Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 3126–3131, March 1999
Medical Sciences

Intravitreous transplantation of encapsulated fibroblasts secreting the human fibroblast growth factor 2 delays photoreceptor cell degeneration in Royal College of Surgeons rats

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Communicated by Thaddeus P. Dryja, Harvard Medical School, Boston, MA, December 31, 1998 (received for review October 27, 1998)

ABSTRACT **We developed an experimental approach with genetically engineered and encapsulated mouse NIH 3T3 fibroblasts to delay the progressive degeneration of photoreceptor cells in dark-eyed Royal College of Surgeons rats. These xenogeneic fibroblasts can survive in 1.5-mm-long microcapsules made of the biocompatible polymer AN69 for at least 90 days under *in vitro* and *in vivo* conditions because of their stable transfection with the gene for the 18-kDa form of the human basic fibroblast growth factor (hFGF-2). Furthermore, when transferred surgically into the vitreous cavity of 21-day-old Royal College of Surgeons rats, the microencapsulated hFGF-2-secreting fibroblasts provoked a local delay of photoreceptor cell degeneration, as seen at 45 days and 90 days after transplantation. This effect was limited to 2.08 mm^2 (45 days) and 0.95 mm^2 (90 days) of the retinal surface. In both untreated eyes and control globes with encapsulated hFGF-2-deficient fibroblasts, the rescued area (of at most 0.08 mm^2) was significantly smaller at both time points. Although, in a few ocular globes, surgical trauma induced a reorganization of the retinal cytoarchitecture, neither microcapsule rejection nor hFGF-2-mediated tumor formation were detected in any treated eyes. These findings indicate that encapsulated fibroblasts secreting hFGF-2 or perhaps other agents can be applied as potential therapeutic tools to treat retinal dystrophies.**

Among various gene-therapy protocols that have been designed in hopes of transferring therapeutically important genes to target tissues, the transplantation of genetically engineered cells has become particularly promising. To protect allogeneic or xenogeneic grafts from immune rejection, cells have been encapsulated successfully with nondegradable biocompatible polymers and used for the experimental treatment of several neurodegenerative disorders (1, 2). Aimed at protecting these grafts against recipients' immune responses, encapsulation is suited particularly for situations in which a permanent release of therapeutic substances into target tissues is desired. Because of the easy accessibility and the highly organized structure of the retina, the eye can serve as an excellent organ to validate such cell-therapy protocols. We, therefore, developed an experimental approach to study the therapeutic effect of the cell-encapsulation technique on the degenerative retina. Currently, one of the most studied animal models for inherited retinal disease is the Royal College of Surgeons (RCS) rat (3, 4). The retinal-pigment epithelial cells of these rats are primarily defective in the phagocytosis of shed outer segments of photoreceptor cells (PRC). The RCS retinal dystrophy is characterized by a secondary degeneration of PRC beginning during the third postnatal week and ending at the age of 2 months. The disappearance of PRC occurs by apoptosis (5) and is accompanied by a significant decrease in the production of fibroblast growth factor 2 (FGF-2; GenBank accession no. M27968) in the retina (6).

The cytoplasmic 18-kDa form of FGF-2 is secreted, despite the absence of a hydrophobic secretory signal sequence (7). FGF-2 either is stored extracellularly by associating with heparan sulfate proteoglycans or binds with high affinity to tyrosine kinase receptors where it exerts pleiotropic effects (8). Human FGF-2 (hFGF-2) can transiently delay PRC degeneration in the RCS rat retina when injected intravitreously or subretinally (9). On the basis of this finding, we developed an experimental approach that allows the intraocular secretion of this trophic factor by encapsulated mouse fibroblasts. The capacity of the encapsulation technique to rescue PRC was evaluated in the dystrophic RCS retina. Among the various biocompatible artificial polymers that have been employed for encapsulation, the copolymer AN69 (polyacrylonitrile–methallylsulfonate) seems to be particularly suited for both the protection of encapsulated cells against the host immune system and the long-term maintenance of cell viability (10). AN69 is used as a semipermeable hemodialysis membrane and fails to activate the complement system. The present study shows that transplantation of encapsulated xenogeneic hFGF-2-secreting cells into the vitreous body can promote the survival of PRC in the RCS rat retina.

MATERIALS AND METHODS

Stable Transfection and Adherent Cell Culture. To produce the hFGF-2-secreting NIH 3T3 PS-FGF18 (FGF18) mouse fibroblasts were stably transfected with the plasmid pSFGF18 (6.43 kb), which was constructed on the basis of the vector pEN (11). pSFGF18 contains the pVC-derived *Sma*I–*Nco*I fragment (12), encoding the amino-terminal secretion signal peptide of the vascular endothelial growth factor (VEGF), which was inserted into the *Sma*I–*Nco*I restriction sites of pF18EN (13). The vector further comprises the cytomegalovirus enhancer/promoter sequence upstream of the chimeric VEGF–hFGF-2 gene, as well as downstream sequences, including the internal ribosomal entry site of the encephalomyocarditis virus, the neomycin-resistance gene, the simian virus 40 polyadenylation signal sequence, the IVS2β intron of the rabbit

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Abbreviations: FGF, fibroblast growth factor; FGF18, NIH 3T3 PS-FGF18 fibroblast; hFGF, human FGF; IR, immunoreactivity; P16, NIH 3T3-P16 fibroblast; PRC, photoreceptor cells; RCS, Royal College of Surgeons.

