An Experimental Model of Rhegmatogenous Retinal Detachment: Surgical Results and Glial Cell Response

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PURPOSE. To modify existing experimental models to simulate the typical clinical presentation of human rhegmatogenous retinal detachment (RRD), namely an RD caused by a retinal break, in a phakic eye, with a posterior vitreous detachment (PVD); to model RRD in a species that is anatomically similar to humans; and to characterize the glial cell response to RRD.

METHODS. Mixed-breed pigs underwent vitrectomy, PVD, subretinal injection of viscoelastic, and creation of a break at the apex of the RD. The crystalline lens was not removed. Follow-up was for 0, 1, and 7 days. Tissue was processed for light and electron microscopy. The glial cell response was characterized using antibodies to glial fibrillary acidic protein (GFAP).

RESULTS. Of 11 RRDs created in seven pigs, 10 increased in size and 1 decreased. Light and electron microscopy demonstrated typical features of RD. There was constitutive expression of GFAP in astrocytes and Müller cells with increased immunoreactivity from day 1.

CONCLUSIONS. This study provided a model of RRD that simulates the typical clinical presentation in humans. It used techniques that most vitreoretinal surgeons are familiar with, and an animal that is widely available and anatomically similar to humans. Anatomic success was high, and the glial cell response established comparability with other species. (Invest Ophtalmol Vis Sci. 2003;44:4026–4034) DOI:10.1167/iovs.02-1264

Rhegmatogenous retinal detachment (RRD) occurs when a retinal break allows vitreous fluid to accumulate between the neurosensory retina and the retinal pigmented epithelium (RPE). Without surgical treatment many RRDs lead to blindness in the affected eye.3 There have been several animal models of RD. These have allowed surgeons to investigate new treatment strategies2–4 and have resulted in a better understanding of the pathogenesis of RD6–8 and the cellular response it produces.7,9–22

Not all models fully replicate the typical clinical features of RRD. Some do not rely primarily on a retinal break to create and maintain an RD7,22–26 and many do not completely detach the posterior vitreous face from the internal limiting membrane (ILM) of the retina.5,20,22–26,29 Many models of RDs remove the crystalline lens,4,11,14,27 yet most patients with RD are have phakic eyes.30

A variety of animal species have been selected to model human RD, including rabbit,5,6,7,11–15,20,22–25,29,31,32 cat,27 dog,2,26 and primate.8,33 Individually, each of these species have important differences from human eyes in terms of either the size of the globe and crystalline lens, scleral rigidity, vitreous turgor, retinal circulation, photoreceptor type and distribution, or features such as a tapetum lucidum.

The purpose of this study was to develop a model of RRD in an animal with anatomy that closely resembles that of humans and to validate this model by establishing that the clinical course and cellular response to experimental RD were comparable. This study also sought to adapt existing models of RD to create one that resembles the usual presentation of human RRD, that is an RD caused by a retinal break, in a phakic eye, with a posterior vitreous detachment (PVD).

METHODS

Anesthesia

All procedures were approved by the Secretary of State (Home Office, Animal Procedures Section) and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Eight to 10-week-old, male Large White pigs were selected (mean weight, 24.5 kg; range, 21.0–30.8 ± 3.4). These incorporated mixed breeds to reduce the risk of malignant hyperthermia that occurs with pure-breds. Animals were sedated with an intramuscular injection of azaperone 2 mg/kg (Janssen Animal Health, Bucks, UK) and ketamine (Pfizer Pharmaceuticals, UK) and maintain an RD22–28 and many do not completely detach the posterior vitreous face from the internal limiting membrane (ILM) of the retina.5,20,22–26,29 Many models of RDs remove the crystalline lens,4,11,14,27 yet most patients with RD are have phakic eyes.30

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Surgery

All operations were performed by a single surgeon (TLJ), using a sterile surgical technique when appropriate. After induction of general anesthesia, 0.4% oxybuprocaine, 1% cyclopentolate, and 10% phenylephrine eye drops (Chauvin Pharmaceuticals Ltd., Essex, UK) were instilled three times into the right eye. The conjunctival sac was irrigated with saline to remove any debris, and the surgical field was prepared with 5% aqueous povidone iodine solution.

A lateral canthotomy was created, and the eyelids and nictitating membrane were retracted with a 5-0 silk stay suture. A 270° with 5% aqueous povine iodine solution.

