Transplantation of Photoreceptors to Light-Damaged Retina

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We investigated the possibility of reconstructing light-damaged retinas by photoreceptor transplantation. The outer nuclear layer containing the photoreceptor cells was eliminated in adult albino rat retinas by exposure to constant high-level illumination. Photoreceptors for transplantation were harvested from neonatal rats using a novel isolation technique that maintained the cellular organization of the outer nuclear layer. Transplantation was accomplished using a transcorneal approach to the subretinal space, which minimized trauma to the eye. The retina reattached to the back of the eye with transplanted photoreceptors interposed between the retina and the overlying tissues. Prelabelling with fluorescent dye enabled positive identification of the transplanted cells. The transplanted photoreceptors appeared to survive transplantation for at least 6 weeks and were immunohistochemically reactive for opsin. The antibody staining for opsin identifies the transplanted cells as photoreceptors and indicates that they are still capable of producing visual pigment and therefore may have the capacity to transduce light. These findings indicate that photoreceptors can be transplanted to form a new outer nuclear layer in a damaged mature retina. Invest Ophthalmol Vis Sci 30:1684-1690, 1989

Several forms of blindness (retinitis pigmentosa, retinal detachment and exposure to intense light) are primarily related to the loss of the retinal photoreceptors.1,2 However, destruction of the photoreceptors does not necessarily lead to loss of the remaining retina or the axons that connect the retina to the brain. In such cases, if the photoreceptors could be replaced and innervate the retina appropriately, some degree of vision might be restored. We therefore investigated the possibility of reconstructing light-damaged retinas by transplantation of photoreceptors.

While major difficulties with technical aspects of photoreceptor transplantation were evident, several factors made such transplantation potentially promising. The retina does not necessarily undergo glial scar formation when damaged, as does the adult central nervous system.3,4 This characteristic may contribute to the potential of retinal cells to regrow severed axons within the eye.4,5 Regrowth of photoreceptor axons also might be facilitated by the close proximity of their postsynaptic targets within the adjacent outer plexiform layer. Thus in order for the transplanted photoreceptors to make appropriate connections with the host's retina, growth across substantial intervening neural (or glial scar) tissue would not be necessary.

The photoreceptor layer of the retina is nonvascularized. The necessity for prompt revascularization, which limits the transplantability of most neural tissue, is therefore not a limitation with photoreceptors. Furthermore, while the eye is considered an immunologically privileged site, rejection of transplanted tissue can occur.7 Nonvascularized tissue (eg, the cornea), however, has shown the least amount of tissue rejection. Since the photoreceptor layer is not vascularized, the possibility of this tissue being rejected may therefore be further decreased. In addition MHC antigens which have been implicated as important components both in the generation and expression of immune response8 are only weakly expressed (Class I) or apparently not expressed at all (Class II) by photoreceptors.9 The limited vulnerability of the photoreceptor layer to transplant rejection opens up the potential for the transplantation of tissue that is genetically dissimilar, which could have considerable clinical utility.
Finally, successful transplantation of other neural tissue, particularly of embryonic and postnatal retina to the optic tectum and to host retina led us to attempt to reconstruct light-damaged retinas by transplanting photoreceptors. The results described here indicate that photoreceptors can be transplanted to retinas lacking photoreceptors. Some of these results have been published in abstract form.

Materials and Methods
Experimental Animals

As an animal model for human blindness resulting from the loss of retinal photoreceptors we used adult albino rats (Sprague-Dawley) that were exposed to constant illumination averaging 1900 lux for 2 to 4 weeks. As shown in Figure 1 this exposure destroys most photoreceptors, eliminating cells of the outer nuclear layer but leaving the remaining neural retina intact. Photoreceptors for transplantation were taken from 8-day-old normal rats of the same strain that had been maintained under colony room illumination (10–20 lux) on a 12 hr/12 hr light/dark cycle. Experimental animals were anesthetized with ketamine and sodium pentobarbital. A preoperative dose of dexamethasone (10 mg/kg IP) was also administered. Animals were handled according to the USPHS Policy for Humane Care and Use of Laboratory Animals and the ARVO Resolution on the Use of Animals in Research.

Photoreceptor Preparation

In order to maintain the organization of the donor photoreceptor layer, we chose not to harvest and purify them by published techniques, which require dissociating the cells, thereby disrupting the organization and cellular polarity of the photoreceptor layer. Instead, we have developed a technique to isolate a more intact photoreceptor layer from the retina.

