Müller Cell Reactivity and Photoreceptor Cell Death Are Reduced after Experimental Retinal Detachment Using an Inhibitor of the Akt/mTOR Pathway

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Purpose. To test the effect of Palomid 529, an inhibitor of the Akt/mTOR pathway, on Müller cell proliferation, subretinal glial scar formation, and photoreceptor survival after experimental retinal detachment (RD).

Methods. Palomid 529 (600 μg) in balanced salt solution or balanced salt solution alone was injected intravitreally immediately after RD into the right eyes of 12 rabbits. Ten micrograms of BrdU was injected intravitreally on day 3. Animals were killed on day 3 or 7, at which time retinal sections were labeled with anti-BrdU to detect dividing cells, with anti-vimentin to identify Müller cells, and with the isolectin B4 to identify microglia and macrophages. Outer nuclear layer (ONL) thickness was measured from fluorescence-labeled nuclear-stained sections. Labeling was imaged using confocal microscopy. Six additional animals received either drug or balanced salt solution injections into normal eyes, and paraffin sections were stained with hematoxylin and eosin.

Results. In the drug-treated eyes there was a significant decrease in the number of anti-BrdU–labeled Müller cells, the number and size of subretinal scars, and the number of isolectin B4-labeled cells. The ONL was also significantly thicker, and there was no evidence of toxic effects.

Conclusions. Palomid 529 is an effective suppressor of Müller cell proliferation, glial scar formation, and photoreceptor cell death in a rabbit model of RD. This suggests that inhibiting the Akt/mTOR signal transduction pathway may be an effective strategy to decrease proliferation and photoreceptor cell death induced by detachment and perhaps represents a novel therapy for related human diseases such as proliferative vitreoretinopathy. (*Invest Ophthalmol Vis Sci.* 2009;50: 4429–4435) DOI:10.1167/iovs.09-3445

Proliferative vitreoretinopathy (PVR) and subretinal fibrosis are two potentially devastating complications of retinal detachment that occur in a small but consistent population of patients. When the retina becomes separated from the retinal pigment epithelium under the conditions of a rhegmatogenous detachment (i.e., a detachment with a tear or hole in the retina), all nonneuronal cell types undergo proliferation[1] and, consequently, have the potential for participating in the formation of scars or "membranes" characteristic of these diseases. To date, Müller cells, astrocytes, RPE cells, and macrophages have been convincingly identified in the membranes formed after detachment.[2–5] When the membrane is present in the subretinal space (subretinal fibrosis), the regeneration of photoreceptor outer segments is prevented,[6] most certainly causing loss of vision in that region. The incidence of subretinal fibrosis in humans, however, is unknown, presumably because of the difficulty of visualizing a thin layer of tissue between the retina and the retinal pigment epithelium. When a cellular membrane grows on the vitreal surface of the retina, the cells can contract causing redetachment of the retina. Cellular membranes on the subretinal (posterior) or epiretinal (anterior) surface of the retina are considered part of the spectrum of PVR.[7] PVR remains the most common (approximately 5%–12% of cases) cause of the failure of retinal reattachment surgery.[8–13] Although advances in the surgical management of PVR have improved chances for reattachment of the retina, the visual prognosis of patients remains poor, with only 11% to 25% of patients achieving visual acuity of 20/100.[14] Presumably, the decrease in visual acuity that results from retinal detachment and PVR results, in part, from the loss of photoreceptors because apoptosis is a clear consequence of detachment[15–21] and can continue at low levels even after the retina is reattached.[22] Although numerous attempts have been made to reduce the incidence of PVR and subsequent membrane formation, to date no effective pharmacologic treatment has been found.[23,24]

In animal models of retinal detachment, Müller cells appear to form the primary scaffold underlying the formation of membranes outside the retina.[25,26] Beginning within hours of detachment, transcription factors involved in proliferation and hypertrophy increase their expression in Müller cells.[27] By 3 days after detachment, Müller cells are actively proliferating within the retina[1,28] and are beginning to grow preferentially into the subretinal space. After 1 week, subretinal scars can be observed covering large areas along the photoreceptor surface. Although the timing is unknown, a similar process of glial reactivity and growth into the subretinal space has been shown to occur following retinal detachment in humans.[29] Intraretinal proliferation, though reduced after 3 days of detachment, continues at low levels, presumably as long as the retina remains detached.[22] Retinal reattachment effectively stops Müller cells from growing into the subretinal space, but they then begin to extend processes onto the vitreal surface of the retina, forming epiretinal membranes. The low, but consistent, levels of proliferation observed in retinas that were surgically reattached for 28 days[22] may explain why it often takes months after reattachment surgery before PVR is observed in patients. Thus strategies to prevent PVR or subretinal fibrosis will probably

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have to include treatment that is prompt and that lasts for an extended time.

