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Successful Cotransplantation of Intact Sheets of Fetal Retina with Retinal Pigment Epithelium

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PURPOSE. Many retinal diseases, such as macular degeneration, affect both retinal pigment epithelium (RPE) and photoreceptors. Therefore, retinal repair may require transplantation of both tissues together as a cograft.

METHODS. As recipients of retina–RPE cografts, 7- to 10-week-old albino Royal College of Surgeons rats that lose their photoreceptors because of a pigment epithelium defect were used. Freshly harvested intact sheets of RPE with neural retina from pigmented normal rat fetuses were gel embedded for protection and transplanted into the subretinal space.

RESULTS. After 6 to 7 weeks, with the support of the cografted RPE sheet, transplanted photoreceptors developed fully in organized parallel layers in the subretinal space. Immunohistochemistry for rhodopsin, rod α-transducin, and S-antigen and peanut agglutinin labeling for cone interphotoreceptor matrix domains suggested that the photoreceptors in the graft were capable of normal function.

CONCLUSIONS. Freshly harvested intact sheets of fetal RPE and retina, transplanted together into the subretinal space, can develop a normal morphology. Such transplants have the potential to benefit retinal diseases with dysfunctional RPE and photoreceptors. (*Invest Ophthalmol Vis Sci.* 1999;40: 1557-1564)

Interactions between photoreceptors and retinal pigment epithelium (RPE) are crucial to retinal function. For example, the RPE is responsible for daily phagocytosis of shed tips of photoreceptor outer segments.[1] In the Royal College of Surgeons (RCS) rat mutant, RPE cells cannot phagocytize the shed photoreceptor outer segment tips, leading to accumulation of outer segment debris and subsequent photoreceptor degeneration.[2,3] In humans, diseases of RPE, such as age-related macular degeneration, a leading cause of blindness,[4,5] also lead to photoreceptor degeneration.

One approach to the treatment of these diseases is to replace diseased cells with healthy cells. To date, most retinal transplantation studies have involved transplanting only RPE[6-10] or neural retina separately.[11-20] Based on experiments showing that photoreceptors in the RCS rat can be rescued by injecting freshly harvested RPE cells,[6-8,21,22] clinical trials of RPE transplants have been performed in patients with macular degeneration.[23,24] However, such transplantations can only be

effective if there are some remaining photoreceptors in the diseased retina that are not already committed to cell death. For example, RCS photoreceptors can be rescued by RPE transplantation only up to 4 weeks of age.[6-8] When the photoreceptors have irreversibly degenerated at later stages of the disease, transplantation of either RPE or photoreceptors alone will have no effect on the time course of retinal degeneration. Transplant photoreceptors develop fully and maintain outer segments only if supported by a healthy RPE.[25-28] Therefore, in some diseases or stages of disease, retinal repair may require the transplantation of intact sheets of both RPE and neural retina. Another precondition for a functional transplant is that the inner retina of the recipient still be intact[29,30] and connect either directly or indirectly with the photoreceptors of the transplant.

Earlier experiments of retinal transplantation involved transplanting retinal tissue as cell aggregates[12-14,18] or cell suspensions.[15] However, this consistently resulted in the formation of photoreceptor rosettes. Gouras et al.[16] injected retinal microaggregates (i.e., <0.2-mm^2 pieces) of postnatal mouse retina into the subretinal space of *rd* mice. A portion of these microaggregates was arranged in the right orientation and small patches of transplant photoreceptors could develop with outer segments in contact with host RPE. If postnatal or mature photoreceptors are transplanted as intact sheets, rosette formation can be avoided.[11] Cografting of intact sheets of RPE with retina has not been achieved before, probably because of the extreme difficulty of dissection and implantation. Especially in the rat that offers several good retinal degeneration models,[2,31-33] the implantation of intact retina/RPE sheets into the subretinal space is very delicate because of the small eye and the large lens.

Our laboratory has developed a unique method for transplanting intact sheets of fetal donor tissue in rats.[25-28] The

procedure involves a special technique of gel-embedding the tissue, and the use of a custom-made implantation instrument to place the tissue with minimal trauma into the subretinal space. Such transplants can "repair" an area of a light-damaged retina by development of a parallel photoreceptor layer with outer segments in contact with the host RPE and can become integrated with the host retina.[28] In that article,[28] the term "integration" was used to describe the apposition of the transplant toward the host retina in the absence of glial barriers. This means intermingling of transplant and host cell processes so that they cannot be distinguished, thus creating a potential for connections.

Our laboratory has consistently and successfully used fresh fetal neural retinal donor tissue (reviewed in Ref. 26). Fetal or embryonic tissue has a high capacity to develop different cell types, sprout neuronal processes, and produce trophic factors. Most important, because of a lack of antigenic sites, embryonic cells are well tolerated immunologically by a host of the same species when placed into the central nervous system or the eye.[34-36]

The goal of the present study was to investigate the feasibility of transplanting sheets of freshly harvested fetal RPE and retina together and the development of these tissues in a dysfunctional host retina, the RCS rat, at an age when photoreceptors could no longer be rescued by RPE cell transplants. We have shown previously that aggregates of fetal retinal cells transplanted to a retinal lesion site develop most retinal cell types, including photoreceptors[12,13,17] and send out neuronal processes to form synapses in the host retina.[37]

Part of this cografting study has been published as meeting abstracts.[38,39]

METHODS

In this study, 11 albino RCS rats were used as recipients. The rats received a transplant in one eye at 48 to 67 days of age, when the majority of the photoreceptors have degenerated and the subretinal space is filled with outer segment debris.[3] The animals were treated according to the regulations in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Donor tissue was obtained from pigmented embryonic day (E)18 to E20 Long–Evans rat fetuses prelabeled in utero by injecting a timed-pregnant rat with 40 mg/kg bromo-deoxy-uridine (BrdU), a thymidine analogue, on 2 to 3 gestational days before harvesting the embryos.[18] Donor eyes were incubated in dispase (Collaborative Biomedical Products, Bedford, MA) for 10 minutes at 37°C to enable the RPE and retina to be dissected from surrounding tissues. To enter the subretinal space, a small incision (~1 mm) was cut just behind the *pars plana* of the host eye. Retinal pieces with attached RPE sheets (width, 0.5-0.7 mm; length, 1.0-1.5 mm) were protected with a thin layer of gel (Fig. 1A; note: All figures are oriented with the ganglion cell layer toward the top and the photoreceptor layer toward the bottom of the micrograph) and grafted as intact sheets to the subretinal space, using a custom-made implantation tool according to a published procedure.[28] The tissue was usually placed in the superior quadrant.

