Exp. Eye Res. (2002) **75**, 115–125
doi:10.1006/exer.2002.2001, available online at http://www.idealibrary.com on IDEAL

Transplanted Sheets of Human Retina and Retinal Pigment Epithelium Develop Normally in Nude Rats

ROBERT B. ARAMANT[ab] AND MAGDALENE J. SEILER[ab]*

[a]*Departments of Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY, U.S.A. and* [b]*Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY, U.S.A.*

(*Received 26 November 2001 and accepted in revised form 21 February 2002*)

This study investigated whether transplanted sheets of human fetal retina together with its retinal pigment epithelium (RPE) could develop and maintain their cytoarchitecture after long survival times. Transplant recipients were nine albino athymic nu/nu rats with a normal retina. The donor tissue was dissected from fetuses of 12–17 weeks gestational age. Transplants were analyzed at 5–12 months after surgery by light and electron microscopy, and immunohistochemistry with various antibodies specific for rhodopsin, S-antigen, transducin, neurofilament and synaptophysin. In 4 of 11 transplants, the RPE stayed as a monolayer sheet and supported the development of the retinal sheet with a normal lamination, including photoreceptor inner and outer segments. Cones and rods in the organized transplants were labeled with different photoreceptor markers. Inner and outer plexiform layers, containing cone pedicles and rods spherules, were immunoreactive for synaptophysin. As the recipients had a normal retina, transplant/host integration was not expected. However, at the transplant/host interface, there were sometimes areas without glial barriers, and neurofilament-containing processes could be observed crossing between transplant and host. In other, more disorganized transplants, the RPE cells were partially dispersed or clumped together in clusters. Such transplants developed photoreceptors in rosettes, often with inner and outer segments.

In conclusion, sheets of human fetal retina transplanted together with its RPE to the subretinal space of nude rats can develop and maintain perfectly laminated transplants after long survival times, indicating the potential of applying cotransplantation to human patients with retinal diseases.

© 2002 Elsevier Science Ltd.

Key words: retinal transplantation; retinal degeneration; fetal.

1. Introduction

Retinal function depends on the interactions between photoreceptors and retinal pigment epithelium (RPE). For example, the RPE is responsible for daily phagocytosis of shed tips of photoreceptor outer segments (Bok, 1993). In humans, diseases of RPE, such as age-related macular degeneration, also lead to photoreceptor degeneration (Segato et al., 1993; Oshinskie, 1996). In photoreceptor diseases, such as in retinitis pigmentosa, degeneration of rod photoreceptors leads to degeneration of RPE (Milam et al., 1998). Inability of RPE cells to phagocytize the shed photoreceptor outer segment tips, such as in the Royal College of Surgeons (RCS) rat mutant leads to accumulation of outer segment debris and subsequent photoreceptor degeneration (LaVail, 1981; D'Cruz et al., 2000).

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 (Li and Turner, 1988; Lopez et al., 1989; Sheng et al., 1995; Little et al., 1996) or neural retina separately (Silverman and Hughes, 1989; Aramant et al., 1990a,b; Del Cerro et al., 1991; Gouras et al., 1994; Seiler and Aramant, 1994, 1995, 1998; Ghosh et al., 1998; Huang et al., 1998; 1998; Kwan et al., 1999). Based on experiments showing that photoreceptors in the RCS rat can be rescued by injecting freshly harvested RPE cells (Li and Turner, 1988; Lopez et al., 1989; Li et al., 1990; LaVail et al., 1992; Yamamoto et al., 1993), clinical trials of RPE transplants have been performed in macular degeneration patients (Algvere et al., 1994, 1997). However, such transplants can only be effective if the diseased retina still contains some remaining photoreceptors that are not already committed to cell death. For example, photoreceptors in the RCS rat can be rescued by RPE transplants if the surgery is performed at an age of 28 days, but not at 38 days (Li and Turner, 1991). When the photoreceptors have irreversibly degenerated at later stages of the disease, transplantation of either RPE or photoreceptors alone will have no effect. Therefore, in some diseases or stages of disease, retinal repair may require the transplantation of both RPE and

* Address correspondence to: Magdalene J. Seiler, Department of Ophthalmology & Visual Sciences, University of Louisville, School of Medicine, 301 E. Muhammad Ali Blvd., Louisville, KY 40202, U.S.A. E-mail: magdalene.seiler@louisville.edu

0014-4835/02/$35.00/0 © 2002 Elsevier Science Ltd.

neural retina. Another precondition for a functional transplant is that the inner retina of the recipient be still intact (Santos et al., 1997; Milam et al., 1998; Humayun et al., 1999) and able to connect either directly or indirectly to the transplant photoreceptors.

A unique method has been developed to transplant intact sheets of fetal donor tissue in rats (Aramant and Seiler, 1995, 2002; Seiler and Aramant, 1998; Aramant et al., 1999). The procedure involves a special technique of embedding and flattening the tissue without touching it, and the use of a custom-made implantation instrument to place the tissue with minimal trauma into the subretinal space. Provided the retinal fetal sheets are placed in the correct polarity, such transplants can morphologically repair an area of a damaged retina by developing a parallel photoreceptor layer with outer segments in contact with the host RPE, and can become integrated with the host retina (Seiler and Aramant, 1998). However, transplant photoreceptors still depend on contact with functional RPE to develop fully and maintain outer segments (Aramant and Seiler, 1995; Seiler and Aramant, 1998). Recently, it has been shown that intact sheets of retina with RPE can be transplanted to and develop normally in the RCS rat at an age when photoreceptors can no longer be rescued by RPE transplants (Aramant et al., 1999). Because of the small eye and the large lens in the rat, the implantation of intact-retina/RPE sheets into the subretinal space is very delicate.

The purpose of this study was to investigate whether transplanted sheets of human fetal retina with RPE can be maintained as sheets and develop normal lamination, as has been previously shown in the RCS rat (Aramant et al., 1999). The ultrastructural development of human retina (Hollenberg and Spira, 1973; Spira and Hollenberg, 1973; Rhodes, 1984; Johnson et al., 1985; Linberg and Fisher, 1990; Van Driel et al., 1990; Narayanan and Wadhwa, 1998) and of human fetal aggregate transplants (Ehinger et al., 1991) has been described in relatively few publications, so this would also give the opportunity to study the ultrastructure of the human retina at this early stage of development.

Transplant recipients were athymic nude rats, which have been used previously to study the development of human fetal aggregate transplants (Aramant and Seiler, 1994). Since these rats have no T-cells, they can tolerate xenografts without immunosuppression. Since nude rats cannot be effectively light damaged (unpublished observations), transplants were performed in nude rats with normal retina.

Part of this study has been published as meeting abstracts (Seiler and Aramant, 2000a,b).

2. Material and Methods

Experimental Animals

Nine athymic nu/nu albino rats were obtained from Taconics (Germantown, NY, U.S.A.) at the age of 5 weeks. They were housed in autoclaved microisolator cages with autoclaved food and water. The surgery was performed in a sterile environment in a 'bio-bubble' (Bio Bubble Inc., Ft. Collins, CO, U.S.A.). Animals were 64–86 days of age at the time of surgery. All animals were treated according to the regulations in the ARVO and NIH guidelines. An overview of the experiments is shown in Table I.