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β-globin gene, and the ampicillin-resistance gene. Thus, plasmid transcription gives rise to a bicistronic mRNA encoding hFGF-2 and the aminoglycoside phosphotransferase. To obtain NIH 3T3-P16 fibroblasts (P16), NIH 3T3 cells were stably transfected with a modified pSFGF18 plasmid that lacks the hFGF-2 transgene sequence. Cells were cultured in growth medium containing F12 medium and DMEM (1:1, vol/vol), 10% decomplemented fetal calf serum, 0.7 mg/ml geneticin, and a prefabricated mixture of antibiotics and antimycotics (with final concentrations of 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 μg/ml amphotericin B).

Encapsulation and Cell Viability. FGF18 or P16 that had been treated with trypsin were resuspended in growth medium and mounted by capillarity into Dialox-treated 5-cm-long AN69 polymer fibers (50-kDa cut-off; 300-μm external diameter and 200-μm internal diameter; Hospal R & D, Meyzieu, France). Fibers were heat-sealed at both ends and kept in culture for 1 day to allow cell aggregation. Then, the capsules were segmented into 1.5-mm or 4.0-mm microcapsules. They were kept in culture for at least 14 days before transplantation to allow elimination any microcapsule displaying signs of cellular leakage. Cell viability was determined by trypan blue exclusion in isolated microcapsules kept in growth medium as well as in explanted microcapsules. Encapsulated P16 and FGF18 were released by treating them with trypsin from 4.0-mm-long capsules after 2, 15, 30, and 60 days in culture ($n = 6$ for each cell type and time point) and from 1.5-mm-long microcapsules explanted 45 days after *in vivo* transplantation ($n = 4$ for each cell type). Cell viability was also measured in microcapsules containing P16 or FGF18 90 days after transplantation ($n = 1$ for each cell type). Fig. 1*D* exemplifies the integrity of an explanted 1.5-mm microcapsule with a few particles at its outer surface.

Biopolymer Permeability and Bioassay. Capsules (1.0-cm long) were preincubated for 24 h in growth medium and subsequently filled with 125 ng of recombinant hFGF-2 ($n = 3$). After a 24-h incubation in Technoplastic-Products (Trasadingen, Switzerland) culture plates, the concentration of hFGF-2 released from capsules was measured by ELISA (R & D Systems). Detachment of hFGF-2 from the culture plates was carried out at 37°C by successive 20-min incubation steps. Microcapsules (1.5-mm and 4.0-mm long), which were either empty or filled with FGF18 or P16 and kept in culture for 15, 30, or 60 days ($n = 6$ per capsule length, cell type, and time point), were transferred to 6-well Technoplastic-Products culture plates (one microcapsule per well) containing adherent NIH 3T3 fibroblasts (5×10^4 cells per well). After 10 days, the NIH 3T3 cell density was determined. The rate of cell proliferation was analyzed statistically by using the Mann–Whitney *U* test.

hFGF-2 Production by FGF18. Recombinant hFGF-2 and crude FGF18 or P16 cell extracts were separated on a denaturing SDS/12% polyacrylamide gel and blotted onto an Immobilon P membrane (Millipore). After an overnight incubation of the membrane with primary polyclonal rabbit anti-hFGF-2 antibodies (1/200; Santa Cruz Biotechnology), hFGF-2 was visualized by enhanced chemiluminescence (Amersham Pharmacia). The secretion of hFGF-2 from increasing numbers of FGF18, cultured in 6-well Technoplastic-Products culture plates for 24 h, was measured by ELISA.

Intravitreous Transplantation. Microcapsules (1.5-mm long), filled with either P16 or FGF18, were kept in culture for 14 days. Leak-proof capsules were then transplanted into vitreous cavities (Fig. 1*C*) from 21-day-old dark-eyed RCS rats ($n = 21$ rats). The microcapsules were introduced into the vitreous cavity of anesthetized rats through the pars plana. Eyes were covered postoperatively with a paste containing dexamethasone (0.13 mg per eye) and oxytetracycline (0.67 mg per eye). Rats were kept for 45–90 days under standard housing conditions. In our first series, four groups of randomly constituted rats received intravitreous transplants. In groups I (6 rats) and II (4 rats), the eyes were analyzed 90 days after transplantation. In groups III (6 rats) and IV (5 rats), the eyes were studied 45 days after transplantation. In all groups, the

