Abstract  Purpose: To examine the effects of saline-induced bleb detachments in rabbit retina.  

Methods: Retinal bleb detachments were produced by the injection of 50 µl of balanced salt solution (BSS) into the subretinal space of one eye of each of six rabbits using a glass pipette with a flat tip, 50 µm in diameter. The retina was examined by biomicroscopy, scanning laser ophthalmoscopy (SLO), auto-fluorescence and simultaneous fluorescein and indocyanine green (ICG) angiography. Histological examination was carried out at 1, 2, 3 and 4 months after surgery.  

Results: All rabbits showed leakage of fluorescein for at least a day after detachment, but within 1 month the leakage ceased. ICG staining developed gradually at the level of the RPE or Bruch’s membrane near sites of previous staining. Lipofuscin fluorescence also developed gradually around areas of staining. Lipofuscin also developed gradually around areas of staining. Histology revealed the source of the excessive lipofuscin to be in the RPE layer, especially in cells migrating away from Bruch’s membrane.  

Conclusions: Short-term bleb detachments cause a transient breakdown in the blood–retinal barrier, long-term ICG staining at or deep to the RPE layer, hyperlipofuscinosis and migration of the RPE. The abnormal lipofuscin accumulation is apparent on fluorescence ophthalmoscopy and can be confused with markers such as green fluorescent protein.

Introduction  

Transplants of retinal pigment epithelium into the subretinal space, as allografts or xenografts, encounter rejection [5, 15] even though this is a relatively sequestered immunological site [16, 21]. The mechanism by which foreign transplants are detected and destroyed in the subretinal space by the immune system is poorly understood. Initially, the immune system must detect foreign proteins from the transplants by means of antigen-presenting cells. Subsequently, the antigen-presenting cells transfer this foreign antigen to lymphocytes, presumably in lymph nodes, causing activation and clonal expansion of naive lymphocytes. In a third phase, activated lymphocytes locate and destroy the transplant in the subretinal space. The factors responsible for antigen-presenting cells finding foreign proteins from the transplant, on the one hand, and for allowing activated lymphocytes to detect the transplant in the subretinal space, on the other, are unknown. 

Factors that can influence the detection of foreign transplants within the subretinal space may be related to the surgical technique used for transplantation. For this reason, we examined the consequences of producing short-term bleb detachments in rabbit retina where transplants are usually placed in order to determine what could influence transplant detection. Such bleb detachments are thought to reattach within hours after they are produced and cause relatively little retinal damage [12]. Our experiments complement earlier ones by Marmor and collaborators [25, 27], who monitored the progress of retinal bleb detachments in rabbits by fluorescein angiography and histology. We have combined simultaneous fluorescein and indocyanine green (ICG) angiography and histology.
graphy with scanning laser ophthalmoscopy (SLO) to follow in vivo changes in the retina and choroid following short-term bleb detachments. The results reveal that such detachments transiently damage the blood–retinal barrier and cause later changes in the RPE and choroid, which can be identified ophthalmoscopically.

**Methods**

Six adult pigmented rabbits anesthetized with ketamine 20 mg/kg and xylazine 10 mg/kg i.m. were used in these experiments. The pupils were dilated with 2% cyclopentolate and 1% tropicamide. The conjunctiva was cut at the limbus and a scleral incision was made 2 mm from the limbus with a 15-degree knife (Alcon Ophthalmic). A self-retaining plano-convex lens was placed on the cornea on a cushion of hyaluronic acid (Healon) to facilitate viewing of the retina. A glass micropipette, having a flat tip, 50 µm in diameter, and filled with balanced salt solution (BSS; Alcon Surgical) was inserted through the scleral incision and guided through the vitreous with the aid of a surgical microscope. The pipette was brought to the retinal surface at a point just below the myelinated optic disc. As the pipette gently touched the neural retina, approximately 50 µl of BSS was slowly injected into the subretinal space, producing a circular bleb detachment of the neural retina having a diameter of about 3 mm. We tried to avoid touching the RPE layer with the tip of the pipette and slowly withdrew the pipette as the detachment formed. The pipette was then removed from the eye and the scleral and conjunctival incisions were sutured with 6–0 ethilon.

The retina was examined at 15 min to 18 h after surgery by SLO (Rodenstock, Munich) and fluorescein and ICG angiography and weekly thereafter. The ICG system uses a diode laser at wavelength 780 nm and a barrier filter transmitting wavelengths greater than 810 nm. Rabbits were killed for histological examination at 1, 2, 3 and 4 months after surgery. The eyes were removed and fixed by 3% glutaraldehyde in phosphate-buffered saline. A segment including the retina, choroid and sclera at and around the detachment was dissected as a block with the aid of a surgical microscope. One corner of this segment was cut for orientation before being dehydrated, osmicated and embedded for sectioning. Serial sections were examined through the detachment site, including unaffected areas on both sides of the detachment, by light and in selected cases by electron microscopy.

In the carrying out of these experiments, we followed the “Principles of laboratory animal care” (NIH publication no. 85-23, revised 1985) and the OPRR Public Health Service Policy on the Humane Care and Use of Laboratory Animals.

**Results**

Figure 1 shows the retina of a normal rabbit viewed by blue (Fig. 1A) and infra-red (Fig. 1B) light. An early-phase fluorescein angiogram (Fig. 1C) reveals the retinal vessels and small areas of hyper- and hypo-pigmentation revealed by choroidal fluorescence. An early-phase ICG angiogram (Fig. 1D) reveals the choroidal circulation. The earlier appearance of ICG in the thin vessels identifies them as the arteries; the vessels of larger diameter, filling slightly later, are the veins.

Figure 2 shows a bleb detachment produced 15 min earlier. Blue light (Fig. 2A) reflected from the surface of the detachment produces more reflectance than the untouched retina and highlights the circular, sharply demarcated detachment. Small light spots at one side of the detachment show the retinotomy site. ICG angiography shows staining in the late phase of the angiogram at the retinotomy site (Fig. 2B, arrows). Fluorescein angiography shows leakage along the edge of the detachment and at the retinotomy site, which increases with time (Fig. 2D). In areas not blocked by leakage there is no evidence of increased window defects. At this time the ICG angiogram is normal in both early and late phases.

Figure 3 shows a bleb detachment 18 h after surgery. The detachment is hardly identifiable with blue light (Fig. 3A). ICG angiography, however, shows staining in the late phase of the angiogram at the retinotomy site (Fig. 3B, arrows). Fluorescein angiography (Fig. 3C, D) reveals leakage at the retinotomy site (arrow), which increases with time (Fig. 3D).