Donor Tissue

Human fetal tissues derived from elective abortions were collected after donor consent in accordance with a protocol approved by the Human Studies Committee, University of Louisville, following the procedure of the protocol of Helsinki. Donor eyes from three human fetuses of 12, 14 and 17 weeks gestational age were incubated in dispase for 30 min at 37°C to enable the RPE and retina to be dissected free from surrounding tissues. The donor tissue was protected during transplantation by a 4 % MVG alginate gel (Pronova, Oslo, Norway). An example of the histology of an embedded retina with RPE is shown in Fig. 1(A) and (B). Before

TABLE I

Overview of experiments

Donor age (weeks post-conception)	Animals	Eyes with transplant surgery	Survival time after transplant surgery (weeks)	Laminated transplants		Disorganized transplants
				Large area	Small area	
12	4	5*	42–50	2	1	2
14	3	3	23–38	1	1	1
17	2	3*	37	1	–	2
Total	9	11	ave. 39·5 ± 8 weeks	4	2	5

*Some animals received transplants in both eyes.

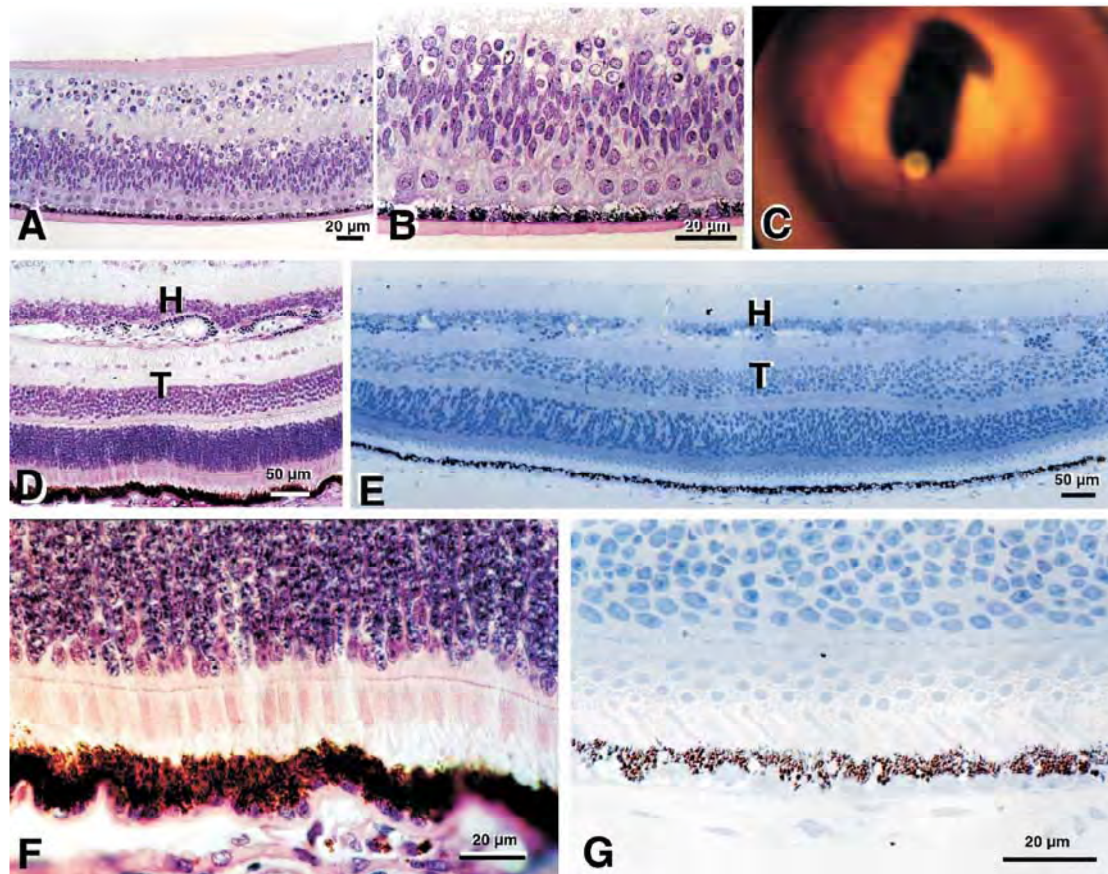

FIG. 1. Donor tissue and morphology of laminated transplants. (A, B) Fetal human retina, 13 weeks gestation, with RPE. (C) Pigmented transplant in the back of an albino nude rat eye, 3 months after surgery. Fundus photograph. This transplant showed a good lamination when killed at 8·9 months after surgery (see Fig. 1(E)). (D–G) Histology of laminated transplants. Two examples. (D) donor 17 weeks, 8·5 months after transplantation; (E) donor 14 weeks, 8·9 months after transplantation. (F,G) Enlargements. Bars: (A,B,F,G) = 20 μm, (D,E) = 50 μm.

transplantation, a small piece (in average 0·7 × 1·2 mm) was cut out, and taken up in a custom-made implantation tool (Aramant and Seiler, 2002). The donor tissue was taken from different areas of the fetal retina. In general, the macula region could not be dissected because the RPE easily detached in this area.

Transplantation Procedure

Rats were anesthetized by intraperitoneal injection of ketamine/xylazine in sterile saline (37·5 mg kg^{-1} ketamine and 5 mg kg^{-1} xylazine), and their pupils dilated by topical application of 1 % atropine sulfate. A small incision (approximately 1 mm) was cut behind the pars plana of the host eye. Using a custom-made implantation tool as described previously (Seiler and Aramant, 1998; Aramant et al., 1999; Aramant and Seiler, 2002), the transplant was placed into the subretinal space, in the superior nasal quadrant of the host retina. Transplant placement was verified by an eye exam immediately following the surgery. Eye exams were also performed at several time points after surgery (example in Fig. 1(C)).

Tissue Processing

Rats were perfusion-fixed with 4 % paraformaldehyde/0·4 % glutaraldehyde in 0·1 M Na–phosphate buffer. The retinal area containing the transplant was either embedded in 5 % agar and cut on a vibratome at 80 μm, embedded in paraffin and cut at 5 μm, or cryoprotected overnight with 30 % sucrose and cut on a cryostat at 15 μm. Selected vibratome sections were osmicated and flat embedded in epon.

Immunohistochemistry and PNA Staining

Sections were blocked in 20 % horse serum. Monoclonal antibodies used: rhodopsin (clone rho1D4 1 : 50; R. Molday, Vancouver, BC, Canada), S-antigen (clone A9C6 1 : 20 000; Donoso et al., 1985), rod α-transducin (clone TF 15, 1 : 20 000) and γ-transducin (clone TF 28, 1 : 20 000) (Navon and Fung, 1988), neurofilament 68 kDa (1 : 20 000) (ICN), synaptophysin (1 : 100 000) (Sigma), microtubule-associated protein 1A (MAP 1A) (1 : 20 000) (Amersham), human nuclei (1 : 10) (MAB1281, Chemicon; Vescovi et al., 1999), and human neurofilament 70 kDa (1 : 50) (MAB5294-30UL, Chemicon; Julien et al.,

1987). Most sections were incubated in primary antibody overnight at 4°C. The primary antibody was detected using the Vector Elite ABC kit for mouse antibodies (Vector Laboratories, Burlingame, CA, U.S.A.) and DAB; or with a fluorescent secondary antibody (AF488-anti-mouse IgG; Molecular Probes). As a control, the primary antibody was omitted.

On paraffin sections, biotinylated peanut agglutinin (PNA) lectin (Vector Laboratories, Burlingame, CA, U.S.A. 1:1000) was used to detect cone interphotoreceptor matrix. It was detected by the Vector Elite ABC (Vector Laboratories, Burlingame, CA, U.S.A.) and DAB.