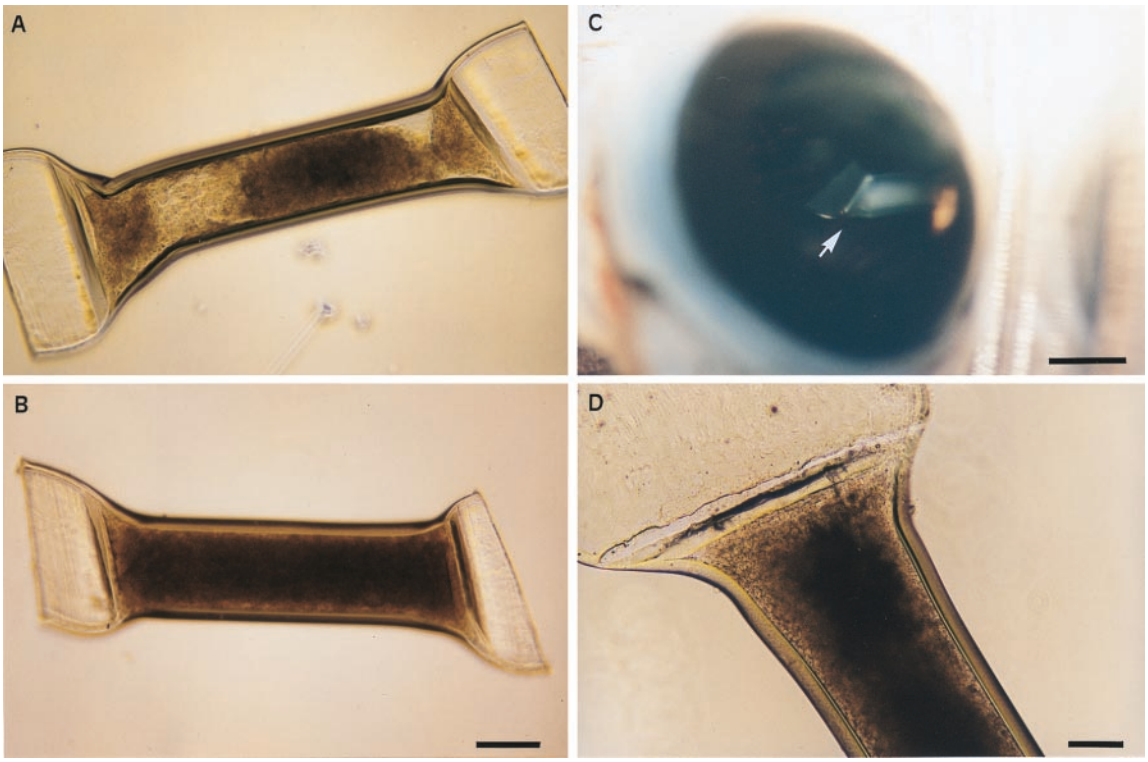

FIG. 1. Microencapsulation and transplantation of encapsulated FGF18 to the eye of an RCS rat. (*A* and *B*) Cells are packed into a 1.5-mm microcapsule and kept in culture for 1 day (*A*) or 14 days (*B*). (*C*) A 1.5-mm microcapsule (arrow) implanted into the vitreous cavity. (*D*) Explanted 1.5-mm microcapsule after being in the vitreous cavity for 90 days. [(*A* and *B*) Bars = 200 μm; (*C*) bar = 1 mm; (*D*) bar = 100 μm.]

surgeon operated on a randomly chosen eye. In groups I and III, microcapsules containing hFGF-2-producing cells were allocated to a randomly chosen eye, and the contralateral eye received P16 microcapsules. In groups II and IV, hFGF-2-releasing microcapsules were allocated to a randomly chosen eye, and the contralateral eye received no treatment. In group I, one eye, which received an FGF18 microcapsule, presented endophthalmitis. In group IV, one rat displayed endophthalmitis in the untreated eye. The treated eye of the same rat could not be analyzed because of difficulties encountered during the cutting procedure. Thus, of 42 eyes involved in our first series, only 39 eyes could be subjected to detailed histological and statistical analyses. In the second series, five RCS rats unilaterally received a microcapsule filled with either FGF18 ($n = 3$) or P16 ($n = 2$), which were transplanted subretinally. This type of implantation caused surgically induced local tissue necrosis. Of the two eyes containing a P16 microcapsule, one developed endophthalmitis.

Tissue Staining and Histological Analysis. Ocular globes were embedded in Tissue-Tek O.C.T. compound (Miles), frozen, and cut on a cryotome. Serial 16-μm sections were mounted onto slides, postfixed for 20 min with 2% (wt/vol) paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 48°C, and stained with toluidine blue. In each eye, the number of PRC layers was counted on sections about 50 μm apart under a light microscope. The number of PRC rows in the outer nuclear layer was determined at field points 125 μm apart. According to the chosen criterion, cell rescue was indicated when a field point showed a row number of more than four PRC layers. The field of delayed cell degeneration was determined on two-dimensional schematic representations of the retina. Differences in the rescued retinal area between the experimental groups were analyzed statistically by using the Mann–Whitney *U* test.

Immunohistochemistry. Ocular globes were fixed for 2 days in Davidson's fixative and embedded in paraffin. Serial 5-μm sections were mounted onto ChemMATE capillary gap microscope slides (Dako). They were subjected to a standard immunostaining procedure (ChemMATE Detection kit) with 10 ng/μl monoclonal mouse anti-bovine basic FGF IgGs as primary antibody (Upstate Biotechnology, Lake Placid, NY) and subsequently stained with Harris' hematoxylin.

RESULTS

Viability of Encapsulated Fibroblasts. The viability of encapsulated P16 and FGF18 was examined first under culture conditions. In 4.0-mm microcapsules, cells reaggregated and attached to the biomaterial within 24 h (Fig. 1*A*). As determined 15 days after encapsulation, both cell types filled the lumen of the fibers (Fig. 1*B*) by reaching an average density of 2.16×10^5 cells per microcapsule. At this time point, an average of 75% of the FGF18 and 67% of the P16 were viable inside the microcapsules (Fig. 2). When encapsulated cells were cultured for 30–60 days, cell survival declined to 60–65% of the FGF18 and to 2% of the P16. In explanted microcapsules retrieved after 45 days *in oculo*, 58% of the FGF18 and 69% of the P16 were viable. The cell number per microcapsule varied between 2×10^3 and 1×10^4. A few FGF18 retained their ability to attach to the culture plate after being released from the capsules. As found in microcapsules explanted after 90 days *in oculo*, 29% of the FGF18 were viable. On the other hand, P16 had changed their morphology and had fissioned to apoptotic bodies. The FGF18 appeared homogeneously distributed within the microcapsules, as observed *in situ* on frozen ocular tissue sections. In contrast, P16 formed isolated aggregates. The expression of the hFGF-2 transgene by encapsulated and transplanted FGF18 was verified by immunohistochemistry. At 90 days after transplantation, hFGF-2-like immunoreactivity (IR) was detected in the cytoplasm of 30% of