The recipient rats were killed 43 to 155 days after implantation with an overdose of sodium pentobarbital (300 mg/kg).

Most rats were perfusion fixed with 4% paraformaldehyde, 0.18% picric acid in 0.1 N Na-phosphate buffer (pH 7.2); eyecups were embedded in paraffin. The eyes of three rats were immersion fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 N Na-phosphate buffer (pH 7.2), and retinal pieces were embedded in Epon. Epon sections (0.75 μm) were stained with toluidine blue. Paraffin sections (8 μm) containing the transplant and the surrounding host retina were stained with hematoxylin-eosin, peanut agglutinin (PNA) lectin (1: 1000; Vector, Burlingame, CA), and monoclonal antibodies for BrdU (Dako, Carpinteria, CA), rhodopsin,[40] rod α-transducin,[41] and S-antigen (arrestin).[42] The immunohistochemical procedures have been described previously.[17,28]

RESULTS

Transplantation Successes and Failures

In 4 of 11 animals (43 and 51 days after transplantation), the transplanted eyes contained cografts of fully laminated neural retina supported by the cotransplanted sheet of RPE (Figs. 2, 3, 4). In the remaining seven animals (data not shown), the RPE had separated from the neural retina during implantation, the neural retinal transplants had formed rosettes, and/or the transplanted RPE cells had dispersed and migrated into the retinal cografts. In three of these animals, transplants had been misplaced into the choroid and showed infiltration of macrophages.
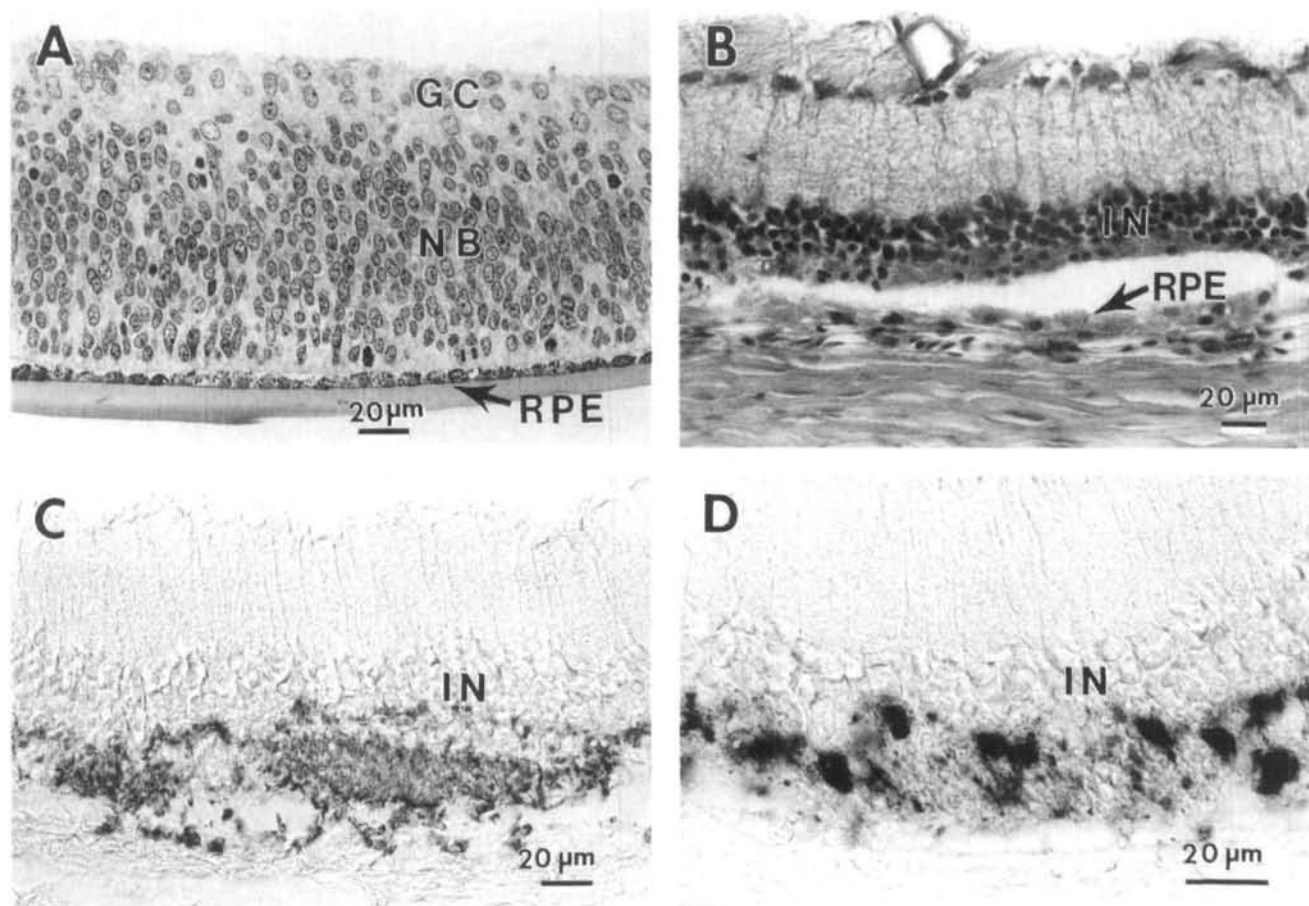
Recipient Retina

At the earliest time of death (host age 3 months), the recipient retina had lost almost all rod photoreceptors (Figs. 1B, 1C, 1D). Although remnants of the outer segment debris stained for rhodopsin, no rhodopsin-stained photoreceptors could be seen (Fig. 1C). The sparse staining for the phototransduction protein S-antigen in the recipient retina revealed only a few remaining cell bodies of photoreceptors, most likely cones (Fig. 1D).