3. Results

All 11 transplants survived. In 4 of 11 experiments, the RPE stayed as a monolayer sheet and supported the development of the retinal sheet with a normal lamination, including photoreceptor inner and outer segments (see Table I; Fig. 1(D)–(G)). Often, the host RPE could not be seen in the transplant area. A very small laminated area, surrounded by rosettes on both sides, was seen in two additional transplants. In the other transplants, the RPE cells were partially dispersed or clumped together in clusters. Such transplants developed photoreceptors in rosettes, often with inner and outer segments, but without contact to RPE cells. Most photoreceptors of the host retina overlaying the transplants had degenerated because of the separation from the host RPE cells by the transplant (Fig. 1(D) and (E)).

Photoreceptor Markers

Photoreceptors in well-laminated areas stained for the rod markers rhodopsin, S-antigen, and rod α- and γ-transducin (Fig. 2(A)–(D)). Transducin immunoreactivity was much lower in photoreceptors arranged in rosettes (data not shown). PNA labeled the cone inter-photoreceptor matrix (Fig. 2(E)). Profiles of cones were prominent in the transplant.

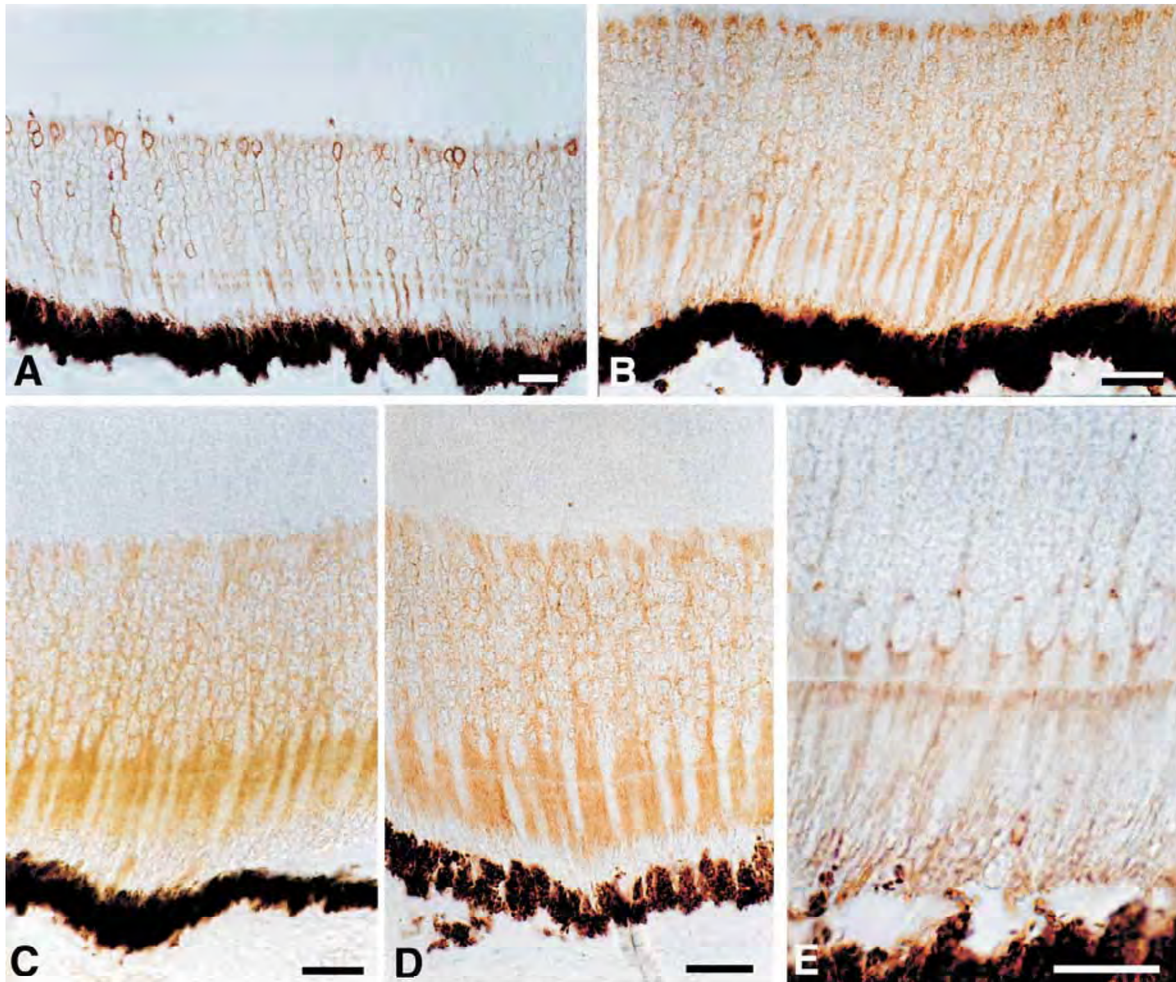

FIG. 2. Photoreceptor markers. Same transplant as in Fig. 1(D). Relatively short outer segments. (A) Rhodopsin (rho1D4). Label of rod cell bodies and outer segments. (B) S-antigen (A9C6). Label of rod terminals in the outer plexiform layer, and of outer segments. Rod cell bodies are more faintly labeled. (C) Rod α-transducin (TF15), (D) Rod γ-transducin (TF28): main immunoreactivity in inner segments; note the unstained cones. (E) PNA labeled cone interphotoreceptor matrix. Bars = 20 μm.