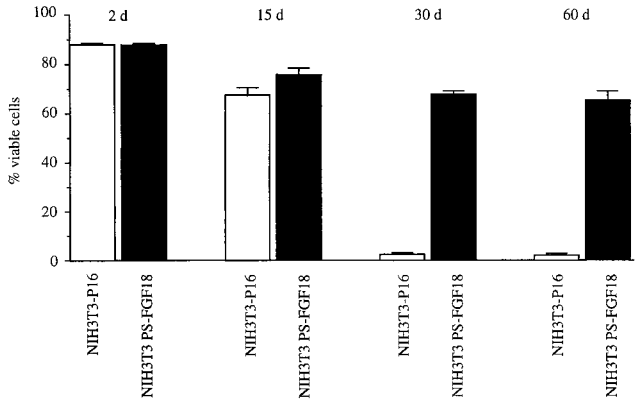

FIG. 2. Survival rate of P16 (white bars) and FGF18 (black bars) in 4.0-mm microcapsules measured 2, 15, 30, and 60 days after encapsulation. Viable cells were identified by trypan blue exclusion assays and are indicated as the mean percentage of the total number of encapsulated cells. (Bars = SEM; $n = 6$.)

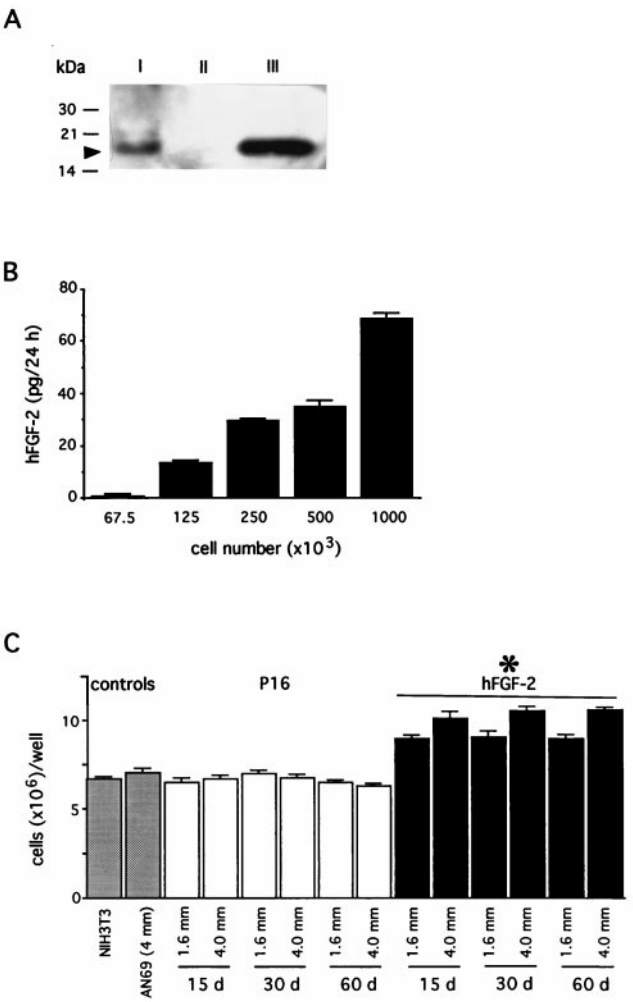

FIG. 3. Production of hFGF-2 in stably transfected fibroblasts. (*A*, lane I) This Western blot of crude cell extracts (10^5 cells/lane) indicates the 18-kDa hFGF-2 in FGF18 (arrow head). (*A*, lane III) Its apparent molecular mass corresponds to that of hFGF-2 (10 ng). (*A*, lane II) P16 cell extract. (*B*) Secretion of hFGF-2 by FGF18 at different cell densities. Data are expressed as mean values (pg per 24 h) ±SEM ($n = 6$). (*C*) Bioassay of hFGF-2 secretion from 1.5-mm and 4.0-mm capsules filled with either P16 or FGF18 and kept in culture for 15, 30, or 60 days. Data are expressed as mean values ± SEM. (*, $P < 0.001$; $n = 6$.)