Organization of Successful Cografts

In the four successful transplantations, a full lamination had developed in the neural retinal transplant with parallel retinal layers, including mature photoreceptors in contact with the cotransplanted RPE sheet (Figs. 2A, 2B, 2C). The donor tissue could be unequivocally identified by immunohistochemistry for BrdU. BrdU-labeled cells were seen in all retinal layers (Fig. 2D). In transplanted RPE cells, the BrdU-label was mostly obscured by the pigment and could only be seen in some less pigmented cells (Fig. 2E). Some ganglion or amacrine cells were misplaced in the inner plexiform layer of the transplant (Figs. 2A, 2B). The photoreceptor layer contained 5 to 10 rows of photoreceptor nuclei (Figs. 2A, 2B, 2C). Parallel inner and outer segments developed in transplant photoreceptors in contact with the cotransplanted RPE (enlargements shown in Figs. 2F, 3C, 3D, 4B, 4C, 4D). A sectioning artifact was seen in Figures 2A, 2D, 2E, 2F, 3A, 3B 4A, 4B, 4C, and 4D (breakup of outer segments) which can also occur in sections from normal retina.

Interface between Transplant and Host Retina

The apposition of the transplants toward the host retina varied. In three of four transplants, partial absence of glial barriers and

FIGURE 1.   (A) Donor tissue. Gel-embedded E18 rat retina with RPE. The neural retina contains a ganglion cell layer (GC) and an outer neuroblastic layer (NB). (B, C, D) Recipient RCS rat retina. (B) Central area of recipient retina close to transplant. The inner nuclear layer (IN) is immediately adjacent to the RPE. Hematoxylin-eosin staining. (C) Rhodopsin staining of outer segment debris in the subretinal space. (D) S-antigen staining of cone cell bodies close to the IN and of outer segment debris in the subretinal space.

interdigitation of transplant and host tissue could be seen (Fig. 2B). However, an apparent glial limiting membrane was seen separating part of the transplants from the host retinas (Figs. 2A, 2C). The fourth transplant was completely separated from the host retina by a glial limiting membrane. No remnants of host photoreceptor debris were seen in the transplant area. The neural retinal transplant and the overlaying host retina contained some microglial cells, but no major immune response was seen (Fig. 2B). Some pyknotic ganglion cells were seen in the host retina overlying the transplant (Fig. 2B) but also in other areas.

RPE Cells in Cografts

The cotransplanted RPE sheets had been placed either onto the host Bruch's membrane (Figs. 3A, 3C), or the transplanted RPE cells apparently had produced a second Bruch's membrane on top of presumably remnant host RPE (Figs. 3B, 3D). These cells were suggested to be of host origin because of the intact sheet of pigmented donor RPE overlaying it. The remnant albino host cells had taken up some pigment granules from the donor cells (Fig. 3D). In either case, the transplanted RPE sheet maintained its characteristic monolayer. In one area of a transplant (not shown), the RPE sheet appeared to have folded on itself and formed a double layer. This did not interfere with the full development of the adjoining photoreceptors, although the

outer segments were shorter in one case (Fig. 3D). One transplanted RPE sheet had a hole at the time of implantation. This hole appeared to have been sealed at the time of the animal's death, presumably because of proliferation and/or migration of the transplanted RPE cells.

Photoreceptor Markers in Transplants

As a measure of photoreceptor organization and viability, transplanted photoreceptors were stained for the phototransduction proteins: rhodopsin (Fig. 2F), rod α-transducin (Figs. 4A, 4B), and S-antigen (Fig. 4C). Intense transducin immunoreactivity was seen in inner segments of fully developed photoreceptors organized in parallel layers and in contact with the transplanted RPE (Fig. 4A). The retinal edges of the transplants sometimes formed rosettes (Fig. 4A). In rosettes, photoreceptors had only weak transducin immunoreactivity (Fig. 4A). Cone interphotoreceptor matrix domains were labeled with PNA, suggesting a normal level of RPE–photoreceptor adhesion (Fig. 4D).