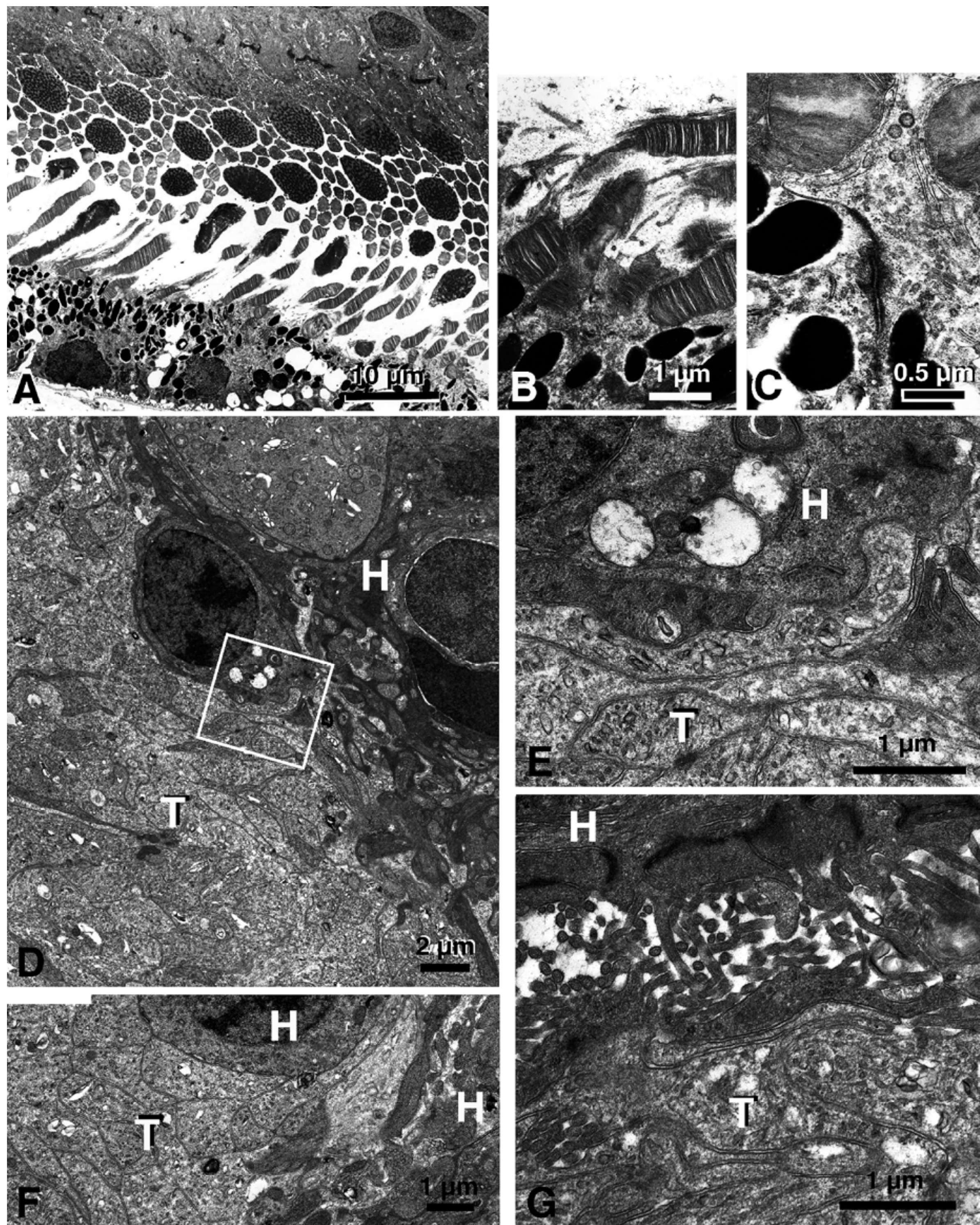

FIG. 3. Electron microscopy of cografts. Same transplant as in Fig. 1(E). (A–C): Transplant photoreceptors and RPE. (A) Low-power electron micrograph showing the subretinal space of the transplant. This section has been cut tangentially. Note the large cone inner segments interspersed with rod inner and outer segments, and relatively short outer segments contacting the heavily pigmented transplant RPE. (B) Outer segments in contact with RPE. (C) Junctional complex between cotransplanted RPE cells. (D–G): Interface between human transplant (T) and rat host (H). (D) In this area, the host photoreceptors have degenerated. Host processes appear more electron-dense than transplant processes. No glial barrier recognizable at this magnification. One host cone is adjacent to the inner plexiform layer of the transplant. Area in box is enlarged in (E). (E) Contact area between host cone (H) and transplant processes (T). (F) Another adjacent area showing integration. (G) Host separated from transplant by a glial barrier formed by host Müller cells. Note the adherent junctions of the host outer limiting membrane, and Müller cell microvilli in the space between transplant and host. Bars: (A) = 10 µm, (B, E–G) = 1 µm, (C) = 0·5 µm, (D) = 2 µm.

Cotransplanted RPE in Contact With Outer Segments of Transplant Rods and Cones

In the well-laminated areas (Fig. 3(A)), the cotransplanted RPE were in apparently normal contact with transplant photoreceptor outer segments (Fig. 3(B)) and were maintained as a monolayer with epithelial characteristics and tight junctional complexes (Fig. 3(C)).

Synaptic Layers

Synaptic layers of transplant and host were immunoreactive for synaptophysin and MAP 1A (Fig. 4(A)–(C)). Cone pedicles stained well for synaptophysin in vibratome sections. Normal synaptic structures with ribbon synapses were observed in the inner plexiform layer of the transplant (Fig. 4(D)).

Transplant/Host Integration

Because the recipient's retina was normal at the time of surgery, integration between transplant and host was not expected to occur. In most cases, the transplants appeared to be separated from the host retina (example in Figs. 1(D) and 3(G)). In such areas, the host outer limiting membrane was still maintained, and Müller cells microvilli filled the space between transplant and host (Fig. 3(G)). However,

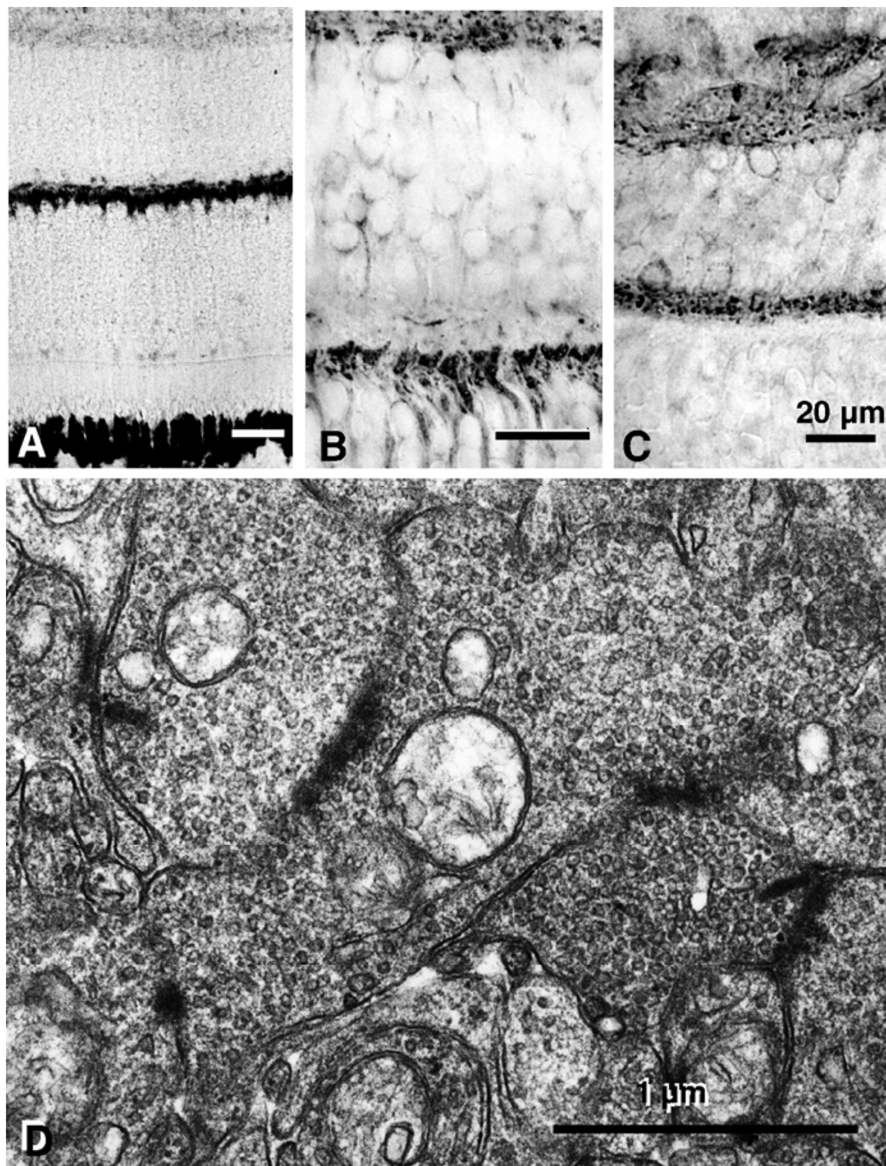

FIG. 4. Synaptic layers. (A,B) Synaptophysin, a marker for synapses. (A) Outer plexiform layer heavily labelled. Paraffin section. (B) Inner and outer plexiform layers are well labeled. Individual cone pedicles are distinctly outlined. Vibratome section. (C) Microtubule-associated protein (MAP) 1A. Vibratome section. Label of plexiform layers and cell bodies of horizontal, amacrine and ganglion cells. There was less immunoreactivity in paraffin sections. (D) Ribbon synapses of bipolar cells in inner plexiform layer. Bars: (A–C) = 20 µm, (D) = 1 µm.