Palomid 529 (Paloma Pharmaceuticals, Jamaica Plain, MA) is a wholly synthetic, nonsteroidal, small molecule drug of the dibenzochrome class that has been shown to inhibit TORC1 and TORC2 of the Akt/mTOR pathway by reducing levels of TORC1 and TORC2 complexes.[30] In addition, it has been shown to reduce the phosphorylation of Akt (Ser473), GSK (indicative of a TORC2 inhibitor), and S6 (indicative of a TORC1 inhibitor).[30] The physical properties of the drug are consistent with a long ocular half-life. Indeed, preliminary studies show that it is still detectable in the vitreous using liquid chromatography–mass spectrometry methodology (LC/MS/MS) at 5 months after a single injection into the eye in a rabbit pharmacokinetic study (D. Sherris, personal communication, 2009). Recently, Palomid 529 has been shown to be an effective inhibitor of new vessel growth in experimental models of ocular angiogenesis, including oxygen- and laser-induced retinopathy (Sherris D, et al. *IOVS* 2008;49:ARVO E-Abstract 3766). Because the drug is an inhibitor of the Akt/mTOR pathway, which is involved in numerous cellular events including metabolism, cell growth and survival, apoptosis, and cell cycle progression[31,32] and because its physical properties are consistent with a long ocular half-life, we undertook a series of studies to examine the effects on Müller cell proliferation, subretinal scar formation, and photoreceptor survival induced by retinal detachment. We show here that a single injection of Palomid 529 can significantly reduce photoreceptor cell death, the amount of intraretinal proliferation, and the number and size of subretinal scars, indicating that it may be useful for treating proliferative diseases associated with retinal detachment surgery and ensuing photoreceptor cell death.

Materials and Methods

Tissue Preparation

Retinal detachments were created in adult New Zealand Red pigmented rabbits as described by Eibl et al.[33] Briefly, intramuscular injections of the combined drugs xylazine (3 mg/kg) and ketamine (15 mg/kg) were used for anesthesia and analgesia. Additional analgesia was provided by topical eye drops of proparacaine. A 0.25% solution of sodium hyaluronate (Healon; Pharmacia, Piscataway, NJ) in balanced salt solution (BSS; Alcon, Fort Worth, TX) was infused via a glass pipette between the neural retina and the retinal pigment epithelium. The sodium hyaluronate is necessary to prevent spontaneous reattachment of the retina, and 0.25% is the most dilute solution that maintains the detachment for extended periods. The pipette tip, with an external diameter of approximately 100 μm, was inserted into the eye by an incision that was made several millimeters below the pars plana to prevent the pipette from touching the lens. Approximately half the inferior retina was detached in the right eye, leaving the superior attached regions as internal controls. The left eyes served as uninjected controls. Immediately after producing the detachment, either 600 μg Palomid 529 in 50 μL balanced salt solution or balanced salt solution alone was injected intravitreally through the incision in the sclera. On day 3 after detachment, the experimental (right) eyes were given an intravitreal injection of 10 μg BrdU (Sigma, St. Louis, MO) in 50 μL balanced salt solution. BrdU was given on day 3 for both the 3-day and the 7-day detachment experiments because it has been shown previously that the proliferative response is at its maximum at this time.[28] Attached retinal regions within the eyes with the detachment served as control retinas labeled with BrdU. Animals were humanely killed either 4 hours after BrdU injection on day 3 (n = 3 BSS treated and 3 drug treated) or on day 7 (n = 3 BSS treated and 3 drug treated) using sodium pentobarbital (120 mg/mL intravenously). After enucleation, the eye was fixed in 4% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4; Electron Microscopy Sciences, Fort Washington, PA) for at least 24 hours. To determine potential toxic effects of the drug, six additional animals received intravitreal injections of either vehicle (BSS, n = 3) or drug (Palomid 529, n = 3) into normal eyes. Daily ophthalmoscopic examination of the retina did not reveal any signs of inflammation resulting from the presence of Palomid 529. These animals were humanely killed on day 15, the eyes were fixed in 4% paraformaldehyde, and the whole eyes were embedded in paraffin for light microscopic evaluation. All experimental procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, the tenets of the Declaration of Helsinki, and the guidelines of the Animal Resource Center of the University of California, Santa Barbara.