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Histology

Eyes were enucleated with animals under terminal anesthesia at the end of surgery on days 0, 1, or 7. Globes were positioned cornea uppermost, and the anterior segment was dissected. The weight of fluid in the vitreous cavity maintained the eyecups, which were viewed under the operating microscope. Areas of attached and detached retina were isolated, as were those containing retinal breaks. Tissue for light and transmission electron microscopy (TEM) were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 10 g/L calcium chloride. Tissue was then postfixed in 2% osmium tetroxide in 0.2 M sodium cacodylate buffer for 1 hour, dehydrated in a graded series of ethanol, and embedded in Araldite epoxy resin. Semithin (1-μm) sections were stained with methylene blue and viewed and photographed (Ektachrome 64T; Eastman Kodak, Rochester, NY) on a light microscope. Ultrathin (70-nm) sections for TEM were cut with a diamond knife (Leica, Milton Keynes, UK) on a microtome (Ultratcut E; Reichert Jung, Vienna, Austria) and stained with uranyl acetate and lead citrate (Agar, Cambridge, UK). Sections were examined and photographed by microscopy (1200EX; JEOL, Tokyo, Japan).

Immunolabeling for Glial Fibrillary Acidic Protein

In addition to light microscopy, the glial cell response to RD was assessed by immunolabeling for the intermediate filament, glial fibrillary acidic protein (GFAP). Tissue was fixed in 4% paraformaldehyde in 0.1 M sodium phosphate buffer at pH 7.2. for 1 hour. Specimens were transferred to 30% sucrose in phosphate buffer and then to embedding compound (Bright Instruments, Huntingdon, UK), before being frozen in a eutectic solution of isopentane, cooled in a liquid nitrogen bath. A cryostat (Anglia Scientific Instruments, Cambridge, UK) was used to cut 7- to 10-μm frozen sections that were mounted on polylysine-coated microscope slides (BDH, Poole, UK). Sections were incubated with 1:25 Cy3-tagged, monoclonal mouse anti-pig antibody to GFAP (clone G-A-5; Sigma-Aldrich, St. Louis, MO) for 30 minutes at 37°C. Phosphate-buffered saline (PBS) was used as the diluent (Sigma-Aldrich). Preliminary blocking studies conducted in the presence of XS soluble GFAP (Calbiochem, La Jolla, CA) confirmed that binding was antigen specific. Slides were rinsed with PBS, covered with a glycolerol-based mounting medium (CitiFluor Ltd., London, UK), viewed, and photographed (Ektachrome 320T film; Eastman Kodak) on a fluorescence microscope with a 546/580-nm filter set (Leitz).

RESULTS

Surgical Results

The initial size of the experimental RDs was 3 to 4 DD. Two pigs were used for day 0 experiments (five RDs). Three were followed up for 1 day. Of seven RDs in this group: two increased in size to 7 DD, 1 to 6 DD, and 2 to 5 DD. One showed a small increase in size from 3 to 4 DD, and one decreased to 1.5 DD. Two pigs were followed up for 1 week. Of four RDs in this group, two increased to 8 DD, 1 to 5 DD, and 1 to 6 DD. Of the 11 RDs followed up for 1 or 7 days, 6 were bullous (Fig. 1). The other five showed a more shallow elevation of 1 to 3 DD.

Mydriasis was maintained throughout surgery in all animals, without the instillation of additional topical mydriatics. No animal developed clinical or histologic evidence of proliferative vitreoretinopathy (PVR) or intraocular infection. In the first and third cases, intraocular hemorrhage developed during creation of RD (variable degree of air analysis), and the fellow eye was used with the animal under terminal anesthesia for day-0 experiments. Pig two had a 4- to 5-DD posterior pole hemorrhage that was cleared with the vitrectomy cutter. One case of lens touch at the time of initial surgery resulted in a lens opacity that precluded an adequate fundal view, and a pars plana lensectomy was performed with the vitrectomy cutter.

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Light Microscopy

Compared with attached retina (Fig. 2A), detached retina at day zero (Fig. 2B) demonstrated a moderate reduction in the density of photoreceptor outer segments, with mild to moderate vacuolation in the inner plexiform layer. Retinal breaks had a ragged margin and were turned inward, toward the vitreous cavity. By day 1 this inward pouting of the retinal break was more pronounced, the density of photoreceptor outer segments had reduced further, and those that remained appeared disorganized and shortened (Fig. 2C). The inner segments appeared rounded and swollen with some outer segment elements evident within the cell bodies. Fluid spaces were seen around the cone nuclei and between the external limiting membrane (ELM) and outer plexiform layers. Some inflammatory cells were seen in and around retinal blood vessels. There were increased spaces occupied by pale-staining cells, consistent with the appearance of glial cells. This apparent increase in glial elements was most noticeable at the edge of the retinal break, which appeared rounded compared with the ragged edges at day 0. By day 7 (Fig. 2D) the retinal break showed a further rounding and more pale-staining areas. There were fewer fluid-filled spaces. Only occasional outer segments were evident, and the outer retinal border was formed predominantly by the ELM. Pigment was scattered throughout the retina and in association with focal areas of subretinal glial proliferation. Retina primed with a subretinal injection of BSS was not noticeably different from retina primed with viscoelastic.