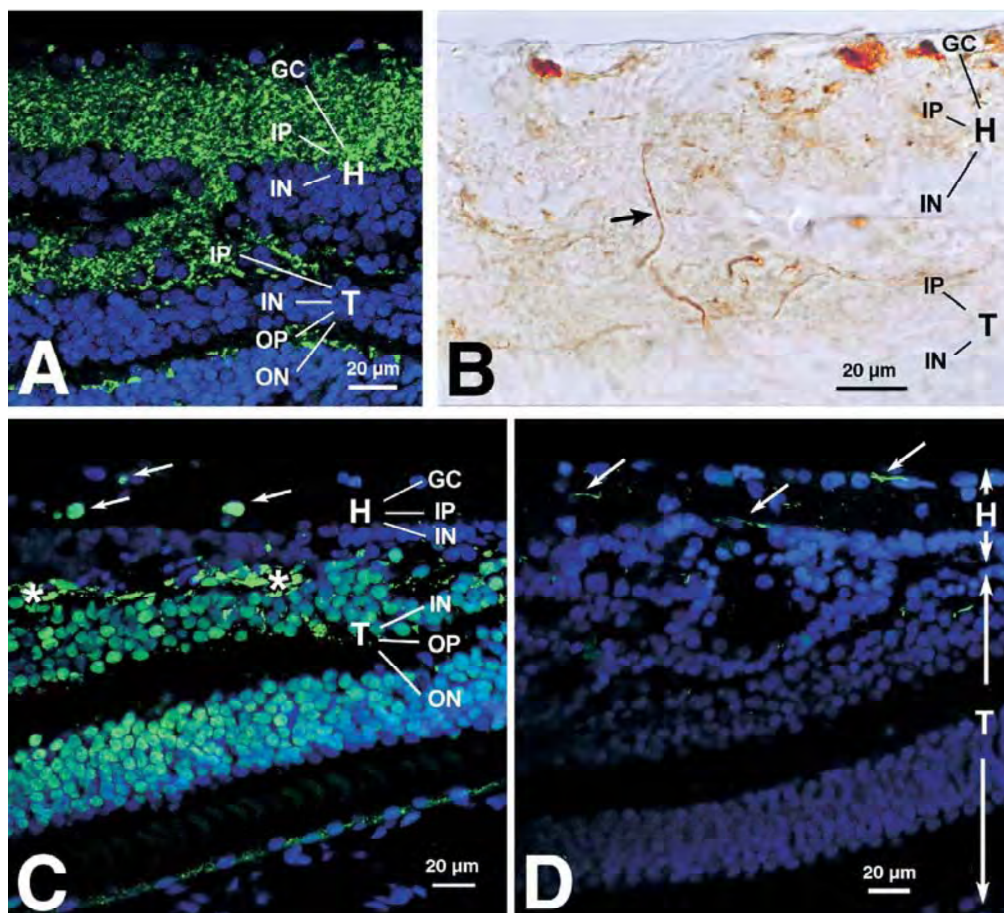

FIG. 5. Connectivity and integration. (A) Synaptophysin (green), DAPI counterstain (blue) for nuclei. Continuous plexiform layers between transplant and host. 8 μm projection of confocal scan. (B) Neurofilament 68 kDa labeled process (arrow) between transplant (T) and host (H). (C) Immunohistochemistry with monoclonal antibody for human nuclei. Several nuclei of human transplant cells (arrows) can be seen in the rat host retina. Asterisks: autofluorescent nuclei of degenerated host cells (non-specific fluorescence). 8 μm projection of confocal scan. (D) Immunohistochemistry with antibody for human neurofilament. 7.4 μm projection of confocal scan. Bars:(A,C,D) = 20 μm. Donor 12 weeks, 11.7 months after transplantation. (B) Same transplant as in Fig. 1(E): donor 14 weeks, 8.9 months after transplantation. Abbreviations: GC = ganglion cell layer; IP = inner plexiform layer; IN = inner nuclear layer; OP = outer plexiform layer; ON = outer nuclear layer.

occasionally, areas of the plexiform layers of the transplant appeared to fuse with the host (Figs. 1(E), 3(D)–(F) and 5(A)). In the electron microscope, host processes appeared more electron dense than transplant processes at the transplant/host interface (Fig. 3(D)–(F)). Neurofilament immunoreactive processes could be observed passing between transplant and host (Fig. 5(B)). Using human-specific antibodies that do not crossreact with rat tissue, it was possible to observe nuclei of transplant cells (Fig. 5(C)) and transplant-derived neuronal processes, immunoreactive for human neurofilament, in the host retina (Fig. 5(D)).

Photoreceptor Synapses

Normally appearing cone pedicles and rod spherules were observed in the outer plexiform layer of the transplant (Fig. 6(A)–(D)). The morphology of the outer plexiform layer was distinctively different from the outer plexiform layer of the host retina outside the transplant area (data not shown).

4. Discussion

The present study shows for the first time that sheets of human fetal neural retina together with its RPE can develop laminated transplants in the subretinal space. The recipients were normal, albino nude rats.

Our laboratory has consistently used fresh fetal donor tissue (reviewed in Aramant and Seiler, 2002). Fetal or embryonic tissue has a high capacity to develop different cell types, sprout neuronal processes, and produce trophic factors. Fetal retinal tissue is likely less immunogenic than adult tissue because it contains fewer microglia than older tissue (Ashwell et al., 1989; Provis et al., 1997). Many studies