DISCUSSION

This study is the first to show that freshly harvested intact sheets of fetal RPE with neural retina can be transplanted to
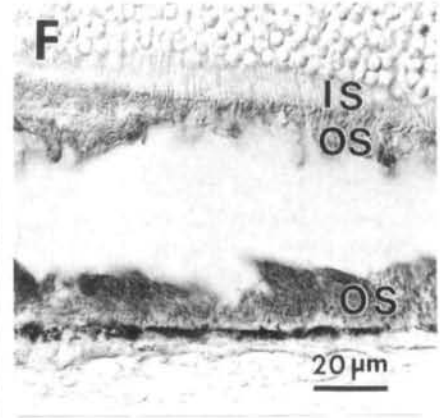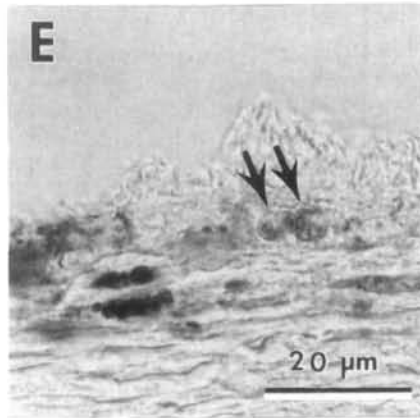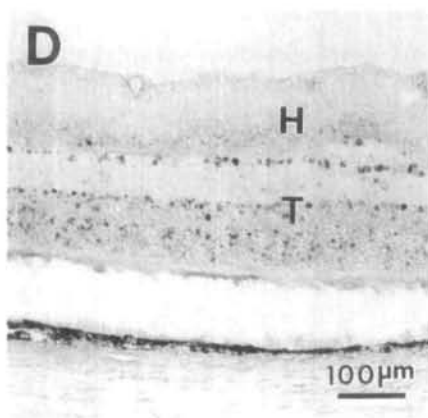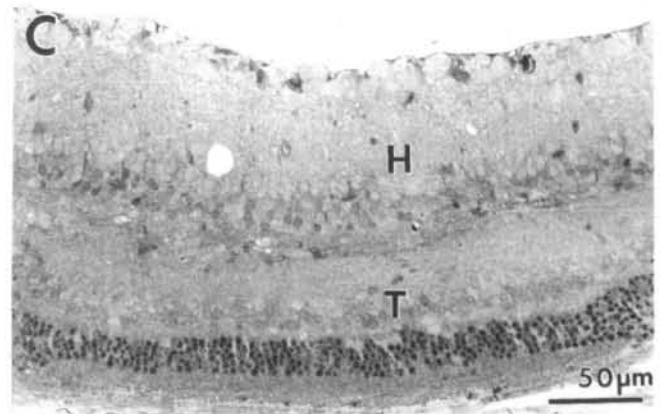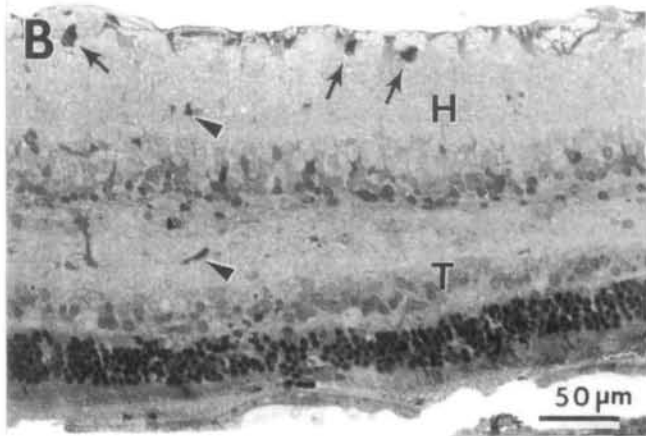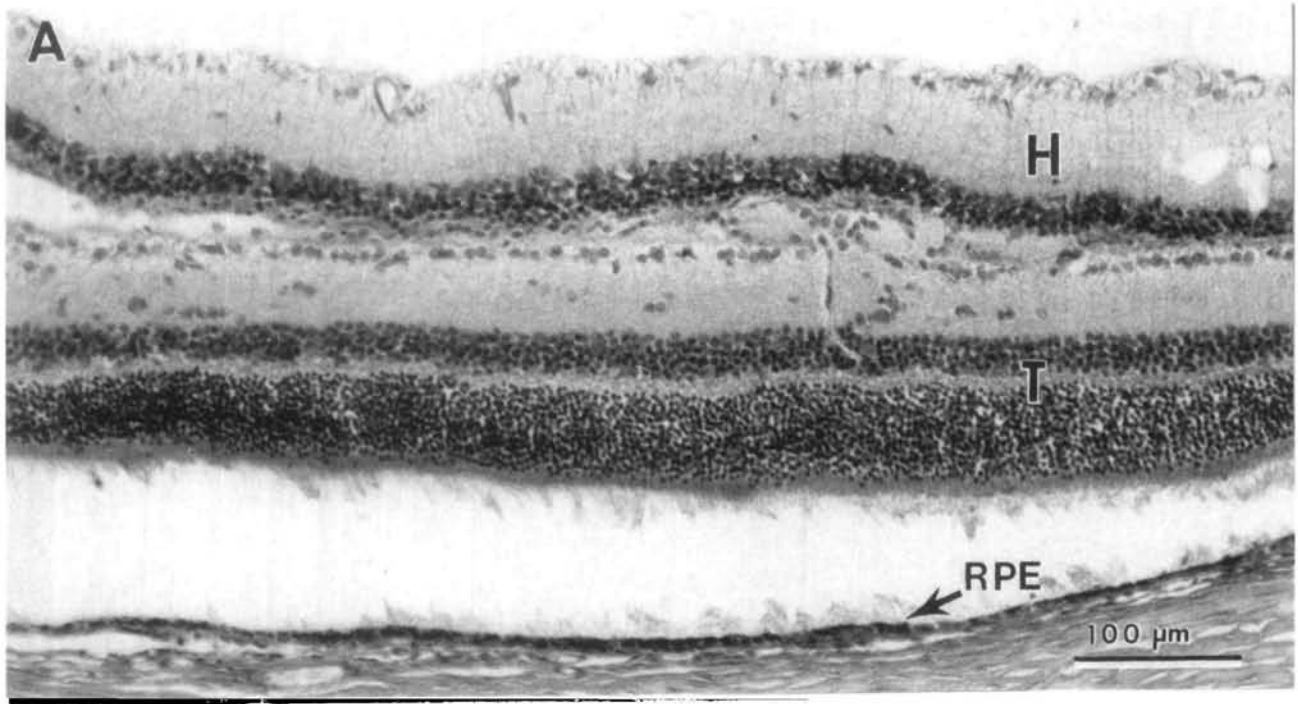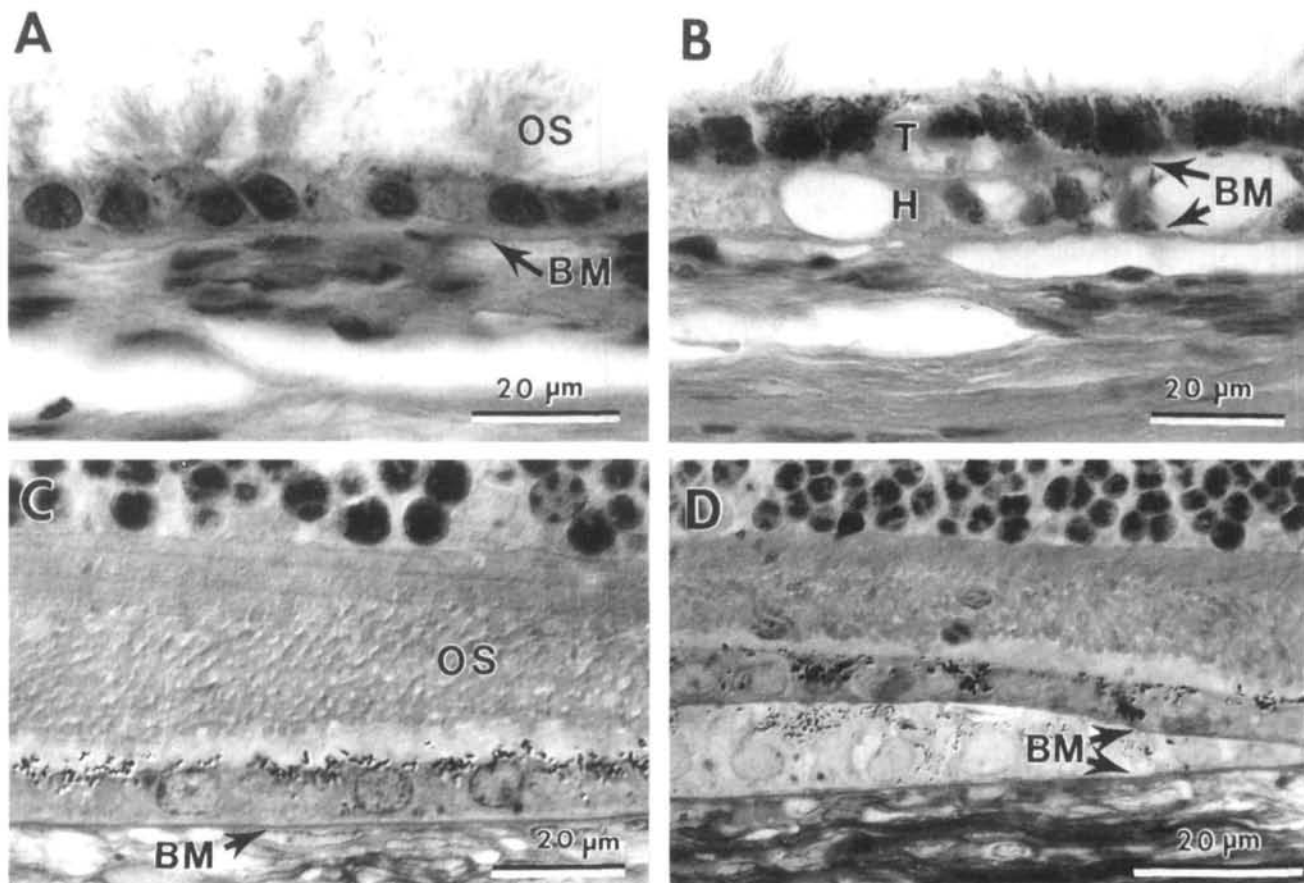
FIGURE 3. Retinal pigment epithelium of retinal cografts. (A, B): Donor age, E18; host age at time of transplantation, 