The retina from the anesthetized 8-day-old rat is removed, flattened with radial cuts and placed with the receptor side down on a gelatin slab secured to the vibratome chuck. It is coated with a 4% gelatin solution and cooled to 4°C with ice-cold Ringer’s solution. The retina is sectioned at 20 to 50 μm until the photoreceptor layer is reached. Vibratome section thickness calibration and previous histological mea-
measurements of the thickness of the developing retina provide guides to depth of sectioning. The appropriate sectioning depth is further determined by microscopic observation of each section. When the photoreceptor layer is reached, the stage is advanced and a thick (200 to 300 μm) section is taken, undercutting the photoreceptor layer secured to the gelatin base.

The technique, which takes advantage of the planar configuration of the retinal layers in the flat-mounted preparation, allows us to separate the photoreceptor layer from the retina. The photoreceptor layer, secured to a thin gelatin substrate, can be manipulated and transplanted with minimal damage and cellular disorganization. We chose a gelatin substrate because of its flexibility, apparent lack of toxicity to neural tissue and because it dissolves at body temperature. These characteristics allow the photoreceptor layer to be transplanted on a mechanically stabilizing substrate that dissolves after insertion and thus does not appear to interfere with tissue growth and interactions between the retina and underlying retinal pigment epithelium (RPE) following transplantation.

**Dil Labeling**

For labeling of transplanted tissue the isolated outer nuclear layer was cultured overnight with 40 μg/ml of dil (1,1'-dioctadecyl-3,3,3',3'-tetramethylinodocarbocyanine perchlorate; Molecular Probes, Inc., Eugene, OR) in Earle's MEM containing 10% fetal calf serum, incubated under 95%/5% oxygen/carbon dioxide mixture at room temperature. Labeling techniques and fluorescent microscopy were otherwise those of Honig and Hume. Dil was removed from sections that were to be counterstained with FITC-labeled RET-P1 opsin antibody by prior washing in acetone.

**Surgical Procedure**

To reduce bleeding and surgical trauma we used a transcorneal surgical approach to the subretinal space. This procedure entails making a transverse incision in the cornea sufficient to allow insertion of a spatulated acrylic carrier that is 2.5 mm wide with side rails 0.5 mm high. The carrier is advanced under the iris (dilated with topical atropine) to the ora serrata, detaching the retina. The carrier is then advanced under the retina into the subretinal space to the posterior pole of the eye. The channel provided by the head carrier allows a piece of the photoreceptor layer attached to the gelatin substrate (up to 2.5 × 4 mm) to be placed on the carrier and guided into the subretinal space by fine forceps. The carrier is then removed while the gelatin is held in position by the forceps. Following removal of the forceps, the edges of the corneal incision are abutted to allow rapid, sutureless healing. The eye is patched during recovery and a prophylactic dose of penicillin is administered. Upon removal of the patch, a veterinary ophthalmic antibiotic ointment (Pharmaderm Inc., Melville, NY) is applied.

Transplant recipients were maintained on a 12 hr/12 hr light/dark cycle with an average light intensity of 50 lux. Following appropriate survival times, the animal was overdosed with pentobarbital and perfused transcardially with phosphate buffered 3% paraformaldehyde-2% glutaraldehyde solution. Cryostat sections of both the light-blinded eye (control) and the eye receiving the photoreceptor transplant were then cut (20 μm).

**Immunohistochemistry**

Antibody labeling for opsin was performed on retinas fixed with 3% paraformaldehyde and cryosectioned at 20 μm. Immunohistochemical methods were otherwise those of Hicks and Barnstable. Elimination of the primary antibody eliminated specific labeling for opsin.

**Results**

The photoreceptor layer is the outermost layer of the retina and is apposed to the RPE. Optimal positioning of the transplanted photoreceptor layer therefore could be obtained by insertion into the subretinal space, thus placing the transplanted photoreceptor layer between the retina and the RPE. The standard surgical approach to the subretinal space is through the highly vascularized sclera and choroid. We initially tried this approach and found that although the photoreceptor layer could be positioned correctly, considerable bleeding into the subretinal space frequently occurred. This bleeding and resulting inflammatory reaction severely compromised survival of the transplants (three survived out of 22 cases). In addition, these complications appeared to inhibit reattachment of the retina to the underlying RPE.