Immunocytochemistry/Light Microscopy

Immunocytochemistry was performed as described by Eibl et al.[33] with slight modifications. Pieces of retinal tissue approximately 3 mm square were excised from three regions within each eye. Regions included attached and detached retinas from within the eyes with the detachment and attached retinas from the contralateral uninjected eyes. The tissue was rinsed in phosphate-buffered saline (PBS), embedded in low-melt agarose (5%; Sigma), and sectioned at 100 μm using a vibratome (Technical Products International, Polysciences, Warrington, PA). Sections were incubated in normal donkey serum (1:20) in PBS, 0.5% bovine serum albumin, 0.1% triton X-100, and 0.1% azide (PBTA) overnight at 4°C on a rotator. The following day the sections were pretreated with 2 N HCl for 1 hour as an antigen retrieval step for BrdU. After rinsing in PBTA, the primary antibodies and lectin were added and incubated overnight at 4°C on a rotator in PBTA. Anti-BrdU (1:200; Accurate Chemical and Scientific Corp., Westbury, NY) was used to detect dividing cells, anti-vimentin (1:500; Dako, Carpinteria, CA) was used to identify Müller cells, and the isolectin B4 *Griffonia simplicifolia* (1:50; Vector Laboratories, Burlingame, CA) was used to label microglia and macrophages. After rinsing of the primary antibodies in PBTA, the secondary antibodies (streptavidin CY5, donkey anti-rat CY3, and donkey anti–mouse CY2 [Jackson ImmunoResearch, West Grove, PA]) were added together, each at 1:200 in PBTA, overnight at 4°C on a rotator. The next day the sections were rinsed in PBTA, mounted on glass slides using 5% n-propyl gallate in glycerol with the nuclear stain Hoechst (1:5000; Invitrogen), and viewed on an laser scanning confocal microscope (Fluoview 500; Olympus, Tokyo, Japan). Paraffin sections of whole eyes (nondetached but drug or BSS treated) were cut at 4 μm, counterstained with hematoxylin and eosin, and photographed with a digital camera on a microscope (BX60; Olympus). Given that whole eyes from three different animals within each group were embedded and sectioned, examination of the entire length of the retina was performed in a single section, and similar retinal regions from control and experimental animals were chosen to be photographed.

Quantitation

Quantitation was performed using confocal images labeled with anti-BrdU, -vimentin, and isolectin B4. At least 60 single-plane confocal images at 1024 × 1024 pixel resolution were captured from three animals within each of the experimental groups (vehicle and drug treated). Similar retinal locations were examined in all animals. To quantify the effect of drug treatment on proliferation, the number of anti-BrdU–labeled Müller cell nuclei was determined per millimeter of retina using the confocal images from the 3- and 7-day time-points. To quantify the effect of drug treatment on subretinal glial scar formation, scars were defined as areas of continuous cellular growth located sclerad to the outer limiting membrane that were also labeled with anti-vimentin. The number of scars was counted, and their lengths were measured per millimeter of retina in the 7-day animals because no scars were observed at 3 days. To determine the width of the ONL, sections were stained with a fluorescent nuclear dye (Hoechst), and the ONL was automatically segmented by a statistics-based clustering method.[34] ONL widths (i.e., the distance between the outer plexiform

layer and the outer limiting membrane) in the control retina were then compared with the widths in the vehicle- and drug-injected eyes as a measure of photoreceptor survival.[35] Only the 7-day point was examined because more photoreceptor cell death occurred at this time than at day 3. The same sections were used to quantify all the responses described. To determine statistical significance, a pairwise Student's *t*-test was calculated between control and experimental animals, and $P < 0.05$ was considered significant.

Results

Analysis of Attached Retinas

Light microscopy of paraffin-embedded normal retinas and confocal microscopy of attached retinal regions labeled with various antibodies from within the detached eyes were examined to determine possible adverse effects of the drug (Fig. 1). No differences in retinal organization between vehicle- and drug-treated normal retinas were observed in the paraffin sections (Figs. 1A, 1B). In addition, antivimentin and isolectin B4 labeling appeared similar between the vehicle- and drug-treated attached retinas at 3 and 7 days; anti-vimentin labeling (green) extended within Müller cells from the ganglion cell layer (GCL) to the ONL, and microglia (blue) were present only in the inner portion of the retina in all groups (Figs. 1C–1F). The labeling pattern was similar to that observed in an untouched normal eye (data not shown).[33] The only difference was the presence of macrophages in the vitreous in vehicle- and drug-injected eyes. Finally, no anti-BrdU labeling was observed in the vehicle- or drug-treated attached retinas. These data indicate that the drug did not cause gross adverse effects to the morphology of the retina and did not stimulate the proliferation of Müller cells or microglia.