1.8 months; age at time of death, 3.2 months. (C D) Donor age, E20; host age at time of 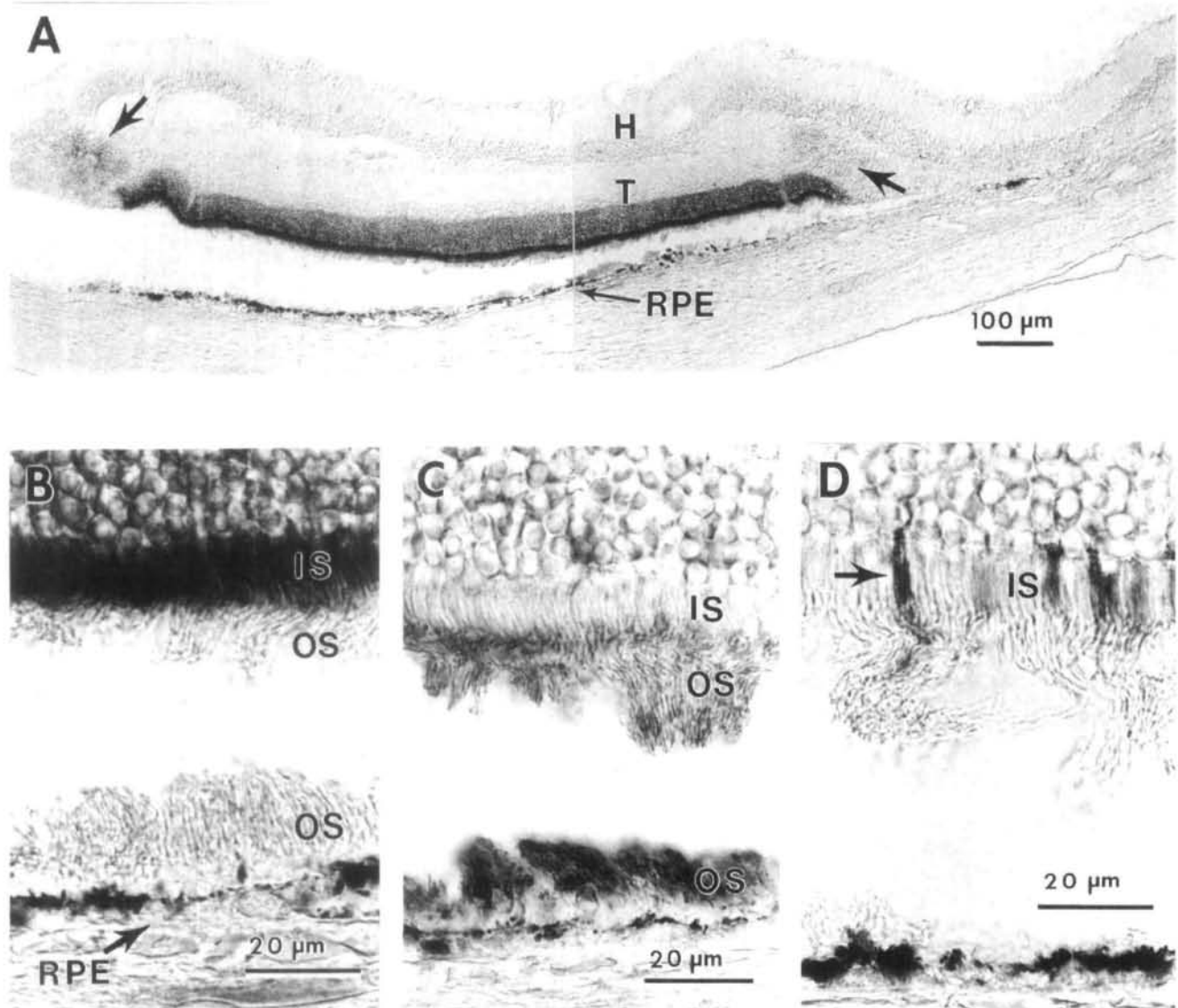transplantation, 2.2 months; age at time of death, 3.9 months. (A, C) Enlargement of cotransplanted RPE monolayer in contact with photoreceptor outer segments (OS). Note the clearly demarcated Bruch's membrane (BM). (B, D) Transplanted pigmented RPE sheet (T) on top of presumable host RPE cells (H). There are two Bruch's membranes (BM). (D) The abnormal-appearing host cells have taken up some pigment.