By using the transcorneal approach to the subretinal space of the eye, we found that the positioning of the photoreceptor layer between the host's retina and the adjacent epithelial and choroidal tissue layers of the eye could be accomplished while minimizing vascular damage and subsequent bleeding into the eye. In addition, we have found that this approach does not appear to disrupt the integrity of the retina, which reattaches to the back of the eye with the transplanted photoreceptors interposed between the retina and the RPE. As seen in Figure 2, retinal reattachment appears to be facilitated in the immediate area of the transplant. At the present time the reason for this is not known.
Fig. 2. Cryostat section 4 weeks post-transplantation showing host retina which has spontaneously become reattached to the back of the eye with the transplanted photoreceptors interposed between the retina and the RPE. (A) Low-power photomicrograph showing the location of the photoreceptor transplant (between arrowheads) at the posterior pole of the host eye. Bar = 0.5 mm. (B) Higher-power photomicrograph showing the interface between the transplant and the adjacent retina devoid of outer nuclear layer. Arrows indicate the extent of the transplant (T). Arrowheads indicate three possible residual photoreceptors that survived constant illumination. Note their fusiform shape contrasts with the rounder, more normal shape of the transplanted photoreceptors. H&E stain. Bar = 100 μm. (C) FITC fluorescent micrograph of antibody Ret P-1 specific for opsin in a section adjacent to that shown in panel (B). Arrows indicate the extent of the transplant. Transplanted cells are labeled for opsin indicating that they are photoreceptors. Some nonspecific fluorescence is evident adjacent to the transplant. Bar = 100 μm.

Using this insertion method it is possible to position the photoreceptors at the posterior pole of the retina (Fig. 2A). Posterior positioning might be thought to be the most difficult, but in practice this does not seem to be the case as most of the transplants directed toward the posterior pole were in fact found in this location. Since high visual acuity requires the central retina, the ability to make transplants to various portions of the retina and most importantly to the posterior pole is significant if this procedure is to be of eventual clinical utility.

To determine the viability of the transplanted photoreceptors, cryostat sections (20 μm) were made from both the blinded eye (control) and the eye receiving the photoreceptor transplant at 2, 4, or 6 weeks after transplantation. We found that the photoreceptors survived transplantation at all times tested (36 out of 54 transplants). In most instances the surviving transplant approximated its size at the time of transplantation. More importantly, there was no apparent reduction in transplant size with longer survival times, suggesting that the transplants were stable.

As a control we examined the contralateral eyes that did not receive a photoreceptor transplant. In these eyes, the retinas possess very few residual photoreceptors which are located adjacent to the outer plexiform layer and the RPE. However, these residual cells are abnormal in appearance, having flattened, pyknotic cell bodies instead of the rounded cell bodies of normal photoreceptors. Furthermore, the residual photoreceptors do not form an outer nuclear layer composed of columnarly stacked cell bodies, but instead are found in isolation, or at most appear as a single or double layer of cells (see Fig. 1B) located mainly in the peripheral retina.

The transplanted cells are easily distinguished from the residual photoreceptors by a number of parameters. First, they are found in discrete patches and have the characteristic columnar stacking arrangement of up to about 12 cell bodies that is characteristic of photoreceptor cells in the outer nuclear layer of the
normal retina. They do not have the flattened appearance of the residual native photoreceptors, but instead have the round, nonpyknotic cell body typical of normal photoreceptor cells. Furthermore, the transplanted photoreceptors can form rosette configurations, a characteristic of transplanted and cultured retina, while residual photoreceptors are not found in these configurations.

In every case we found that some portion of the transplanted outer nuclear layer contained at least one rosette formation. To quantify the occurrence and characteristics of the rosettes, we classified each transplant as having only small (composed of six to eight cells) and infrequent (up to two per section) rosettes, as transplants in which the rosettes were larger (composed of eight to 15 cells) and more frequent (three or more per section), and those where rosettes were large (composed of over 15 cells) and occurred in over half of the transplant. We found that in 48% of the cases rosettes were small and infrequent, in 19% they were intermediate in size and frequency, while in 33% of the cases they were large and frequent in number. The size and occurrence of rosettes within the transplants was not correlated with term of survival.

While a clear description of the condition and number of outer segments in the transplants awaits ultrastructural analysis, even at the level of light microscopy the outer segments are clearly decreased in number in the transplanted cells. Those present (most conspicuously within the rosettes) also appear to be shorter than normal.

To investigate the possibility that our surgical procedure in some manner induced the regeneration of patches of native photoreceptors, we performed sham operations. In these cases all procedures were performed as with the photoreceptor transplants except that no photoreceptors were attached to the inserted gelatin slab. While the retina reattached to the back of the eye in no instance did we find patches of photoreceptors.

To positively identify the photoreceptor patches in experimental animals as transplanted tissue, in several cases we labeled the donor outer nuclear layer with the fluorescent marker diI prior to transplantation. As shown in Figure 3B the photoreceptor patches were fluorescently labeled while the host retina did not show dil fluorescence.