Analysis of Detached Retinas

In detached retinal regions of the eyes injected with the vehicle at day 3, anti-vimentin labeling (green) extended to the outer limiting membrane, giving the Müller cells a thickened, distorted appearance within the retina (Fig. 2A; compare to 3-day attached retina + vehicle, Fig. 1C). No Müller cell growth was observed into the subretinal space. As reported previously,[36] isolectin B4-labeled microglia (blue) appeared rounded and were dispersed throughout the retina, whereas macrophages (blue) were observed only in the subretinal space. Most anti-BrdU–labeled Müller cell nuclei (red) occurred in the inner nuclear layer (INL; Fig. 2A). At 7 days, the anti-vimentin–labeled Müller cell processes frequently extended into the subretinal space (Fig. 2B, bracket). The anti-BrdU–labeled nuclei (labeled on day 3) generally occurred in the outer retina (i.e., distal to the INL) and in the subretinal glial scars, suggesting that these nuclei had migrated from the inner retina to the outer retina between days 3 and 7.

At 3 and 7 days after detachment in the drug-treated eyes, the occurrence of anti-BrdU–labeled nuclei was greatly reduced (Figs. 2C, 2D). Anti-vimentin labeling was elevated compared with nondetached vehicle-treated retinas, and the Müller cells appeared thickened (compare with Figs. 1C, 1D). They rarely extended into the subretinal space at either day; if they did, they were much smaller (Fig. 2D, bracket). In addition, the Müller cells did not show the distorted appearance and lateral branching characteristically observed in vehicle-injected control detachments, perhaps contributing to the overall more organized appearance of the retina. Certainly, the retinas did not show any evidence of pathology beyond that associated with detachment. Finally, there were fewer microglia and macrophages in the drug-treated eyes (Figs. 2C, 2D).

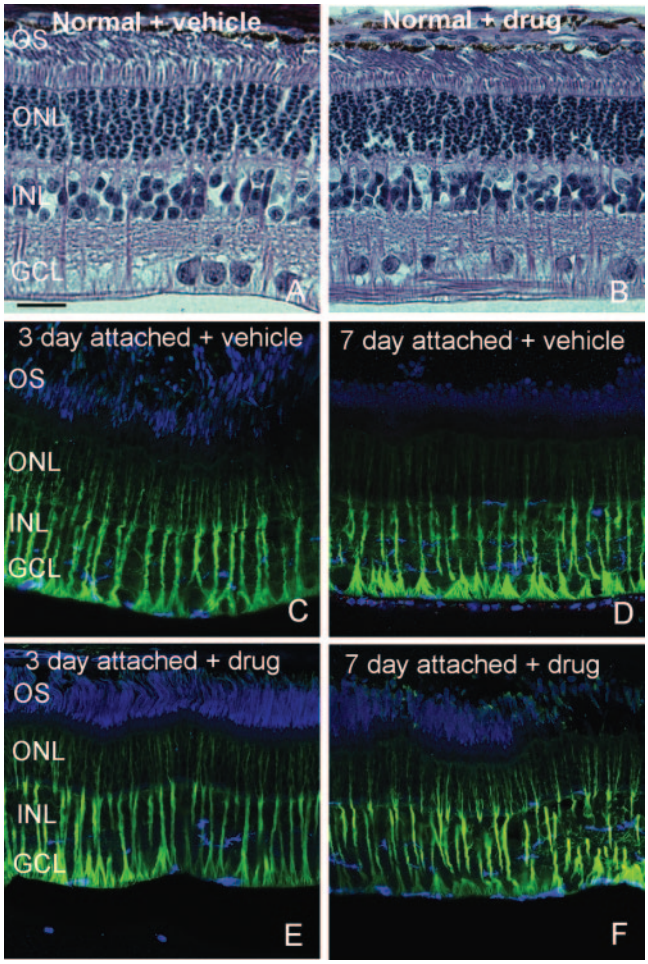

Figure 1. Control attached retinas. (**A**, **B**) Light micrographs of hematoxylin and eosin-stained paraffin sections illustrating the normal retinal morphology in vehicle-injected (**A**) and drug-injected (**B**) normal eyes. (**C**, **D**) Laser scanning confocal images of attached retinal regions from within the eyes of animals with 3- or 7-day detachments receiving intraocular injection of vehicle (**C**, **D**) or drug (**E**, **F**). Sections were labeled with anti-vimentin (Müller cells, *green*), anti-BrdU (dividing cells, *red*), and isolectin B4 (microglia and macrophages, *blue*). Anti-vimentin labeling extended from the GCL into the ONL, no anti-BrdU labeling was observed, and the isolectin B4 labeled the fine processes of microglia in the inner retina and macrophages in the vitreous (the *blue* labeling of the OS is nonspecific). OS, outer segment. Scale bar, 20 μm.