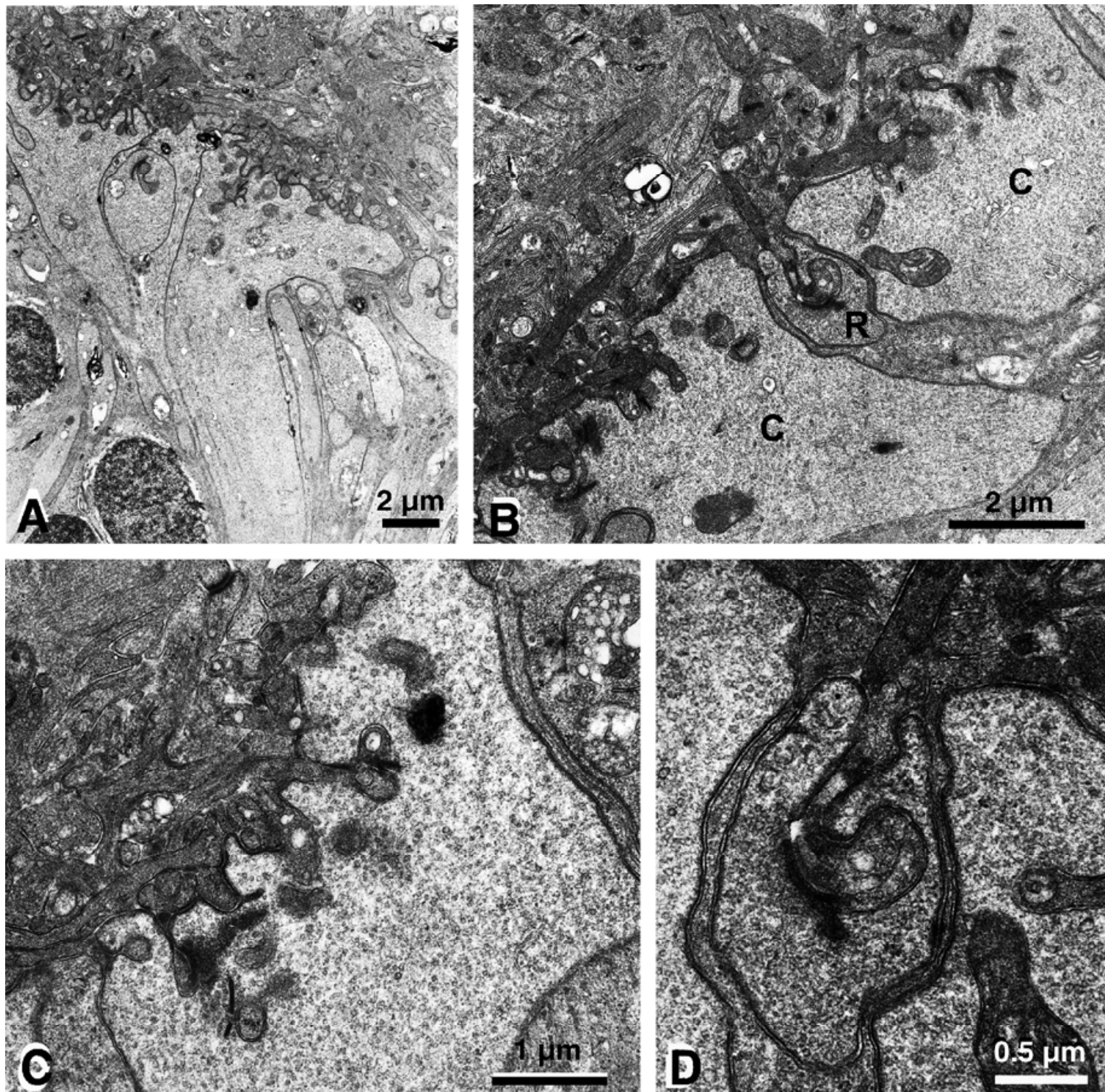

FIG. 6. Photoreceptor synapses in transplant. (A) Outer plexiform layer. (B) Two cone pedicles (C) and rod spherule (R). (C) Cone pedicle. (D) Rod spherule [enlargement of (B)]. Bars: (A,B) = 2 μm, (C) = 1 μm, (D) = 0·5 μm.

indicate that transplants of dissociated RPE cells to the subretinal space undergo chronic rejection (Algvere et al., 1999) and express MHC class I and II antigens after transplantation (Zhang and Bok, 1998). However, transplantation of intact sheets might have immunological advantages: allografted sheets of RPE, in contrast to dissociated cells, have been shown to be immunologically privileged — they are not rejected when transplanted to the kidney capsule (Wenkel and Streilein, 2000). Postnatal retinal tissue, however, was rejected.

Another group has also transplanted fetal retinal sheets (without RPE) to the subretinal space (Ghosh et al., 1998,1999b; Ghosh and Ehinger, 2000) using a different procedure. They used the rabbit as a host animal, which unfortunately does not provide a model of retinal degeneration. Since it is not vascularized, the rabbit host retina overlying the transplant almost completely degenerates with longer survival times in contrast to normal or degenerated rat hosts (Aramant and Seiler, 2002).

The preparation of human fetal cografts was simpler than the preparation of rat cografts in that the cells were easier to manipulate and to dissect. The transplants could be seen well in eye exams because of the contrast between the intensive pigmentation of the donor RPE sheet and the albino host eye. The transplants appeared to increase in size in the first months after transplantation, and grew much larger than corresponding transplants of fetal rat retina.

However, only 4 of 11 transplants developed large areas of lamination whereas the other transplants developed mostly rosettes, and the RPE sheet had dissolved. The reason for this might be the technical difficulty of performing surgery in the small rat eye. If the tissue is inserted in the wrong angle, damage to the

Bruch's membrane might occur. Although nude rats do not have T-cells, exposure to the choroid will lead to invasion of macrophages (data not shown). The donor RPE sheet is very fragile, and can loosen easily since the fetal retina has not yet developed outer segments. There appeared to be a storage time limit of ca. 6 hr for successfully dissecting retina/RPE sheets.

In the cases of well-laminated transplants, the donor RPE appeared to be placed directly on Bruch's membrane. In most cases, the host RPE could not be seen in the transplant area. They might have been scraped off during surgery by the implantation instrument, or degenerated after surgery. The present study suggests that the transplanted RPE sheet and the transplanted photoreceptors mutually benefit from each other. Electron microscopical analysis suggested normal interactions between transplant photoreceptors and transplant RPE. The morphologically organized appearance of the transplant photoreceptors suggests that the transplant RPE cells supported the transplant photoreceptors as in a normal retina by providing a barrier towards the host choroid and transporting nutrients from the choroid to the photoreceptors. On the other hand, the presence of organized photoreceptors in the transplant appeared to support the maintenance and maturation of the cotransplanted RPE sheet. Other studies have shown that the RPE is necessary for normal retinal morphogenesis in vivo (Raymond and Jackson, 1995) and influences the lamination of neural retina in vitro by acting on early Müller glial cells via diffusible factors (Rothermel et al., 1997).

Phototransduction molecules have often been used as an indication of photoreceptor organization and viability. The level of α-transducin in photoreceptors has been shown to decrease rapidly after photoreceptor damage, whereas S-antigen and rhodopsin immunoreactivity persisted for 1–2 months (Mirshahi et al., 1991). In the present 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. Remarkably, the antibody concentration needed for demonstration of rod α-transducin immunoreactivity (TF 15, 1:20 000) corresponded to the immunoreactivity of a normal rat retina and of rat cografts of retina with RPE (Aramant et al., 1999). On the other hand, in human fetal aggregate transplants, a 200–400 times higher antibody concentration (1:50–1:100) was needed to demonstrate transducin immunoreactivity (Seiler and Aramant, 1994). This suggests a normal phototransduction function in photoreceptors of laminated transplants (see also Seiler et al., 1999). Photoreceptor morphology also appeared to be relatively normal at the electron microscopic level. In comparison to human fetal aggregate transplants (Aramant et al., 1990b; Ehinger et al., 1991; Aramant and Seiler, 1994; Seiler and Aramant, 1994), photoreceptor inner and outer segments appeared to be better developed in the laminated transplants in this study.

The donor tissue had been transplanted to a normal retina with initially intact photoreceptors. In the rat host, as long as there are some rows of photoreceptors with an outer limiting membrane present as a barrier, less connectivity is expected between transplant and host than when fetal retina is transplanted to a host with retinal degeneration (Seiler and Aramant, 1998; Aramant et al., 1999; and unpublished work). Interestingly, Ghosh et al. (1999a) reported connectivity between long-term laminated embryonic rabbit retinal transplants (sheets, however without RPE) to normal rabbit host retina and observed sprouting of PKC and parvalbumin-immunoreactive cells into an intermediate plexiform layer between transplant and host. In our study, areas of transplant/host 'integration' could occasionally be observed, i.e. an absence of apparent glial barriers with intermingling of transplant and host processes seen at the EM level. In addition, donor cells could be observed in the inner plexiform and ganglion cell layer of the host. It is unclear, however, whether the transplant cells had been mixed with host tissue at the time of surgery, or whether the cells had actively migrated into the host retina. In areas where host photoreceptors had not completely degenerated due to the retinal detachment, the transplants appeared to be separated from the host by a glial barrier formed by host Müller cells, similar to what has been shown for microaggregate retinal transplants to *rd* mice (Gouras et al., 1994).