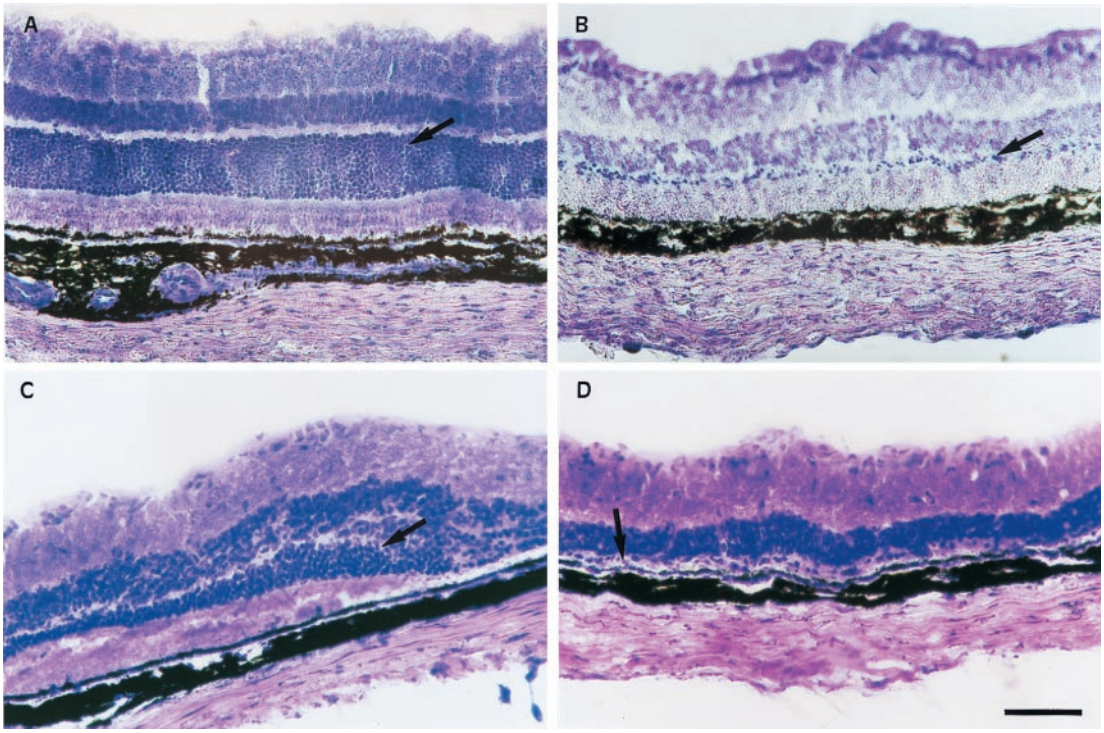

FIG. 4. Delay of PRC degeneration in RCS rat retinas 90 days after transplantation. (*A*) Retina of a 21-day-old rat before the beginning of PRC degeneration. (*B*) Dystrophic retina of a 63-day-old rat. (*C*) PRC rescue 90 days after transplantation in an eye that received microencapsulated FGF18. Note the microcapsule-proximal retina which comprises five or six PRC layers (arrow). (*D*) P16-filled microcapsule close to a dystrophic retina 90 days after transplantation. The PRC layer is indicated by arrows. (Sections were 16 μm thick; toluidine blue staining; Bar = 20 μm.)

the encapsulated cells; immunohistochemical staining of the inner and outer nuclear layers of the retina as well as of the retinal-pigment epithelial cell layer served as a positive control for the antigen specificity of the antibody (see Fig. 7). Unlike the cytoplasmic hFGF-2 IR of encapsulated cells, the retinal cells show mainly nuclear FGF-2 staining.

Release of hFGF-2 from Microcapsules. The production of hFGF-2 in cultured FGF18 was visualized and quantified by Western blotting and ELISA. On immunoblots, cellular hFGF-2 has an apparent molecular mass of 18 kDa, which corresponds to hFGF-2 (Fig. 3*A*). In P16, the concentration of the endogenous FGF-2 was below the threshold of detection. As quantified by ELISA, FGF18 secrete an average of 68.7 ± 2.3 pg SEM of hFGF-2 ($n = 6$; from 10^6 cells over 24 h; Fig. 3*B*). This value represents the overall level of extracellular hFGF-2, 96% of which was found to adhere to the culture plate. The permeability of the biopolymer membrane for hFGF-2 was verified further in 1.0-cm-long capsules. In these capsules, 2% of hFGF-2 diffused through the biopolymer membrane over 24 h at 37°C. The quantity of released hFGF-2 stimulates NIH 3T3 cell proliferation, as determined by a bioassay. During a coculture period of 10 days with either encapsulated P16 or empty microcapsules, NIH 3T3 cells grew from 5×10^4 cells to 6.5×10^6 cells per well (Fig. 3*C*). A similar proliferation rate was found in untreated NIH 3T3 cell cultures. When NIH 3T3 cells were exposed for 10 days to FGF18 capsules of different lengths kept in culture for 15–60 days, we observed a dose-dependent increase in NIH 3T3 cell proliferation by 35 ± 2.1% SEM (1.5-mm capsules; $n = 18$) and 58 ± 2.6% SEM (4.0-mm capsules; $n = 18$), as compared with P16 capsules ($P < 0.001$).

PRC Rescue and FGF-2 IR. The ability of FGF18 microcapsules to delay PRC degeneration was studied by analyzing the number of PRC layers in the RCS rat retina. At postnatal

Table 1. Analysis of photoreceptor cell rescue by encapsulated fibroblasts

Experimental treatment	*n*	Exposure, days	Rescued area, mm²†	SEM	Maximal area, mm²	Minimal area, mm²
Treatment 1. Microcapsules with FGF18s	9	90	0.95*	0.22	1.85	0.33
Treatment 2. Microcapsules with P16s	6	90	0.025	0.03	0.15	0.00
Treatment 3. Without microcapsules	4	90	0.00	0.00	0.00	0.00
Treatment 4. Microcapsules with FGF18s	10	45	2.08**	0.72	4.10	0.64
Treatment 5. Microcapsules with P16s	6	45	0.083	0.041	0.20	0.00
Treatment 6. Without microcapsules	4	45	0.00	0.00	0.00	0.00

†Area of the retina with more than four photoreceptor cell rows.
*, $P = 0.001$ (Treatment 1 vs. Treatment 2 and Treatment 1 vs. Treatment 3); **, $P = 0.002$ (Treatment 4 vs. Treatment 5); Mann–Whitney *U* test.