Immunolabeling for GFAP

GFAP was expressed throughout the fundus in normal attached retina (Fig. 3A). No formal quantitative assessment of regional variations in GFAP immunoreactivity was made; however, there appeared to be more GFAP immunoreactivity in the posterior pole than in the retinal periphery. Müller cells and astrocytes were both GFAP positive. By day 1, GFAP levels had increased (Fig. 3B). GFAP labeling was most evident in the tissue surrounding the large, superficial retinal vessels in the peripapillary area (Fig. 3C). Throughout the fundus, GFAP levels had increased further by day 7 (Fig. 3D). Immunofluorescence was dominated by the well-characterized5 fibrous pattern extending throughout the length of the Müller cell cytoplasm, from the ILM to the ELM. By day 7, there were areas in which the GFAP reactivity extended beyond the ELM. Relative to neighboring tissue, retinal breaks had slight to moderately elevated levels of GFAP expression and the typical ordered fibrillary pattern was replaced by an irregular staining pattern. This altered staining pattern extended up to approximately 300 μm from the edge of the break. A meshwork of fibrils emanated from star-shaped cells within the nerve fiber layer (Fig. 3E). These cells had the appearance of retinal astro-
cytes. A similar meshwork of fibers surrounded retinal blood vessels (Fig. 3F) throughout the fundus.

**Electron Microscopy**

Studies of normal, attached retina showed the characteristic appearance of glial cells, with vertically oriented intracellular microfilaments within the cytoplasm (Fig. 4). In areas of RD surrounding a retinal break, the normal flat line of the ILM became undulant immediately after the break was formed (day 0), perhaps suggesting an alteration in elastic forces. By day 1 (Fig. 5) there were areas of increased electron density immediately below the ILM. This response was somewhat variable, even when comparing adjacent cells. The ordered vertical arrangement of intracellular filaments was disturbed in some cells by day 1, but was more evident by day 7 (Fig. 6). This response was most marked up to 300 µm from the edge of retinal breaks, corresponding to the observations in frozen sections labeled with anti-GFAP. Light microscopy suggested that the degree of tissue edema was most marked at day 1, but TEM showed considerable intra- and extracellular edema persisting at day 7. There was no evidence of any residual vitreous elements, consistent with the clinical impression that a PVD was created during surgery.

**Discussion**

**Surgical Technique**

**Anatomic Success.** RRD usually occurs when the vitreous gel collapses, vitreoretinal adhesion creates a break in the retina, and liquefied vitreous fluid passes through this break into the subretinal space. There have been several reports of experimental RD, but few mimic this combination of causative events. This may explain a wide variation in the reported anatomic success, ranging from 0% to 100%. This study modified existing models of RD to simulate the usual clinical presentation of human RRD. All RDs persisted for the duration of the study and many increased in size. It is not known whether a longer study interval would have resulted in some further enlargement, or alternatively, reattachment. One RD reduced in size over 24 hours and with longer follow-up may have resolved, resulting in a lower anatomic success rate. Longer follow-up would also be necessary to determine the incidence of PVR.

**Retinal Breaks.** The tendency for most RDs to enlarge resembles the usual course of human RRD and suggests that the RDs were maintained by the retinal break, rather than the viscoelastic alone. This hypothesis is supported by the fact that a control RD created using BSS increased in size, without being primed by viscoelastic. This represents an important difference from the feline model this study adapted. Anderson et al. used a subretinal injection of dilute sodium hyaluronate to create an RD. The solution was injected through the retina with a glass micropipette. Given the small size of the micropipette, the RD was probably maintained by the presence of subretinal viscoelastic, rather than egress of vitreous fluid through the break. Indeed, when BSS was used instead of viscoelastic, RDs resolved within 7 days. In this respect, Anderson’s model simulated serious RD, whereas the present model simulated RRD.

Although subretinal viscoelastic did not appear to alter the cellular response to RD in this or other experiments, there are theoretical advantages to using BSS. This study suggests that viscoelastic is not necessary to maintain RDs in the presence of a retinal break. Further studies of RD created with BSS appear worthwhile.

The presence of a retinal break may influence the cellular response to RD. The retina acts as a barrier to the diffusion of...
larger molecular weight molecules and the movement of macromolecular chemical mediators between the subretinal space and vitreous cavity are impeded by the presence of an intact retina. Retinal perforation with a micropipette does not alter this barrier effect.

