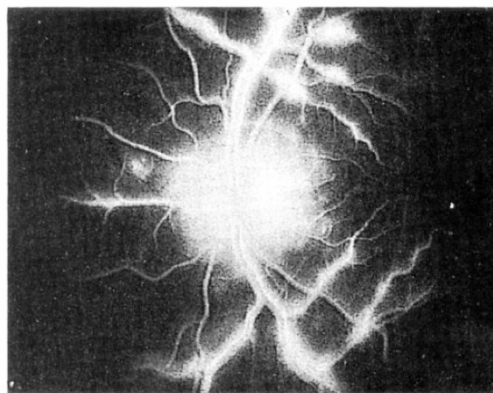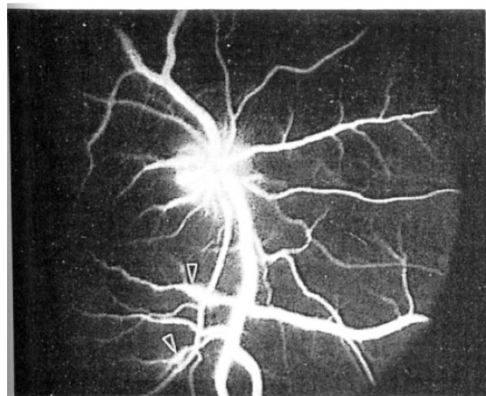

Fig. 4 *Angiogram of primate model of posterior uveitis:*   *B—with sodium fluorescein*
A—with 70,000 mwt fluorescein-labelled dextran
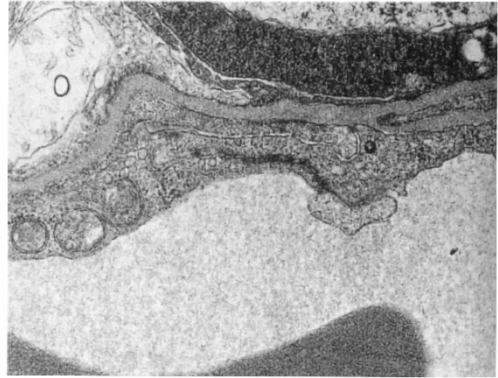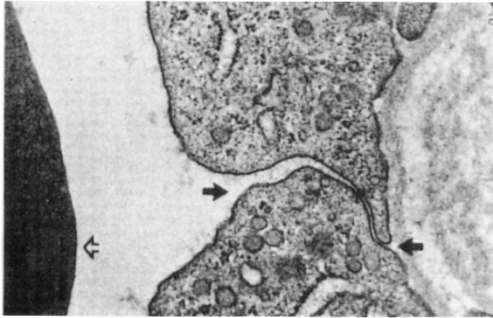
Fig. 5 *Electron microscopy of tight junctions in primate model of uveitis in area demonstrating leak with:*
A—70,000 mwt dextran   B—sodium fluorescein only

cross the tight barriers by passing through the cell whereas substances of greater than 1,000 molecular weight need to go through the tight junctions to cross the barrier. Temporary localised disruption of the tight junctions were seen in the primates but in all eyes, no 70,000 mwt dextran leakage was apparent when the inflammatory process was subsiding several months later although all vessels were patent.

Although no HRP leakage was seen in the inflamed rat eyes, despite every visible junction in every visible vessel in a large number of sections being examined, occasional spot leaks could still have occurred.

It would therefore appear that most of the increased permeability is occurring at the cellular level although areas of damage to the intercellular junctions appear to also occur. The BRB consists of two types of
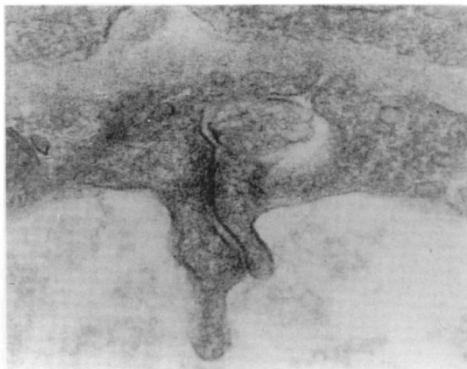

Fig. 6 *Electron microscopy of normal tight junction with HRP in vessel lumen from retina of inflamed rat eye.*

cell—the retinal vascular endothelial cell and the retinal pigment epithelial cell (RPE). In most patients with posterior uveitis, fluorescein leakage from the vessels is much commoner than 'deep leakage' occurring through the RPE but the latter is often difficult to demonstrate. We know that perivasculitis of the retinal vessels occurs and that CD4+ T cells are the main cell involved. It is therefore highly likely that it is the products of these activated cells which results in the damage to BRB.

Cyclosporin, which has its main effect on activated T cells, is very effective in the management of many patients with macular oedema from posterior uveitis.[8] It works by preventing secretion of several different lymphokines, such as interleukin-2 and interferon-gamma, by these T cells. Exactly which lymphokine(s) can damage the endothelial cells and result in increased transcellular permeability is unknown. Work on this subject is now underway since without this information, new and more effective therapeutic regimes for this devastating complication of posterior segment inflammation, cannot be rationally devised.

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Key words: blood-retinal barrier, oedema, retinal antigens, tight junctions, uveitis.

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