Transplantation of cultured human retinal pigment epithelium into rabbit subretina

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Received: 18 September 1992 / Accepted: 16 June 1993

Abstract. Transplantation of normal retinal pigment epithelium (RPE) into a diseased eye holds promise for treatment of several blinding disorders. Previous studies have involved immunosuppression and implantation of freshly isolated cells. We report here the successful transplantation of cultured human RPE cells into rabbits that were not immunosuppressed. A modified pars plana transvitreal technique was used for RPE transplantation. The cultured RPE cells, loaded with carbon as a marker, were transplanted into the denuded Bruch's membrane of albino rabbits. The animals were followed for from 1 week to 3 months. On histologic examination at 2 months, no infiltrating lymphocytes were found in the vitreous cavity or choroid, even though Bruch's membrane was damaged. At about 3 months there were some macrophages in the subretina of transplanted eyes, indicating that an immunoreaction does occur eventually. Electron microscopy of the transplanted RPE showed apical-basal polarity and gap junctions. Restored function was attested to by the presence of phagosomes and phagocytosed outer segments in the transplanted cells. Our findings suggest that there is a weak, delayed immunoreaction to human RPE cells transplanted beneath the retina of the rabbit; however, functional recovery of the transplanted cells occurs before this immune response develops.

Materials and methods

Rabbits

Thirty New Zealand albino rabbits (3 months old) of both sexes, weighing 2-3 kg, were used. The animal experiments conformed to the ARVO Resolution on the Use of Animals in Research, and the animals were maintained in facilities fully accredited by the American Association of Laboratory Animal Science.

Culture of human RPE cells [17]

Human eyes from a 40-year-old donor were obtained from the Lions/Doheny Eye Bank. The eyes were opened circumferentially approximately 2 mm posterior to the ora serrata; the vitreous was removed by aspiration and the retina was gently separated from the RPE layer. The eye cup was washed with Eagle's minimal essential medium (MEM; Irvine Scientific, Santa Ana, Calif.) and incubated at 37°C with 0.005% trypan blue and 0.02 EDTA (Try-EDTA; Irvine Scientific). After 1 h incubation, the Try-EDTA solution was removed from the eye cup and replaced with MEM containing 10% fetal bovine serum (FBS) (Gemini Bioproducts, Calabassas, Calif.), penicillin-streptomycin (100 IU/ml, 100 µg/ml, Irvine Scientific). The RPE cells were transferred to a tissue culture flask and incubated at 37°C in an atmosphere containing 95% air and 5% CO₂.
After the cultured RPE cells became a monolayer, the medium was then passaged by using trypsinization. Third passage cells were used for transplantation. The total elapsed time from cell collection to transplantation was 2 months.

**Labelling RPE cells with carbon particles**

After the cultured RPE cells became a monolayer, the medium was replaced with culture medium to which one drop of india ink per 10 ml of culture medium had been added. Before transplantation, the free carbon was removed from the cells by three rinses in phosphate-buffered saline (PBS), after which the cultures were continued in carbon-free medium. The carbon-loaded RPE cells were detached by incubation for 5 min at 37°C with Try-EDTA, suspended in PBS at a concentration of approximately 5000 cells per 20 μl, and kept on ice for immediate transplantation.

**RPE transplantation [25]**

The rabbits were anesthetized by intramuscular injection of a mixture of ketamine hydrochloride and xylazine hydrochloride. After the pupils had been dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride, 0.5% proparacaine hydrochloride was instilled into the conjunctival sac, and 1 ml 1% lidocaine hydrochloride (10 mg/ml) was injected into the retrobulbar space to immobilize the eye.

A conjunctival flap was formed and pars plana incisions were made with a stiletto blade in both the nasal and temporal areas, 3 mm behind the limbus. A 4-mm infusion cannula attached to a reservoir of balanced salt solution (BSS) was inserted into the vitreous cavity through the nasal incision. A vitrectomy was performed with a Daisy Vitrectomy System (Model D 1700; Storz Instruments, St. Louis, Mo.) through the temporal incision. The previously prepared carbon-labelled RPE cells were drawn up into a glass micropipette connected to a microsyringe. The micropipette, which had a 10-μm tip, was inserted through the temporal pars plana incision under visual control using the operating microscope and a contact lens. The micropipette was gently touched to the retina at a distance of 2-3 disc diameters from the optic disc. An injection was made to produce a small hole in the retina, through which the carbon-loaded RPE cell suspension entered the subretina and formed a bleb. The total injection volume was 30 μl. The pars plana incisions and the conjunctiva were closed with 6-0 dexon sutures. Topical gentamicin sulfate ointment was applied postoperatively.

**Histologic examination**

The rabbits were sacrificed at 1, 2, 3, 4 and 6 weeks, and at 2 months and 3 months after surgery. The eyes were enucleated and fixed in half-strength Karnovsky fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2-7.4) at 4°C for 48 h. The tissue was rinsed three times in PBS and dehydrated in graded alcohol, infiltrated, and embedded in a glycol methacrylate mixture, cut at 2 μm and stained with Richardson's stain. For electron microscopy (EM), the tissue was rinsed for 15 min in cacodylate buffer and post-fixed in 2% osmium tetroxide (Polyscience, Warrington, Pa.) for 2 h. After dehydration and infiltration, the tissue was embedded in epon and thin sections were cut on an ultramicrotome using a diamond knife. The sections were stained with uranyl acetate and lead citrate and examined with an AEI-EM 801 electron microscope.

**Results**

Injection of the carbon-loaded RPE cells into the subretina produced a very lightly pigmented bleb that could be seen immediately (Fig. 1). Within 10 min of injection, the bleb flattened, leaving a discolored area that contained transplanted RPE cells, which were easily distinguished from the host RPE cells that were devoid of carbon. EM revealed that the carbon remained in the cytoplasm of the transplanted RPE cells, where it was surrounded by a pale halo. The appearance of the carbon in the transplanted cells was very similar to that in cultured cells (Fig. 2).

Removal of host pigment epithelium is a basic requirement for RPE transplantation. We found that most RPE cells beneath the bleb had been dislodged (Fig. 3) by injection of the cell suspension, but Bruch's membrane remained intact.

The transplanted carbon-labelled RPE cells were attached to Bruch's membrane in both contiguous and isolated areas (Fig. 3). Shortly after transplantation, the grafted cells loosely adhered to host RPE cells and photoreceptor cells, but by 3–4 weeks following retinal reattachment the anatomic integrity of the retinal structure had been re-established. The transplanted RPE cells showed apical–basal polarity and gap junctions, both with each other and with host RPE cells (Figs. 4, 5). No marked differences in the polarity and gap junctions were noted between host-donor RPE cells. Basal infoldings could be seen once the transplanted RPE attached to Bruch's membrane, although the infoldings of the transplanted RPE were not as straight as those of the host cells. The grafted RPE cells had apical microvilli and processes, and the cell organelles, such as mitochondria and rough endoplasmic reticulum, appeared normal (Fig. 5). Phagosomes also could be identified in the transplanted RPE cells (Fig. 5).