the subretinal space and survive, with development of an apparently normal cytoarchitecture. Previously, it has been shown that cografted cell aggregates of fetal rabbit neural retina with RPE increases the long-term survival of transplanted photoreceptors.[43] However, the organization of these cell aggregate transplants was not comparable to the results presented here using intact sheets. The transplantation of intact sheets of fetal neural retina alone to adult RCS rats results in rosette formation or in transplanted photoreceptor degeneration (Aramant and Seiler, unpublished observations, 1998), whereas transplantations of intact retinal sheets to light-damaged rats with intact RPE show development of photoreceptors with inner and outer segments in

contact with the host RPE.[28] Using a different approach, another laboratory has recently transplanted "full-thickness" fetal retina to rabbits with normal retinas.[19]

The success rate of our experiments was relatively low (4/11) probably for technical reasons, because the surgeon could not see where the tissue was placed in the small rat eye. In addition, because fetal RPE and retina are extraordinarily loosely attached, delicate dissection and transplantation procedures were required. The implantation would be easier in larger animals. However, in contrast to the RCS rat, photoreceptor degeneration proceeds very slowly in the models available in cats, dogs, and pigs. Such animals are expensive and difficult to maintain, and the number of experiments is limited.

FIGURE 2. Retina-RPE cografts. (A, D, E, F) Donor age, E18; host age at time of transplantation, 1.8 months; age at time of death, 3.2 months. (B, C) Donor age, E20; host age at time of transplantation, 2.2 months; age at time of death, 3.9 months. (A) Transplant (T) of neural retina and retinal pigment epithelium (RPE) in the subretinal space of an albino RCS rat host retina (H). Parallel retinal layers developed in the neural retina transplant. There appears to be a glial barrier between transplant and host retina. No photoreceptor debris zone is seen. The breakup of the outer segments of the transplant photoreceptors is a sectioning artifact. Hematoxylin-eosin stained paraffin section. (B) Example of partial absence of glial barriers between recipient retina and retinal cograft. Note some degenerating host ganglion cells (*arrows*) and the presence of a few microglia (*arrowheads*). Toluidine blue-stained Epon section. Breakup artifact in RPE area. (C) Another area of same transplant as shown in (B). Part of the transplant is separated from the host by a glial barrier. (D) BrdU-labeled donor cells clearly distinguish transplant from host tissue. The donor mother had been injected with BrdU on E13, E14, and E17. Labeled cells can be seen in all retinal layers of the transplant. (E) BrdU-labeled nuclei (*arrows*) provide evidence of donor-derived RPE. (F) Outer segments of transplant photoreceptors stained for rhodopsin.

FIGURE 4. Photoreceptors of retinal cografts. (**A, B, C, D**) Host age at time of transplantation, 1.8 months; age at time of death, 3.2 months. (**A**) Rod α-transducin immunoreactivity is high in the parallel photoreceptor layer of the transplant but very weak in photoreceptor rosettes (*arrows*) at the edges of the transplant. No stain in the host retina. (**B**) Enlargement of (**A**) showing main α-transducin immunoreactivity in rod photoreceptor inner segments. (**C**) Outer segments of transplant rod photoreceptors are immunoreactive for S-antigen. (**D**) Interphotoreceptor matrix surrounding transplant cones (*arrow*) identified with the lectin PNA.

In the present study, the transplanted RPE sheet formed a coherent monolayer that appeared to be continuous with the host RPE at the edges of the transplant area. In contrast, transplantation of injected, dissociated RPE cells often leads to formation of multilayered aggregates that do not show the typical RPE morphology.[7,44] Tight junctions between RPE cells throughout the retina are critical to the function of the outer blood–retinal barrier.[45] Because in vitro studies showed that the RPE in RCS rats is capable of forming tight junctions,[46] it is possible that the RPE of the host RCS rat formed junctions with the edges of the transplanted RPE sheet. This should be investigated by electron microscopy.

In most areas of the transplant, the transplanted RPE cells were directly apposed to the Bruch's membrane adjacent to the host choroid, and no host RPE could be seen. The host RPE cells may have been scraped off during implantation, or they may have died and have been subsequently removed by mac-

rophages. In some areas, abnormal-looking host RPE cells could be seen underneath a second Bruch's membrane formed by the transplanted RPE sheet. Therefore, it appears that a monolayer could develop in the fetal transplanted RPE sheet without removal of the dysfunctional host RPE.

The normal morphology and organized appearance of the transplant photoreceptors in the present study suggests that transplanted RPE sheets support the development and maintenance of photoreceptor outer segments in the retinal cografts, as in a normal retina, by providing a barrier toward the host choroid and transporting nutrients from the choroid to the photoreceptors. Other studies have shown that the RPE is necessary for normal retinal morphogenesis in vivo[47] and influences the lamination of neural retina in vitro by acting on early Müller glial cells through diffusible factors.[48] The present study confirms that RPE influences retinal organization in vivo and suggests that freshly harvested RPE sheet transplants may

have a larger rescuing effect on remaining photoreceptors than injected dissociated RPE cells. In some areas, donor RPE was placed on top of host RPE or had folded on itself. However, despite the increased distance from the host choroidal blood supply, transplant photoreceptors in these areas showed development of outer segments. Electron microscopic analysis is needed for closer examination of transplanted RPE and photoreceptor ultrastructure.

Not only is the RPE supportive to photoreceptors, but RPE cells are also affected by photoreceptor activity. For example, the barrier properties and the polarity of embryonic RPE in vitro are dependent on diffusible factors from the neural retina.[49,50] Therefore, the presence of the cografted neural retina may be important for the development and maintenance of a polarized and functional RPE monolayer.

A healthy RPE is necessary to produce and maintain the integrity of Bruch's membrane. In older patients with eye diseases the diffusion properties of Bruch's membrane are often compromised, resulting in a decreased supply of nutrients to the RPE through Bruch's membrane.[51,52] Transplanted RPE would have to be able to change the properties of the recipient's Bruch's membrane back to a healthy state.

As a measure of photoreceptor organization and viability, phototransduction molecules are often identified. The level of α-transducin in photoreceptors was shown to decrease rapidly after photoreceptor damage, whereas S-antigen and rhodopsin immunoreactivity persisted for 1 to 2 months.[53] In our study, the retinal transplant photoreceptors appeared to have a normal distribution of signal transduction proteins, such as rhodopsin, S-antigen, and transducin, and a normal cone interphotoreceptor matrix as shown by PNA staining. The strong staining for α-transducin in organized transplant photoreceptors suggests a normal phototransduction function. The weak α-transducin staining of photoreceptors in rosettes suggested that photoreceptors in rosettes have no or very low light sensitivity. This confirms our previous results that only the photoreceptors supported by RPE and transplanted as fetal intact retinal sheets contain the α-transducin that is necessary to respond to light (see also Ref. 54).