Quantitation

At 3 days of detachment, the average number of anti-BrdU–labeled Müller cell nuclei was significantly reduced from 10.79/mm of retina in vehicle-treated eyes to 1.19/mm retina after drug treatment (Fig. 3). At 7 days, the number was reduced from 9.83/mm retina in the vehicle-treated eyes to 1.49/mm retina after drug treatment (Fig. 3). The average number of subretinal scars was reduced from 2.03/mm retina in controls to 0.39/mm retina in drug-treated retinas (Fig. 4). Only the 7-day time point was quantified because no scars were present at 3 days in vehicle- or drug-treated animals. Average lengths of subretinal scars at day 7 were 131.28 μm in vehicle-treated eyes and 11.01 μm in drug-treated eyes (Fig. 5).

Few immune-related cells (microglia and macrophages) incorporated BrdU in vehicle or treated retinas; therefore, to quantify this response, the total number of isolectin B4-labeled cells was counted per millimeter of retina. At 7 days, the total

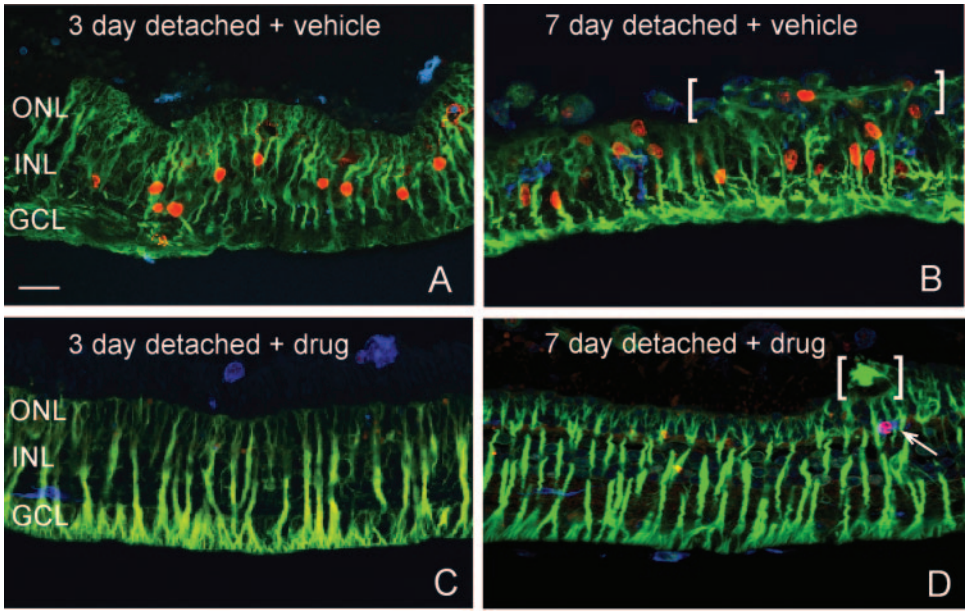

FIGURE 2. Experimental detached retinas. Laser scanning confocal images of retinal sections labeled with anti-vimentin (Müller cells, *green*), anti-BrdU (dividing cells, *red*), and isolectin B4 (microglia and macrophages, *blue*). (**A**) In the 3-day detachments injected with vehicle, anti-vimentin labeling extended through the ONL to the outer limiting membrane, anti-BrdU labeling was present primarily in Müller cell nuclei in the INL, and isolectin B4 labeling was present in microglia throughout the retina and in macrophages in the subretinal space. (**B**) In the 7-day detachments injected with vehicle, anti-vimentin labeling of Müller cells extended into the subretinal space (*brackets*), anti-BrdU labeling was present in nuclei scattered throughout the retina and in the subretinal space, and isolectin B4 labeling appeared in numerous microglia in the retina and macrophages in the subretinal space. In the 3-day and 7-day Palomid 529–treated retinas (**C**, **D**), anti-vimentin labeling extended through the ONL and only occasionally into the subretinal space (**D**, *bracket*), anti-BrdU labeling was low, and isolectin B4 labeling was present throughout the retina and subretinal space. Occasional microglial cells can be seen labeled with anti-BrdU and lectin (**D**, *arrow*). Note that the drug-treated detached retinas (**C**, **D**) also retained a more normal morphology in comparison with the vehicle-treated detached retinas. Scale bar, 50 μm.

number of cells decreased from an average of 43.9/mm retina in vehicle-treated eyes to 24.5/mm retina in drug-treated eyes ($P < 5.7 \times 10^{-5}$; Fig. 6).