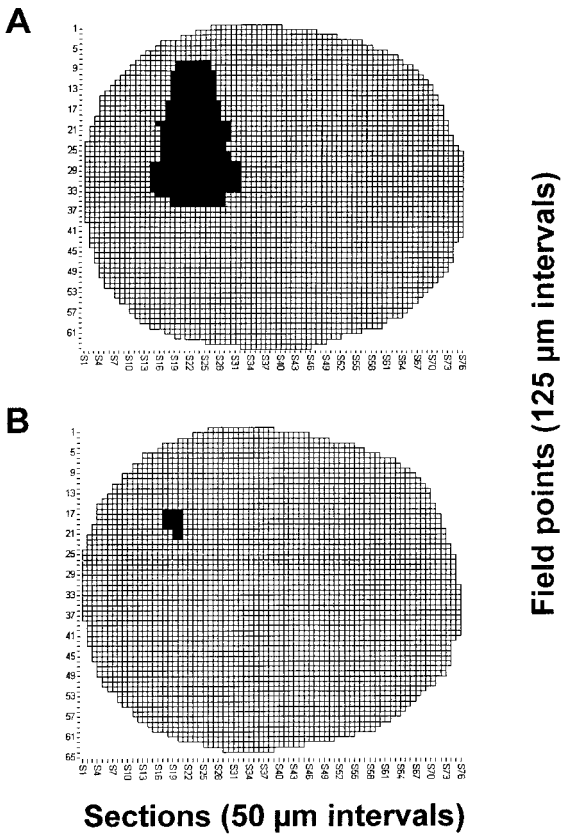

FIG. 5. The retinal surface of treated RCS dark-eyed rats showing maximal rescue effects are represented in these two-dimensional reconstructions. Retinal areas with at least five PRC layers are indicated. (*A*) Region (4.10 mm^2) of delayed PRC degeneration in the retina exposed for 45 days to a FGF18-filled microcapsule. (*B*) Area (0.15 mm^2) of delayed cell degeneration proximal to a P16-filled microcapsule as seen in the only eye that showed a cell-rescue effect 90 days after transplantation.

day 21, the RCS retina normally shows an average of 10 PRC layers (Fig. 4*A*). After 45 days, these layers typically are reduced to one or two rows in untreated eyes (Fig. 4*B*). At 45 and 90 days after transplantation, P16 microcapsule implants caused a delay in PRC degeneration, covering an average retinal area of 0.08 ± 0.04 mm^2 SEM and 0.025 ± 0.03 mm^2 SEM, respectively ($n = 6$; Table 1). In the majority of globes with P16 microcapsules, as well as in all the untreated eyes, PRC layers were reduced to a single cell row. Eyes that

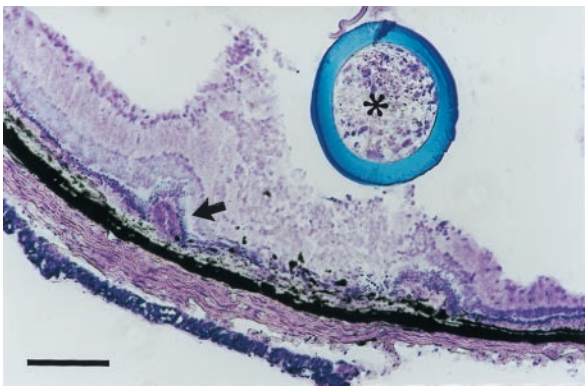

FIG. 6. PRC rosette formation in an RCS rat retina 45 days after transplantation. The microcapsule contains P16 (asterisk). Cellular reorganization of the retina led to the appearance of a PRC rosette structure (arrow). The section (16 μm) was counterstained with toluidine blue. (Bar = 40 μm.)

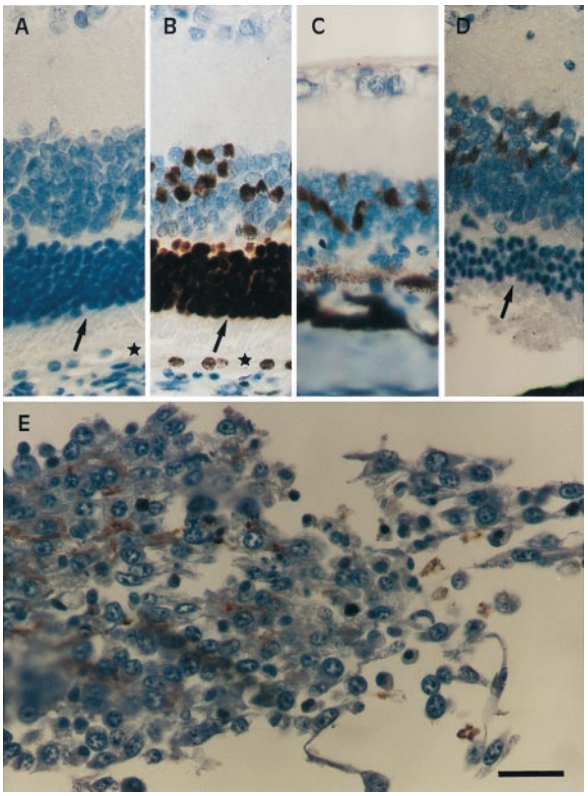

FIG. 7. FGF-2 IR in RCS rat retina. (*A–D*) PRC (arrows) and retinal-pigment epithelial cells (asterisks) of normal 3-month-old RCS retina (*A* and *B*) and dystrophic 111-day-old RCS rat retina (*C* and *D*). Immunostaining without primary antibody (*A*; control) and with anti-FGF-2 antibodies (*B–D*). (*D*) Note the lack of immunostaining in the PRC of the rescued retina. (*E*) Cytoplasmic FGF-2 immunostaining in encapsulated FGF18 90 days after transplantation. The sections (5 μm) were counterstained with hematoxylin. (Bar = 10 μm.)