**Posterior Vitreous Detachment and Lens Status.** Many models of RD attempt to liquefy or remove some of the vitreous gel through chemical or mechanical means, but few perform a complete vitrectomy or create a PVD. In the pig it was possible to create a PVD, and this may have contributed to a high anatomic success rate.

The proportion of patients with aphakic RD has declined from 14% to 1% over the past 20 years, yet many models of RD remove the crystalline lens. Lensectomy was not required in the pig because the lens did not usually obstruct access to the vitreous cavity. This provided a phakic model of RD and unlike many studies, animals underwent only one operation to create an RD.

**Surgical Difficulties and Disadvantages.** As noted by other investigators, we encountered some problems with intraoperative hemorrhage. This may reflect surgical technique or species variation or may be peculiar to the juvenile animals chosen by most researchers. Retinal hemorrhage may occur after both RD and vitreoretinal surgery in humans, but appeared to occur more easily in pigs.

The relatively young age of these animals may also have influenced other aspects of surgery. Compared with mature adults, they may have decreased scleral rigidity, increased vitreoretinal adhesion, an altered healing response, and perhaps greater neural plasticity. Without repeating experiments in older animals, the effect of developmental age cannot be fully determined.

**Anesthesia.** Although anesthesia is possible without intubation, it allows more precise control of the depth of anesthesia and blood oxygen saturation. Given that hypoxia results in increased retinal GFAP expression and modifies the glial response to RD, prevention of intraoperative hypoxia may be important.

**Species Selection**

Several species have been used to model human RD, including rat, rabbit, dog, and primate.
and cat.\textsuperscript{14,27,51} For this study, we chose Large White pigs for several reasons. These animals are affordable, widely available, and need not be reared in dedicated research facilities. Porcine eyes are similar to human eyes in globe and lens size, scleral thickness and rigidity, and retinal ultrastructure.\textsuperscript{52} Unlike rabbit eyes, porcine eyes have a duplex retinal circulation and unlike eyes of cattle, cats, or dogs, they do not have a tapetum cellulosum. Pigs differ from the cone-dominant ground squirrel\textsuperscript{49,53} and rod-dominant primates and cats, in having a high density of both rods and cones. The porcine area centralis has a photoreceptor density of up to 200,000 cells/mm\textsuperscript{2},\textsuperscript{54} comparable to 199,000 in humans.\textsuperscript{55} We are aware of porcine models of PVR,\textsuperscript{56} but not of RRD.

A disadvantage of this species is that they grow rapidly, and problems in handling them may make long-term experiments more difficult. Despite having an area centralis with a high photoreceptor density, they lack the foveolar specialization of humans.

**Glial Cell Response to Retinal Detachment**

Animals were followed up for 1 week, because studies in rabbit,\textsuperscript{11,12} cat,\textsuperscript{14} rhesus monkey,\textsuperscript{16,57} and owl monkey\textsuperscript{59} all indicate that the principal histologic responses to RD occur within this period. The light and electron microscope findings in porcine RD are consistent with findings in other species.\textsuperscript{9,11,12,46,58,59}

Studies in attached cat retina\textsuperscript{15,19} suggest GFAP is expressed in normal Müller cells. There are conflicting reports regarding constitutive expression in rat\textsuperscript{55,47,60,61} and rabbit.\textsuperscript{62–65} Consis-
tent with previous reports, this study found that there was constitutive expression in porcine Müller cells. Astrocytes were also GFAP positive.

Levels of GFAP increase markedly in response to many types of neuronal injury, including RD. This response is well established by 24 to 48 hours. The up-regulation of GFAP observed in this study was similar to reports in the cat in anatomic distribution and temporal response.

The pattern of staining was different at the edge of retinal breaks, where GFAP levels were moderately increased and the ordered fibrillary arrangement disturbed. Similar observations were made with other focal retinal injuries.

Developmental changes in GFAP expression are not well characterized in the pig and may act as a confounding variable in this study. The rapid changes in GFAP expression after RD could not easily be attributed to developmental change, but it

**FIGURE 6.** Electron photomicrograph of porcine retina seven days after creation of a retinal detachment. There is marked intra- and extracellular edema, mitochondrial swelling, and increased electron density throughout the cell cytoplasm. Original magnification, ×8000.
is possible that adult animals would manifest a different response to the juveniles used in these experiments.

CONCLUSIONS

This study provides a porcine model of RRD designed to simulate the clinical presentation of this condition in humans. In particular, animals had a PVD, the RD was maintained by a retinal break, and the crystalline lens remained in situ. The anatomic success using this technique was high. The glial cell changes evident during a 1-week follow-up suggest that the pig may be an appropriate model for the study of human retinal glosis. This model uses techniques and equipment that most vitreoretinal surgeons are familiar with, and an animal that is affordable, widely available, and anatomically similar to humans.

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References