Given that the outer retina appeared more organized in drug-treated eyes, as shown by the antivimentin labeling of Müller cells (compare Figs. 2A and 2B with Figs. 2C and 2D), we sought to determine whether more photoreceptors were present by measuring the ONL width. Seven days of detachment resulted in a statistically significant decrease in the average thickness of the ONL, from 34.45 μm in the normal retina to 19.40 μm in the vehicle-treated detached retinas ($P < 1.27 \times 10^{-11}$; Fig. 7). There was also a statistically significant decrease in the thickness of the ONL in the drug-treated detached retinas to 27.21 μm compared with the normal attached retina ($P < 0.001$). However, the thickness of the ONL was significantly greater in the detached retinas treated with drug than those treated with vehicle (27.21 μm vs. 19.40 μm; $P < 8.81 \times 10^{-6}$), suggesting that Palomid 529 may exert some rescue effect on photoreceptors after detachment.

DISCUSSION

Two potentially blinding conditions that can result from retinal detachment are PVR and subretinal fibrosis, which is the formation of cellular membranes on the vitreal or photoreceptor surface of the retina. The only treatment is surgical removal of the membranes, though it is frequently not possible to adequately peel subretinal membranes. In addition, surgical intervention is often not a permanent solution because recurrence

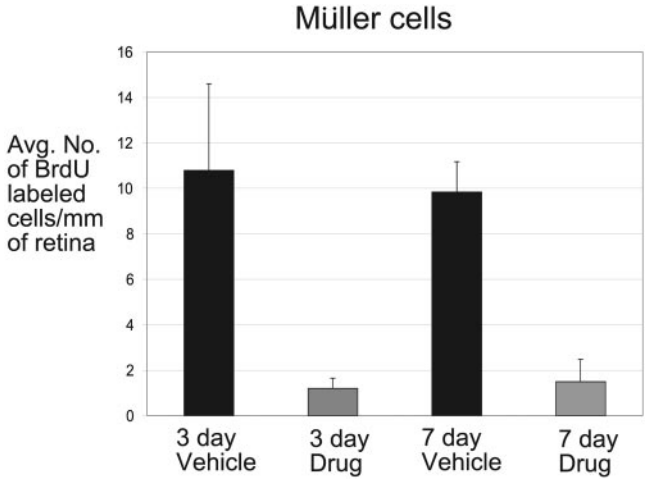

FIGURE 3. Graph illustrating the number of Müller cells proliferating after retinal detachment. The average number of anti-BrdU–labeled Müller cells per millimeter of retina decreased significantly after drug treatment at the 3-day and 7-day detachment points compared with control detachments. Error bars, SD.

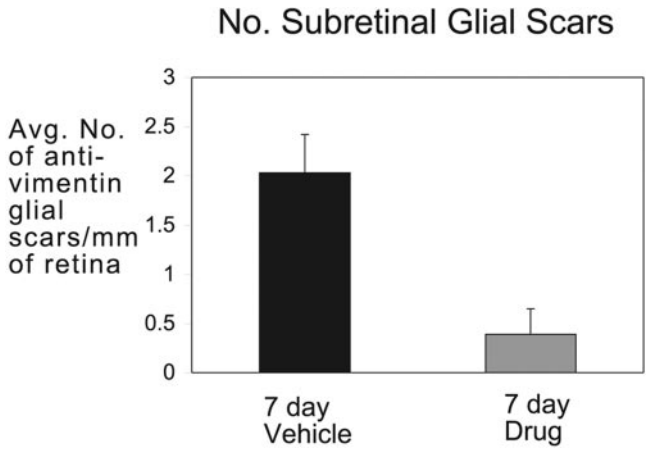

FIGURE 4. Graph illustrating the number of subretinal glial scars after retinal detachment. The average number of anti-vimentin–labeled subretinal glial scars per millimeter of retina decreased significantly in the drug-treated retinas 7 days after detachment compared with vehicle-treated retinas. Error bars, SD.