No immune reaction of the recipient was observed around the cografts of RPE with retina when the transplant had been placed correctly without injuring the host Bruch's membrane. This may be because the subretinal space, like the central nervous system, has been considered an immunologically privileged site; that is, it is partially protected from the peripheral immune system.[55] In contrast to photoreceptors, RPE cells can express major histocompatibility complex (MHC) antigens after transplantation and be rejected slowly when they are mismatched in MHC antigens.[56] However, fetal rat RPE cells may not yet express MHC antigens. During transplantation, junctions between the host RPE cells that form the outer blood-retinal barrier were probably broken (in areas where the host RPE had been removed), thus temporarily exposing the transplant to the peripheral immune system in the choroid. At the edges of the transplant, new junctions had to develop between transplant and host RPE cells to restore the outer blood-retinal barrier. The fact that viable transplants were seen at 43 and 51 days after transplantation indicates that no acute rejection had occurred and the outer blood-retinal barrier was most likely intact. However, the possibility of a slow rejection over a longer time frame cannot be excluded. Delayed or slow rejection has been observed in clinical trials of injection of RPE cells

to patients with macular degeneration.[23,24] Future studies should include longer survival times and tissue processing for electron microscopy.

The main conclusions of our study were: first, it is possible to transplant intact sheets of fetal retina and RPE together to the subretinal space; second, both transplanted tissues, retina and RPE, interact to form histologically normal tissue; and third, in the RCS rat, retinal repair requires the cotransplantation of neural retina with RPE to achieve proper photoreceptor transplant development. This procedure offers hope for persons with retinal diseases that affect both RPE and photoreceptors.

The challenge for the future is to achieve functional transplant-host connections and visual improvement.

Acknowledgments

References

1. Bok D. The retinal pigment epithelium: a versatile partner in vision. *J Cell Sci.* 1993;17(suppl):189-195.
2. Mullen RJ, LaVail MM. Inherited retinal dystrophy: primary defect in pigment epithelium determined with experimental rat chimeras. *Science.* 1976;192:799-801.
3. LaVail MM. Photoreceptor characteristics in congenic strains of RCS rats. *Invest Ophthalmol Vis Sci.* 1981;20:671-675.
4. Segato T, Midena E, Blarzino MC. Age-related macular degeneration. *Aging (Milano).* 1993;5:165-76.
5. Oshinskie LJ. Age-related macular degeneration. *Optom Clin.* 1996;5:25-53.
6. Li L, Turner JE. Inherited retinal dystrophy in the RCS rat: prevention of photoreceptor degeneration by pigment epithelial cell transplantation. *Exp Eye Res.* 1988;47:911-917.
7. Lopez R, Gouras P, Kjeldbye H, et al. Transplanted retinal pigment epithelium modifies the retinal degeneration in the RCS rat. *Invest Ophthalmol Vis Sci.* 1989;30:586-588.
8. Li L, Sheedlo HJ, Turner JE. Long-term rescue of photoreceptor cells in the retinas of dystrophic rats by RPE transplants. *Prog Brain Res.* 1990;82:179-185.
9. Sheng Y, Gouras P, Cao H, et al. Patch transplants of human fetal retinal pigment epithelium in rabbit and monkey retina. *Invest Ophthalmol Vis Sci.* 1995;36:381-390.
10. Little CW, Castillo B, DiLoreto DA, et al. Transplantation of human fetal retinal pigment epithelium rescues photoreceptor cells from degeneration in the Royal College of Surgeons rat retina. *Invest Ophthalmol Vis Sci.* 1996;37:204-211.
11. Silverman MS, Hughes SE. Transplantation of photoreceptors to light-damaged retina. *Invest Ophthalmol Vis Sci.* 1989;30:1684-1690.
12. Aramant R, Seiler M, Ehinger B, et al. Transplantation of human embryonic retina to adult rat retina. *Restorative Neurol Neurosci.* 1990;2:9-22.
13. Aramant R, Seiler M, Ehinger B, Bergström A, Adolph AR, Turner JE. Neuronal markers in rat retinal grafts. *Dev Brain Res.* 1990;53:47-61.
14. Del Cerro M, Ison JR, Bowen GP, Lazar E, Del Cerro C. Intraretinal grafting restores function in light-blinded rats. *Neuroreport.* 1991;2:529-532.
15. Juliusson B, Bergström A, van Veen T, Ehinger B. Cellular organization in retinal transplants using cell suspensions or fragments of embryonic retinal tissue. *Cell Transplant.* 1993;2:411-8.

16. Gouras P, Du J, Kjeldbye H, Yamamoto S, Zack DJ. Long-term photoreceptor transplants in dystrophic and normal mouse retina. *Invest Ophthalmol Vis Sci.* 1994;35:3145-53.

17. Seiler MJ, Aramant RB. Photoreceptor and glial markers in human embryonic retina and in human embryonic retinal transplants to rat retina. *Dev Brain Res.* 1994;80:81-95.

18. Seiler MJ, Aramant RB. Transplantation of embryonic retinal donor cells labelled with BrdU or carrying a genetic marker to adult retina. *Exp Brain Res.* 1995;105:59-66.

19. Ghosh F, Arnér K, Ehinger B. Transplantation of full-thickness embryonic rabbit retina using pars plana vitrectomy. *Retina.* 1998; 18:136-142.

20. Huang JC, Ishida M, Hersh P, Sugino IK, Zarbin MA. Preparation and transplantation of photoreceptor sheets. *Curr Eye Res.* 1998; 17:573-85.

21. LaVail MM, Li L, Turner JE, Yasumura D. Retinal pigment epithelial cell transplantation in RCS rats: normal metabolism in rescued photoreceptors. *Exp Eye Res.* 1992;55:555-562.

22. Yamamoto S, Du J, Gouras P, Kjeldbye H. Retinal pigment epithelial transplants and retinal function in RCS rats. *Invest Ophthalmol Vis Sci.* 1993;34:3068-3075.

23. Algvere P, Berglin L, Gouras P, Sheng Y. Transplantation of fetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularization. *Graefes Arch Clin Exp Ophthalmol.* 1994;232:707-16.

24. Algvere P, Berglin L, Gouras P, Sheng Y, Kopp E. Transplantation of RPE in age-related macular degeneration: observations in disciform lesions and dry RPE atrophy. *Graefes Arch Clin Exp Ophthalmol.* 1997;235:149-58.