received FGF18 microcapsules retained five or six PRC layers in the retina close to the implant, 45 days as well as at 90 days after transplantation (Fig. 4*C*). A rescued area of 2.08 ± 0.72 mm^2 SEM ($n = 10$), observed 45 days after transplantation, is restricted to the retinal zone at the vicinity of the microcapsule and is significantly higher than similar areas observed in untreated eyes ($P < 0.001$; Table 1). The maximal rescue effect amounted to 4 mm^2 or 10% of the entire retinal surface and represents 10 times the region covered directly by the microcapsule (Fig. 5*A*). Similarly, an average retinal area of 0.95 ± 0.22 mm^2 SEM ($n = 9$) was rescued by 90-day-old implants. This local rescue effect is significantly lower than that found 45 days after transplantation, but it is significantly higher than the effect of P16 microcapsules on PRC survival ($P < 0.05$; Table 1). In untreated eyes, no more than three PRC layers were found in the retina at both time points (Fig. 4*D*). The distance between the inner surface of the retina and the microcapsule was measured reproducibly as close to 50 μm in paraffin-embedded ocular tissue sections. Rosette formation and abnormal thickness of the retina were detected in 2 of 31 treated globes that received encapsulated FGF18 or P16 (Fig. 6). Furthermore, posterior cataracts were apparent in three eyes with P16 microcapsules as well as in two globes with encapsulated FGF18. In 29 eyes, the microcapsules were entirely intravitreous and showed neither apparent graft rejection by intraocular macrophage infiltration nor fibrous formation around the microcapsule. In the two eyes displaying rosettes, the microcapsules rested on the inner surface of the retina. One of these two globes contained a P16 microcapsule, and the other received a FGF18 microcapsule. In all of the eyes analyzed, we detected neither an increase of invading macro-

phages in the retina nor any signs of local accumulation of new capillary structures protruding into the vitreous cavity or the subretinal space. No evidence for neovascularization could be observed in the uveal tract, the sclera, or the cornea of any of the examined globes. The endogenous production of FGF-2 in the RCS retina was visualized by immunohistochemistry. In the normal retina of a 3-month-old control rats, mainly nuclear FGF-2 IR was apparent in cells of the inner nuclear layer as well as in all PRC of the outer nuclear layer (Fig. 7*B*). Cell nuclei of retinal-pigment epithelial cells also showed strong immunostaining. In dystrophic 111-day-old RCS rats, however, FGF-2 IR was found in cells of the inner nuclear layer (Fig. 7*C*), but FGF-2 IR was absent from degenerating PRC. In several age-matched RCS rats that received FGF18 microcapsules, the locally rescued PRC were also devoid of FGF-2 IR, whereas the inner nuclear layer still had some immunostained cells (Fig. 7*D*).

DISCUSSION

In the present study, we used an intravitreous cell-therapy approach for testing an experimental treatment of retinal dystrophies. The therapeutic transgene hFGF-2 was transferred to eyes via stably transfected and encapsulated FGF18. These cells as well as hFGF-negative P16 can adhere to the biopolymer and proliferate inside the microcapsules under culture and *in oculo* conditions and grow to confluence. However, P16 showed limited cell survival and eventually underwent cell apoptosis. In contrast, encapsulated FGF18 had long-term cell viability, probably resulting from the antiapoptotic action of FGF-2 (14). In these cells, hFGF-2 IR still can be observed in the cytoplasm 90 days after transplantation. Furthermore, encapsulated FGF18 stimulate NIH 3T3 cell proliferation with a dose-dependent effect. This finding indirectly indicates that secreted hFGF-2 can diffuse through the biopolymer membrane. Similarly, hFGF-2 diffusion through the biopolymer also was observed *in vivo*. When transplanted into the vitreous cavity, microcapsules that were filled with hFGF-2-secreting fibroblasts provoked a delay of PRC degeneration. At 45 days after transplantation, this rescue effect was observed clearly in up to 10% of the retinal surface. The average area rescued by 90-day-old microcapsules, however, is about half of that detected at 45 days after transplantation. As the viability of encapsulated hFGF-2-producing fibroblasts gradually declines *in vivo*, PRC degeneration may have resumed by 90 days after transplantation. In eyes with encapsulated P16, the PRC rescue effect was minimal, indicating that secreted hFGF-2 is responsible for the delay of PRC degeneration. This observation shows further that the surgical trauma caused by the microcapsule implantation did not induce cell rescue. In contrast, in other experimental settings such as intravitreous injections of PBS, RCS rats displayed a significant local rescue effect (9, 15). Although hFGF-2-releasing microcapsules lead to a local delay of PRC degeneration in 66- and 111-day-old RCS rats, the rescued retinal PRC had an undetectable level of FGF-2 immunostaining. This finding suggests that the rescued PRC failed to regain the ability of producing endogenous FGF-2. Thus, the rescue effect of PRC seems to depend entirely on the presence or absence of exogenous FGF-2 in the retina. Although we have shown that our cell therapy strategy delays PRC degeneration, it remains to be determined by multifocal electroretinogram analysis whether or not the rescued PRC retain their function.