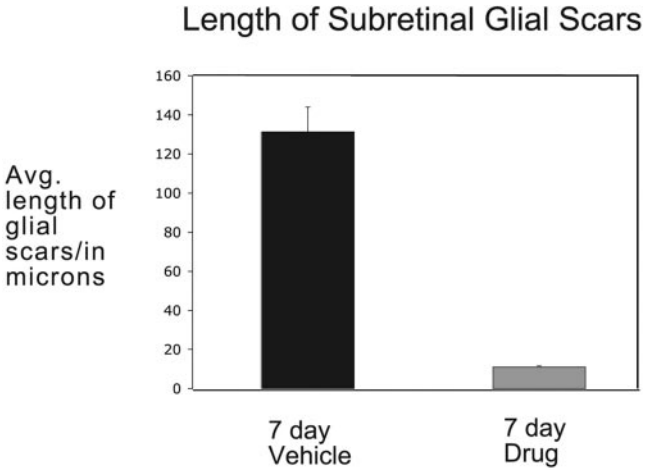

FIGURE 5. Graph illustrating the length of subretinal glial scars after retinal detachment. The average length of antivimentin-labeled subretinal scars decreased significantly in the drug-treated retinas 7 days after detachment compared with vehicle-treated retinas. Error bars, SD.

of secondary membranes is not uncommon. Ideally, a pharmacologic adjunct could be given at the time of reattachment surgery to prevent membrane formation or, alternatively, at the time of membrane removal to prevent continued growth of the tissue. To date no pharmacologic approaches have proven clinically effective at inhibiting this process. We show here that a single injection of Palomid 529 is effective in an animal model at reducing the proliferation induced by detachment, the subsequent hypertrophy and growth of the Müller cells into the subretinal space, and the activation of microglia. In addition, no apparent toxicity is associated with intraocular delivery of the drug based on daily ophthalmoscopic examination, histology of the retina, and lack of Müller cell and microglia activation. In fact, the ONL thickness was greater in the Palomid 529–treated detached retinas, suggesting that it may also have some neuroprotective effect on photoreceptors. Hence, this drug may be useful for treating a variety of related human retinal diseases in which proliferation or photoreceptor cell death is a component.

Typically, PVR is thought to be a condition involving undesirable cell proliferation, cell spreading, and contractility. In-

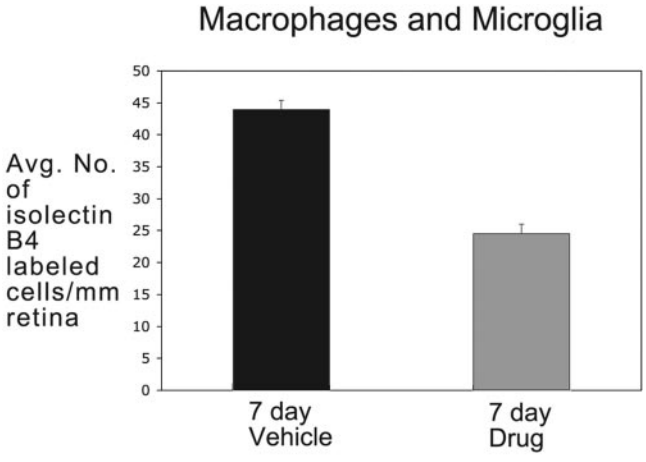

FIGURE 6. Graph illustrating the number of immune-related cells after retinal detachment. The average number of isolectin B4-labeled macrophages and microglia per millimeter of retina decreased significantly in the drug-treated retinas at 7 days after detachment compared with vehicle-treated retinas. Error bars, SD.

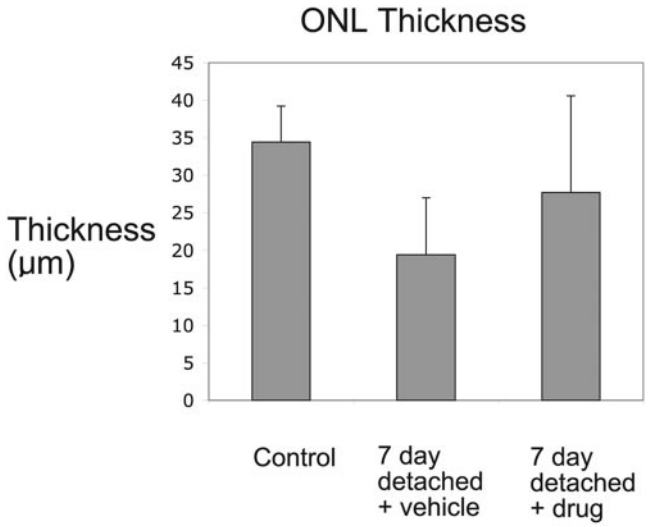

FIGURE 7. Graph illustrating the average thickness of the ONL after detachment. The ONL in 7-day detached retina treated with Palomid 529 was thicker than in 7-day detached retina treated with vehicle, but the ONL in both detachment groups was significantly reduced compared with control retinas. Error bars, SD.