As the human donor tissue was transplanted to a normal retina, testing of function of these transplants was outside the scope of this study. To test the transplant function, it would have been necessary to use recipients with retinal degeneration. Since nude rats appear to be more resistant to light damage than normal albino rats (data not shown), nude rats with normal retinas were used. Since there is a time window for successful transplants after light damage (Seiler and Aramant, 1998; Seiler et al., 2000), the unpredictable availability of the donor tissue would also have been a problem. An alternative, to create a transgenic nude rat with retinal degeneration, was outside the scope of this study, but might be useful in the future. One problem would be the unpredictability of the time point when human donor tissue can be obtained because transplantation in models of retinal degeneration has to be done before the degeneration has progressed too far.

In summary, it is possible to transplant sheets of human fetal retina together with its RPE to the subretinal space of athymic nude rats, and to achieve long-term transplants with normal appearing lamination. This study is one of the steps towards clinical trials of human fetal cografts in patients with retinal degeneration (Radtke et al., 2002).

Acknowledgements

Supported by NIH grant EY08519; the Foundation Fighting Blindness; the Murray Foundation Inc., New York; the Vitreoretinal Research Foundation, Louisville, KY; The Kentucky Lions Eye Foundation; an unrestricted grant from the Research to Prevent Blindness; and funds from an anonymous sponsor. The authors have a proprietary interest in the implantation instrument and method. The authors wish to thank Barbara Kalinowska, Kelly Volk, Lyndsay Tucker, Miranda Messer and Bridgette Boggess for technical assistance. The authors thank Lucinda Schultz, University of Louisville, and Mary Gail Engel, University of Kentucky, Lexington, KY, U.S.A. for cutting ultrathin sections.

References

Algvere, P., Berglin, L., Gouras, P. and Sheng, Y. (1994). Transplantation of fetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularization. *Graefe's Arch. Clin. Exp. Ophthalmol.* **232**, 707–16.

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

Algvere, P. V., Gouras, P. and Dafgard Kopp, E. (1999). Long-term outcome of RPE allografts in non-immunosuppressed patients with AMD. *Eur. J. Ophthalmol.* **9**, 217–30.

Aramant, R. B. and Seiler, M. J. (1994). Human embryonic retinal cell transplants in athymic immunodeficient rat hosts. *Cell Transplant.* **3**, 461–74.

Aramant, R. B. and Seiler, M. J. (1995). Organized embryonic retinal transplants to normal or light-damaged rats. [Neuroscience Abstract]. *Soc. Neurosci. Abstr.* **21**, 1308.

Aramant, R. B. and Seiler, M. J. (2002). Retinal transplantation — advantages of intact fetal sheets. *Prog. Retin. Eye Res.* **21**, 57–73.

Aramant, R. B., Seiler, M. J. and Ball, S. L. (1999). Successful cotransplantation of intact sheets of fetal retinal pigment epithelium with retina. *Invest. Ophthalmol. Vis. Sci.* **40**, 1557–64.

Aramant, R. B., Seiler, M. J., Ehinger, B., Bergström, A., Adolph, A. R. and Turner, J. E. (1990a). Neuronal markers in rat retinal grafts. *Dev. Brain. Res.* **53**, 47–61.

Aramant, R. B., Seiler, M. J., Ehinger, B., Bergström, A, Gustavii, B., Brundin, P. and Adolph, A. R. (1990b). Transplantation of human embryonic retina to adult rat retina. *Restor. Neurol. Neurosci.* **2**, 9–22.

Ashwell, K. W., Hollander, H., Streit, W. and Stone, J. (1989). The appearance and distribution of microglia in the developing retina of the rat. *Vis. Neurosci.* **2**, 437–48.

Bok, D. (1993). The retinal pigment epithelium: a versatile partner in vision. *J. Cell Sci.* (Suppl.): **17**, 189–95.

D'Cruz, P. M., Yasumura, D., Weir, J., Matthes, M. T., Abderrahim, H., LaVail, M. M. and Vollrath, D. (2000). Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. *Hum. Mol. Genet.* **9**, 645–51.

Del Cerro, M., Ison, J. R., Bowen, G. P., Lazar, E. and Del Cerro, C. (1991). Intraretinal grafting restores function in light-blinded rats. *Neuroreport* **2**, 529–32.

Donoso, L. A., Folberg, R. and Arbizo, V. (1985). Retinal S-antigen and retinoblastoma — A monoclonal antibody histopathologic study. *Arch. Ophthalmol.* **103**, 855–7.

Ehinger, B., Bergstrom, A., Seiler, M. J., Aramant, R. B., Zucker, C. L., Gustavii, B. and Adolph, A. R. (1991). Ultrastructure of human retinal cell transplants with long survival times in rats. *Exp. Eye Res.* **53**, 447–60.

Ghosh, F., Arnér, K. and Ehinger, B. (1998). Transplantation of full-thickness embryonic rabbit retina using pars plana vitrectomy. *Retina* **18**, 136–42.

Ghosh, F., Bruun, A. and Ehinger, B. (1999a). Graft-host connections in long-term full-thickness embryonic rabbit retinal transplants. *Invest. Ophthalmol. Vis. Sci.* **40**, 126–32.

Ghosh, F. and Ehinger, B. (2000). Full-thickness retinal transplants: a review. *Ophthalmologica* **214**, 54–69.

Ghosh, F., Johansson, K. and Ehinger, B. (1999b). Long-term full-thickness embryonic rabbit retinal transplants. *Invest. Ophthalmol. Vis. Sci.* **40**, 133–42.

Gouras, P., Du, J., Kjeldbye, H., Yamamoto, S. and Zack, D. J. (1994). Long-term photoreceptor transplants in dystrophic and normal mouse retina. *Invest. Ophthalmol. Vis. Sci.* **35**, 3145–53.

Hollenberg, M. J. and Spira, A. W. (1973). Human retinal development: ultrastructure of the outer retina. *Am. J. Anat.* **137**, 357–85.

Huang, J. C., Ishida, M., Hersh, P., Sugino, I. K. and Zarbin, M. A. (1998). Preparation and transplantation of photoreceptor sheets. *Curr. Eye Res.* **17**, 573–85.

Humayun, M. S., Prince, M., de Juan, E., Jr., Barron, Y., Moskowitz, M., Klock, I. B. and Milam, A. H. (1999). Morphometric analysis of the extramacular retina from postmortem eyes with retinitis pimentos. *Invest. Ophthalmol. Vis. Sci.* **40**, 143–8.

Johnson, A. T., Kretzer, F. L., Hittner, H. M., Glazebrook, P. A., Bridges, C. D. and Lam, D. M. (1985). Development of the subretinal space in the preterm human eye: ultrastructural and immunocytochemical studies. *J. Comp. Neurol.* **233**, 497–505.

Julien, J. P., Tretjakoff, I., Beaudet, L. and Peterson, A. (1987). Expression and assembly of a human neurofilament protein in transgenic mice provide a novel neuronal marking system. *Genes Dev.* **1**, 1085–95.

Kwan, A. S., Wang, S. and Lund, R. D. (1999). Photoreceptor layer reconstruction in a rodent model of retinal degeneration. *Exp. Neurol.* **159**, 21–33.

LaVail, M. M. (1981). Photoreceptor characteristics in congenic strains of RCS rats. *Invest. Ophthalmol. Vis. Sci.* **20**, 671–5.