Despite its satisfactory immunological tolerance, the biopolymer shows limited diffusion characteristics for the hFGF-2 protein. As compared with our approach, which leads to a release of only 1–2 pg of hFGF-2 per day, unique injections of 0.5–1.0 μg hFGF-2 into the vitreous space of RCS rats obtains a more widespread PRC rescue effect (9, 15). However, the injection of high doses of hFGF-2 induces a significant redistribution of retinal macrophages and/or microglia, an important neovascularization of the inner retina, and cataractogenesis in 100% of tested eyes (15). These observations emphasize the need to maintain the amount of hFGF-2 released inside the vitreous cavity below the threshold above which iatrogenic effects can occur. This threshold remains to be determined experimentally.

To obtain a more widespread rescue effect on PRC survival, other stably transfected cell lines that secrete several trophic factors can be used. The release of multiple growth factors even at low concentrations might enhance PRC survival. The use of inducible promoters such as the tetracycline regulation cassette, which allows experimental modulation of transgene transcription, is potentially an additional protection against adverse effects. Furthermore, the use of other biomaterials that are as efficient as AN69 in terms of limiting humoral and cellular immune reactions but allow a better diffusion of therapeutic molecules may widen the field of applications of this therapeutic strategy against neurodegenerative diseases.

Although FGF-2 can potentially induce tumor-like cell proliferation (16), we did not find any signs of tumor formation. A few PRC rosettes were detected in 2 of the 31 ocular globes analyzed; these rosettes emerged independently of the type of microcapsule that was implanted. Despite the local application of antiinflammatory corticosteroid and antibiotics, three eyes developed endophthalmitis. Because ocular atrophy was not restricted to eyes that received a particular type of encapsulated cell, surgically induced infection may be the origin of endophthalmitis. All treated globes were devoid of microcapsule-mediated fibrosis and hyperacute macrophage infiltration into the vitreous cavity, indicating that the biomaterial and hFGF-2 did not provoke any significant cell-mediated immune response. Nevertheless, additional studies remain to be performed to investigate more thoroughly the immune reactions that are possibly triggered by the intraocular transplantation of hFGF-2-releasing microcapsules.

In the present study, we have shown that transplantation of hFGF-2-secreting encapsulated cells can delay PRC degeneration in the RCS rat retina. Intravitreous transplantation of encapsulated xenogeneic cells secreting potential therapeutic factors, therefore, is a promising therapeutic strategy for rescue of PRC.

This study was supported by Retina France. We would also like to thank the Association Valentin Havy, the Fondation pour la Recherche Médicale Appliquée, and the Fondation de France.

1. Aebischer, P., Schluep, M., Déglon, N., Joseph, J. M., Hirt, L., Heyd, B., Goddard, M., Hammang, J. P., Zurn, A. D., Kato, A. C., *et al*. (1996) *Nat. Med.* **2,** 696–699.
2. Emerich, D. F., Winn, S. R., Hantraye, P. M., Peschanski, M., Chen, E.-Y., Chu, Y., McDermott, P., Baetge, E. E. & Kordower, J. H. (1997) *Nature (London)* **386,** 395–399.
3. Bok, D. & Hall, M. O. (1971) *J. Cell Biol.* **49,** 664–682.
4. LaVail, M. M. (1981) *Invest. Ophthalmol. Visual Sci.* **21,** 638–657.
5. Tso, M. O., Zhang, C., Abler, A. S., Chang, C. J., Wong, F., Chang, G. Q. & Lam, T. T. (1994) *Invest. Ophthalmol. Visual Sci.* **35,** 2693–2699.
6. Rakoczy, P. E., Humphrey, M. F., Cavaney, D. M., Chu, Y. & Constable, I. J. (1993) *Invest. Ophthalmol. Visual Sci.* **34,** 1845–1852.
7. Mignatti, P., Morimoto, T. & Rifkin, D. B. (1992) *J. Cell. Physiol.* **151,** 81–93.
8. Fernig, D. G. & Gallagher, J. T. (1994) *Prog. Growth Factor Res.* **5,** 353–377.
9. Faktorovich, E. G., Steinberg, R. H., Yasumura, D., Matthes, M. T. & LaVail, M. M. (1990) *Nature (London)* **347,** 83–86.
10. Honiger, J., Balladur, P., Mariani, P., Calmus, Y., Vaubourdolle, M., Delelo, R., Capeau, J. & Nordlinger, B. (1995) *Biomaterials* **16,** 753–759.
11. Maret, A., Galy, B., Arnaud, E., Bayard, F. & Prats, H. (1995) *Cancer Res.* **55,** 5075–5079.
12. Huez, I., Crèancier, L., Audigier, S., Gensac, M. C., Prats, A. C. & Prats, H. (1998) *Mol. Cell. Biol.* **18,** 6178–6190.
13. Arnaud, E., Touriol, C., Boutonnet, C., Gensac, M. C., Vagner, S., Prats, H. & Prats, A. C. (1999) *Mol. Cell. Biol.* **19,** 505–514.
14. Chow, R. L., Roux, G. D., Roghani, M., Palmer, M. A., Rifkin, D. B., Moscatelli, D. A. & Lang, R. (1995) *Development (Cambridge, U.K.)* **121,** 4383–4393.
15. Perry, J., Du, J., Kjeldbye, H. & Gouras, P. (1995) *Curr. Eye Res.* **14,** 585–592.
16. Rogelj, S., Weinberg, R. A., Fanning, P. & Klagsbrun, M. (1989) *J. Cell. Biochem.* **39,** 13–23.