deed, dividing cells have been observed in membranes removed from patients with PVR.[37,38] However, data from animal models suggest that in addition to proliferation, the growth and hypertrophy of Müller cells may play critical roles in the response perhaps by providing a cellular scaffold on which more complex membranes, involving a variety of cell types, can grow. Both proliferation and hypertrophy of Müller cells begin within the first few days after detachment. After 1 week, proliferation declines to low levels, but processes from these cells continue expanding in the subretinal space, apparently as long as the retina remains detached. This is also true for the growth of epiretinal membranes in the vitreous. Müller cell growth on the vitreal surface appears to begin, however, with retinal reattachment, not detachment.[22] Reattachment reduces the proliferative response significantly,[22] but the growth of membranes along the vitreoretinal surface continues because large membranes are generally observed weeks to months after reattachment. The fact that PVR and subretinal fibrosis are complex combinations of proliferation and cell growth may explain why drugs such as 5-fluorouracil that solely target proliferation were not effective at reducing epiretinal membrane formation in human patients.[23,24] The Akt/mTOR pathway, through which palomid acts, has been shown to regulate proliferation and cell spreading.[30] This may provide a mechanism for Palomid 529's dramatic reduction of proliferation and subretinal gliosis in the rabbit model of detachment, though its precise mechanism of action is unknown. It is presumed that Palomid 529 acts directly on Müller cells because these are the most numerous cell types undergoing division. However, it has been shown that inhibitors of mTOR can affect RPE cells directly by decreasing cell spreading and migration in culture, suggesting that the effects on Muller cells could be indirect, acting through other cell types such as the retinal pigment epithelium.[39] Our data also do not provide an indication of whether the effect of Palomid 529 on increased photoreceptor survival is direct or indirect. It has been shown, however, that activation of the PI3K/Akt signaling pathway can have a neuroprotective effect in the retina by inhibiting light-induced photoreceptor apoptosis,[40] suggesting that the drug may in fact have a direct effect on photoreceptors. Finally, though all the work conducted with this drug, in a variety of cell types and animal models, indicates the effect is specific for

the Akt/mTOR pathway (D. Sherris, personal communication, 2009), the data are only suggestive that the Akt/mTOR pathway is activated after detachment and that Palomid 529 inhibits this pathway in the retina. Future studies are planned to address these issues.

In a pharmacokinetic study of rabbit vitreous, Palomid 529 was shown to have an expected half-life of at least 5 months (D. Sherris, personal communication, 2009), which may explain why we observed an effect on proliferation and scar formation 7 days after detachment. The rapid clearance of drugs such as 5-fluorouracil may contribute to their ineffectiveness in preventing PVR.[23,24] Our data also suggest that subretinal membrane formation may require a critical level of cellular proliferation because Palomid 529 reduced but did not abolish the number of dividing cells. Taken together, these observations suggest that effectively reducing the formation of extraretinal membranes may require an agent that is both rapid and prolonged in its activity and that acts on multiple facets of cellular physiology.

Although the average number of subretinal scars was reduced approximately fourfold with the addition of Palomid 529, their length was reduced more than 20-fold, suggesting that the drug reduced not only Müller cell growth into the subretinal space but also the actual expansion of the processes that reach that space. This correlates with the observation that Müller cells within the retina appear less branched and tortuous in treated eyes (Figs. 2C, 2D), suggesting that we observed a result of Palomid's effect on cell spreading, translated in this case into cell growth. Clinically, Palomid 529 may prevent the formation of scars and reduce the overall growth of scars that are already present. Palomid 529 also appeared to reduce the microglial/macrophage response. Given that these cells become highly migratory when activated, this may also be a result of the anti-spreading effect of the drug. It is unknown, however, whether this is a direct effect on the immune cells or whether the drug acts indirectly through other cell types such as Müller cells and RPE cells.

In summary, the data presented here suggest that Palomid 529 is an effective agent for decreasing proliferation, glial cell growth, and photoreceptor cell death induced by retinal detachment and therefore may represent a novel way to improve the overall outcome of repairing rhegmatogenous retinal detachment.

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