To confirm that the transplants consisted of photoreceptors we used a monoclonal antibody specific for opsin, RET-P1 (provided by C. Barnstable). As opsin is found only in photoreceptors, any cell showing labeling for opsin is therefore identified as a photoreceptor. As can be seen in Figures 2C and 3C, the transplanted cells stain intensely for opsin whereas other retinal cells are unstained. Positive staining for opsin not only identifies these cells as photoreceptors but indicates that these cells are still capable of producing the protein moiety of visual pigment. Retina adjacent to the region of the transplant shows only a

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**Fig. 3.** Cryostat sections 3 weeks post-transplantation. (A) H&E-stained photomicrograph of transplant and host retina. Note the cell-sparse layer that resembles the OPL interposed between the host retina and the transplant. (B) Dil fluorescent micrograph of adjacent section. Transplanted photoreceptors show Dil fluorescence, identifying them as donor tissue. (C) FITC fluorescent micrograph of antibody RET-P1 (specific for opsin) in a section adjacent to that shown in panel (A). Transplanted cells are labeled for opsin, indicating that they are photoreceptors. Bar = 50 μm.
few isolated photoreceptor cell bodies (Fig. 2B) that do not stain for opsin (Fig. 2C), suggesting that they are cones. Their lack of opsin staining, as well as their location and appearance in H&E-stained material, confirms that these cells are the host’s residual photoreceptors (Fig. 2B).

Harvesting the photoreceptor layer from the neonatal retina does not appear to disrupt tissue organization. Once transplanted, the photoreceptor layer can maintain its characteristic columnar arrangement of cell bodies for all survival times examined, thus forming a new outer nuclear layer within the host’s retina. In some cases, strict polarity is lost and rosettes are formed. By light microscopy the new layer appears to be attached to the host’s outer plexiform layer (Figs. 2A, 3A). This layer normally is the site of synaptic contact between the photoreceptors and the retina. Anatomical tracing studies are in progress to determine if the transplanted photoreceptors have formed synapses onto host neurons.

Discussion

While the photoreceptors survive, produce opsin and apparently integrate with the host’s retina, they do not appear completely normal in that the number and length of outer segments is reduced. In this regard photoreceptors lacking outer segments are still capable of phototransduction. The relative scarcity of outer segments has also been noted in retina transplanted to the optic tectum which have also been shown to be functional. This deficiency in outer segments was thought to be the possible consequence of the lack of appropriate apposition of the RPE to the photoreceptors. Since in our preparations RPE is present and in apparently normal apposition to the photoreceptors, the scarcity of outer segments here would not appear to be related to inadequate photoreceptor/RPE contact. This failure of outer segment growth in the presence of photoreceptor apposition to the RPE has also been seen following retinal reattachment. While the reason for the scarcity of outer segments on transplanted photoreceptors remains to be determined, the outer segments are found in highest concentration within rosette configurations in our transplants as well as in transplants of retinal fragments. The geometry of these configurations would of course disallow normal apposition of the photoreceptors with the RPE.

Neonatal photoreceptors were transplanted to their appropriate homotopic site and apparently became integrated with the host’s tissue to reconstruct a portion the host’s blinded retina. To accomplish this we have devised a method to isolate the intact photoreceptor layer. This is significant because it will be necessary to maintain tight matrix organization if coherent vision is to be restored to the retina blinded by the loss of photoreceptors. It should be noted, however, that we found at least some disruption of the organization of the transplant by photoreceptor rosette formations in all cases. While almost half the cases (48%) showed minimal disruption as determined by the size and frequency of occurrence of photoreceptor rosettes, in 33% of the cases this disruption involved more than half the transplant. Since the size and occurrence of rosettes within the transplant did not increase with increasing survival time, it is likely that these formations develop rapidly (prior to our first examination at 2 weeks) following transplantation and then become stable configurations.

We have found that the transplanted photoreceptors not only survive transplantation, but produce opsin, and integrate with the host’s retina. The positive staining for opsin not only identifies the transplanted cells as photoreceptors but indicates that they are still capable of producing the protein moiety of visual pigment and therefore may have the capacity to transduce light.

We have devised methods for isolating the outer nuclear layer from the retina and transplanting pieces of this layer to the subretinal space. The isolation method could be used to prepare other retinal layers so that selected populations of retinal cells can be used in other neurobiological investigations. Our surgical approach minimizes trauma to the eye, avoiding the subretinal bleeding that we and others have found to compromise cellular transplants to this location. In addition, this technique produces physical apposition of the transplanted outer nuclear layer with the host retina, and allows reattachment of the reconstructed retina to the RPE. Together, these methods may also be of use for transplantation of other cell populations to the subretinal space for replacement of damaged, missing or defective cells (ie, RPE).

Key words: transplant, photoreceptor, blindness, neural grafts, retina

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