25. Aramant RB, Seiler MJ. Organized embryonic retinal transplants to normal or light-damaged rats (*Neuroscience* abstract). *Soc Neurosci Abstr.* 1995;21:1308.

26. Aramant R, Seiler M. Retinal cell transplantation. In: RP Lanza, WL Chick, eds. *1996/1997 Yearbook of Cell and Tissue Transplantation.* Dordrecht, The Netherlands: Kluwer Academic Publishers; 1996:193-201.

27. Aramant RB, Seiler MJ. Transplants to restore damaged rat retinas [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1997;38(4):S332. Abstract nr 1548.

28. Seiler MJ, Aramant RB. Intact sheets of fetal retina transplanted to restore damaged rat retinas. *Invest Ophthalmol Vis Sci.* 1998;39: 2121-2131.

29. Eisenfeld AJ, LaVail MM, LaVail JH. Assessment of possible transneuronal changes in the retina of rats with inherited retinal dystrophy: cell size, number, synapses, and axonal transport by retinal ganglion cells. *J Comp Neurol.* 1984;223:22-34.

30. Papermaster DS, Windle J. Death at an early age: apoptosis in inherited retinal degenerations. *Invest Ophthalmol Vis Sci.* 1995; 36:977-983.

31. Organisciak DT, Winkler BS. Retinal light damage: practical and theoretical considerations. *Prog Retinal Eye Res.* 1994;13:1-29.

32. Nishikawa S, Cao W, Yasumura D, et al. Comparing the ERG to retinal morphology in transgenic rats with inherited degenerations caused by mutants opsin genes [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 1997;38(4):S33. Abstract nr 148.

33. Steinberg RH, Matthes MT, Yasumura D, et al. Slowing by survival factors of inherited retinal degeneration in transgenic rats with mutant opsin genes. [ARVO abstract]. *Invest Ophthalmol Vis Sci.* 1997;38(4):S226. Abstract nr 1069.

34. Widner H, Brundin P. Immunological aspects of grafting in the mammalian central nervous system. A review and speculative synthesis. *Brain Res Rev.* 1988;13:287-324.

35. Dunnett SB. Neural transplantation in animals models of dementia. *Eur J Neurosci.* 1990;2:567-587.

36. Kupsch A, Oertel WH, Earl CD, Sautter J. Neuronal transplantation and neurotrophic factors in the treatment of Parkinson's disease: update February 1995. *J Neural Transm Suppl.* 1995;46:193-207.

37. Aramant RB, Seiler MJ. Fiber and synaptic connections between embryonic retinal transplants and host retina. *Exp Neurol.* 1995; 133:244-55.

38. Aramant RB, Seiler MJ, Ball SL. Intact-sheet fetal cografts of RPE with retina to adult RCS rat retina replace both photoreceptors and RPE (*ICER* abstract). *Exp Eye Res.* 1998;67(suppl. 1):S234. Abstract nr 774.

39. Aramant RB, Seiler MJ, Ball SL. Cotransplanting fetal RPE with retina repairs adult degenerated RCS rat retinas. *Soc Neurosci Abstr.* 1998;24:816. Abstract nr 325.9.

40. Molday RS. Monoclonal antibodies to rhodopsin and other proteins of rod outer segments. *Prog Retinal Eye Res.* 1989;8:173-209.

41. Navon SE, Fung BK-K. Characterization of transducin from bovine retinal rod outer segments. *J Biol Chem.* 1988;263:489-496.

42. Donoso LA, Folberg R, Arbizo V. Retinal S-antigen and retinoblastoma: a monoclonal antibody histopathologic study. *Arch Ophthalmol.* 1985;103:855-857.

43. Seiler MJ, Aramant RB, Bergström A. Co-transplantation of embryonic retina and retinal pigment epithelial cells to rabbit retina. *Curr Eye Res.* 1995;14:199-207.

44. Gouras P, Du J, Kjeldbye H, Yamamoto S, Zack DJ. Reconstruction of degenerate rd mouse retina by transplantation of transgenic mouse photoreceptors. *Invest Ophthalmol Vis Sci.* 1992;33:2579-2586.

45. Rizzolo LJ. Polarity and the development of the outer blood-retinal barrier. *Histol Histopathol.* 1997;12:1057-67.

46. Chang C-W, Defoe DM, Caldwell RB. Retinal pigment epithelium cells from dystrophic rats form normal tight junctions in vitro. *Invest Ophthalmol Vis Sci.* 1997;38:188-195.

47. Raymond SM, Jackson IJ. The retinal pigmented epithelium is required for development and maintenance of the mouse neural retina. *Curr Biol.* 1995;5:1286-1295.

48. Rothermel A, Willbold E, Degrip WJ, Layer PG. Pigmented epithelium induces complete retinal reconstitution from dispersed chick retinae in regaggregation culture. *Proc R Soc Lond B Biol Sci.* 1997;264:1293-1302.

49. Rizzolo LJ, Li Z-Q. Diffusible, retinal factors stimulate the barrier properties of junctional properties in the retinal pigment epithelium. *J Cell Sci.* 1993;106:859-867.

50. Gundersen D, Powell SK, Rodriguez-Boulan E. Apical polarization of N-CAM in retinal pigment epithelium is dependent on contact with the neural retina. *J Cell Biol.* 1993;121:335-343.

51. Starita C, Hussain AA, Marshall J. Decreasing hydraulic conductivity of Bruch's membrane: relevance to photoreceptor survival and lipofuscinoses. *Am J Med Genet.* 1995;57:235-237.

52. Starita C, Hussain AA, Patmore A, Marshall J. Localization of the site of major resistance to fluid transport in Bruch's membrane. *Invest Ophthalmol Vis Sci.* 1997;38:762-767.

53. Mirshahi M, de Kozak Y, Tarraf M, Razaghi A, Thillaye B, Faure J-P. Early disappearance of alpha-transducin immunoreactivity in light-induced photoreceptor degeneration. *Curr Eye Res.* 1991;10:993-1000.

54. Seiler MJ, Aramant RB, Ball SL. Photoreceptor function of retinal transplants implicated by light-dark shift of S-antigen and rod transducin. *Vision Res.* 1999;39:2589-2596.

55. Streilein JW. The privilege of immunity in the eye. *Surv Ophthalmol.* 1991;35:67-73.

56. Zhang X, Bok D. Transplantation of retinal pigment epithelial cells and immune response in the subretinal space. *Invest Ophthalmol Vis Sci.* 1998;39:1021-1027.