LaVail, M. M., Li, L., Turner, J. E. and Yasumura, D. (1992). Retinal pigment epithelial cell transplantation in RCS rats: normal metabolism in rescued photoreceptors. *Exp. Eye Res.* **55**, 555–62.

Li, L., Sheedlo, H. J. and Turner, J. E. (1990). Long-term rescue of photoreceptor cells in the retinas of dystrophic rats by RPE transplants. *Prog. Brain. Res.* **82**, 179–85.

Li, L. and Turner, J. E. (1988). Inherited retinal dystrophy in the RCS rat: prevention of photoreceptor degeneration by pigment epithelial cell transplantation. *Exp. Eye Res.* **47**, 911–7.

Li, L. and Turner, J. E. (1991). Optimal conditions for long-term photoreceptor cell rescue in RCS rats: the necessity for healthy RPE transplants. *Exp. Eye Res.* **52**, 669–79.

Linberg, K. A. and Fisher, S. K. (1990). A burst of differentiation in the outer posterior retina of the eleven-week human fetus: an ultrastructural study. *Vis. Neurosci.* **5**, 43–60.

Little, C. W., Castillo, B., DiLoreto, D. A., Cox, C., Wyatt, J., del Cerro, C. and del Cerro, M. (1996). Transplantation of human fetal retinal pigment epithelium rescues photoreceptor cells from degeneration in the Royal College of Surgeons rat retina. *Invest. Ophthalmol. Vis. Sci.* **37**, 204–11.

Lopez, R., Gouras, P., Kjeldbye, H., Sullivan, B., Reppucci, V., Brittis, M., Wapner, F. and Goluboff, E. (1989). Transplanted retinal pigment epithelium modifies the retinal degeneration in the RCS rat. *Invest. Ophthalmol. Vis. Sci.* **30**, 586–8.

Milam, A. H., Li, Z. Y. and Fariss, R. N. (1998). Histopathology of the human retina in retinitis pigmentosa. *Prog. Retin. Eye Res.* **17**, 175–205.

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

Narayanan, K. and Wadhwa, S. (1998). Photoreceptor morphogenesis in the human retina: a scanning electron microscopic study. *Anat. Rec.* **252**, 133–9.

Navon, S. E. and Fung, B. K.-K. (1988). Characterization of transducin from bovine retinal rod outer segments. *J. Biol. Chem.* **263**, 489–96.

Oshinskie, L. J. (1996). Age-related macular degeneration. *Optom. Clin.* **5**, 25–53.

Provis, J. M., Leech, J., Diaz, C. M., Penfold, P. L., Stone, J. and Keshet, E. (1997). Development of the human retinal vasculature: cellular relations and VEGF expression. *Exp. Eye Res.* **65**, 555–68.

Radtke, N. D., Seiler, M. J., Aramant, R. B., Petry, H. M. and Pidwell, D. J. (2002). Transplantation of intact sheets of fetal neural retina with its RPE in retinitis pigmentosa patients. *Am. J. Ophthalmol.* **133** (4), 544–50.

Raymond, S. M. and Jackson, I. J. (1995). The retinal pigmented epithelium is required for development and maintainance of the mouse neural retina. *Curr. Biol.* **5**, 1286–95.

Rhodes, R. H. (1984). Ultrastructure of Müller cells in the developing human retina. *Graefe's Arch. Clin. Exp. Ophthalmol.* **221**, 171–8.

Rothermel, A., Willbold, E., Degrip, W. J. and Layer, P. G. (1997). Pigmented epithelium induces complete retinal reconstitution from dispersed chick retinae in reaggregation culture. *Proc. R. Soc. London* **264**, 1293–302.

Santos, A., Humayun, M. S., de Juan, E., Jr., Greenburg, R. J., Marsh, M. J., Klock, I. B. and Milam, A. H. (1997). Preservation of the inner retina in retinitis pigmentosa. A morphometric analysis. *Arch. Ophthalmol.* **115**, 511–5.

Segato, T., Midena, E. and Blarzino, M. C. (1993). Age-related macular degeneration. *Aging (Milano)* **5**, 165–76.

Seiler, M. J. and Aramant, R. B. (1994). Photoreceptor and glial markers in human embryonic retina and in human embryonic retinal transplants to rat retina. *Brain. Res. Dev. Brain Res.* **80**, 81–95.

Seiler, M. J. and Aramant, R. B. (1995). Transplantation of embryonic retinal donor cells labelled with BrdU or carrying a genetic marker to adult retina. *Exp. Brain. Res.* **105**, 59–66.

Seiler, M. J. and Aramant, R. B. (1998). Intact sheets of fetal retina transplanted to restore damaged rat retinas. *Invest. Ophthalmol. Vis. Sci.* **39**, 2121–31.

Seiler, M. J. and Aramant, R. B. (2000a). Long-term transplants of human fetal RPE sheets together with neural retina develop normally in nude rats. *Invest. Ophthalmol. Vis. Sci.* **41** (**ARVO Suppl.**): S856, program #4550.

Seiler, M. J. and Aramant, R. B. (2000b). Intact-sheet transplants of human fetal retina with pigment epithelium develop normal lamination and survive long-term in nude rats. *Exp. Eye Res.* **71 (Suppl. 1):** S.60, program 209.

Seiler, M. J., Aramant, R. B. and Ball, S. L. (1999). Photoreceptor function of retinal transplants implicated by light–dark shift of S-antigen and rod transducin. *Vision Res.* **39**, 2589–96.

Seiler, M. J., Aramant, R. B., Liu, L., Cooper, N. G. F., Petry, H. M. and Callahan, T. L. (2000). Selective photoreceptor damage in albino rats using continuous blue light. *Graefe's Arch. Clin. Exp. Ophthalmol.* **238**, 599–607.

Sheng, Y., Gouras, P., Cao, H., Berglin, L., Kjeldbye, H., Lopez, R. and Rosskothen, H. (1995). Patch transplants of human fetal retinal pigment epithelium in rabbit and monkey retina. *Invest. Ophthalmol. Vis. Sci.* **36**, 381–90.

Silverman, M. S. and Hughes, S. E. (1989). Transplantation of photoreceptors to light-damaged retina. *Invest. Ophthalmol. Vis. Sci.* **30**, 1684–90.

Spira, A. W. and Hollenberg, M. J. (1973). Human retinal development: ultrastructure of the inner retinal layers. *Dev. Biol.* **31**, 1–21.

Van Driel, D., Provis, J. M. and Billson, F. A. (1990). Early differentiation of ganglion, amacrine, bipolar, and Muller cells in the developing fovea of human retina. *J. Comp. Neurol.* **291**, 203–19.

Vescovi, A. L., Parati, E. A., Gritti, A., Poulin, P., Ferrario, M., Wanke, E., Frolichsthal-Schoeller, P., Cova, L., Arcellana-Panlilio, M., Colombo, A. and Galli, R. (1999). Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. *Exp. Neurol.* **156**, 71–83.

Wenkel, H. and Streilein, J. W. (2000). Evidence that retinal pigment epithelium functions as an immune- privileged tissue. *Invest. Ophthalmol. Vis. Sci.* **41**, 3467–73.

Yamamoto, S., Du, J., Gouras, P. and Kjeldbye, H. (1993). RPE transplants maintain retinal function in RCS rats. *Invest. Ophthalmol. Vis. Sci.* **34**, 1094.

Zhang, X. and Bok, D. (1998). Transplantation of retinal pigment epithelial cells and immune response in the subretinal space. *Invest. Ophthalmol. Vis. Sci.* **39**, 1021–7.