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Retinal Cell Transplantation in the Macula: New Techniques

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Transplantation of RPE allografts into the submacular space after the removal of neovascular membranes in "wet" forms of ARMD usually leads to chronic leakage around the transplant, whereas this seldom occurs in transplants in "dry" forms of ARMD. The former may be the result of chronic rejection because of a compromised blood/retinal barrier. This is supported by the fact that visual function remains over the transplant in the dry forms of ARMD at 6 months after transplantation. Whether this function can slow or reverse the progression of ARMD remains to be seen. The possibility of transplantation of photoreceptors in retinal degenerations is also reviewed.

Macular degeneration Retinal pigment epithelium Photoreceptors Transplantation

INTRODUCTION

We have been attempting to repair the retinal pigmented epithelium (RPE) layer in age related macular degeneration (ARMD). We are assuming that the major cause of this degeneration is the result of RPE dysfunction. Although this hypothesis is unproven, there is much to indicate that the RPE layer is extremely hard-hit early in the course of ARMD (Hogan, 1972; Sarks, 1976; Green, 1991). If we could reconstruct a healthy new RPE layer in the macula of these patients we might have an impact on the progressive blindness that ARMD produces and perhaps influence the treatment of other forms of macular disease.

In order to do this many problems must be solved. First of all are the techniques to move and deliver viable, and functional foreign RPE into the subretinal space without damaging the host photoreceptors, and placing these cells either on top of or displacing and removing the degenerate host RPE layer. Second, we have the question of host/graft rejection threatening the long-term survival of such transplants; although this is probably a lesser problem because immunosuppression could be employed. It would, however, be valuable to know how necessary immunosuppression is. Third, it is also necessary to know exactly what macular or retinal degenerations are due to a primary defect in the RPE layer.

We have been performing RPE transplantation in selected patients with ARMD while we carry out parallel experiments in animals to test each change in technique before we attempt it in human surgery. We have now performed such surgery in 13 patients which can be grouped into three categories reflecting successive changes and/or improvements in our technique. We believe it is essential to do this in the human retina because there is no animal model of ARMD and the results of this procedure are very dependent upon the unique conditions ARMD produces.

Foveal patch transplants in "wet" ARMD

The first group of patients had "wet" forms of ARMD. All had subfoveal neovascular membranes and were candidates for surgical resection of these membranes, a technique that can sometimes be beneficial (Thomas & Kaplan, 1991; Thomas, 1994). In subfoveal neovascularization the adjacent RPE layer is invariably involved by scarring and therefore tends to be removed by the surgery (Lopez *et al.*, 1991; Seregard *et al.*, 1994). Therefore, it is reasonable to try to replace this with a new RPE monolayer.

To do this we used a method that can transfer monolayer patches of cultured human fetal RPE from one culture plate to another (Gouras *et al.*, 1994a). One cuts out a small monolayer patch of RPE growing on a culture plate, sucks it into a glass micropipette and then ejects it into the subretinal space where it tends to unfold to form a quasi-monolayer intimately associated with the host's photoreceptor layer (Sheng *et al.*, 1994). Immediately after the subfoveal neovascular membranes are removed, such a patch of cultured human fetal RPE was injected into the subretinal space (Algvere *et al.*, 1994). Figure 1 illustrates a scanning laser ophthalmoscopic (SLO) view of one such transplant at 2 days (A), 1 month (B) and 3 months (C) after transplantation.

Up to 1 month this patient was detecting light and foveally fixating over the transplant. At 3 months,

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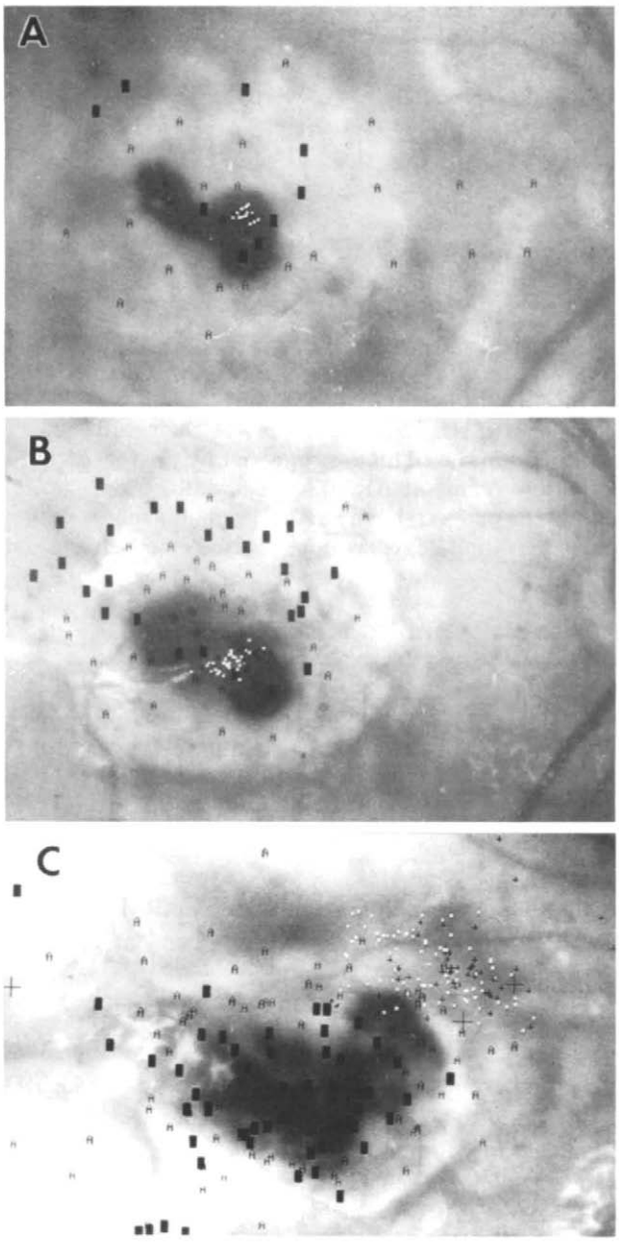

FIGURE 1. Fundus photographs of a 69-year-old subject who had received an RPE patch transplant at 2 days (A), 1 month (B) and 3 months (C) after surgery. The transplant appears as a dark patch in a lighter background, which delineates the area from which a subfoveal neovascular membrane had been removed. The size of the dark (pigmented) patch increases with time. The small white dots indicate fixation and the symbols (A) indicating visibility or ( ) lack of it, as determined by scanning laser ophthalmoscopy.

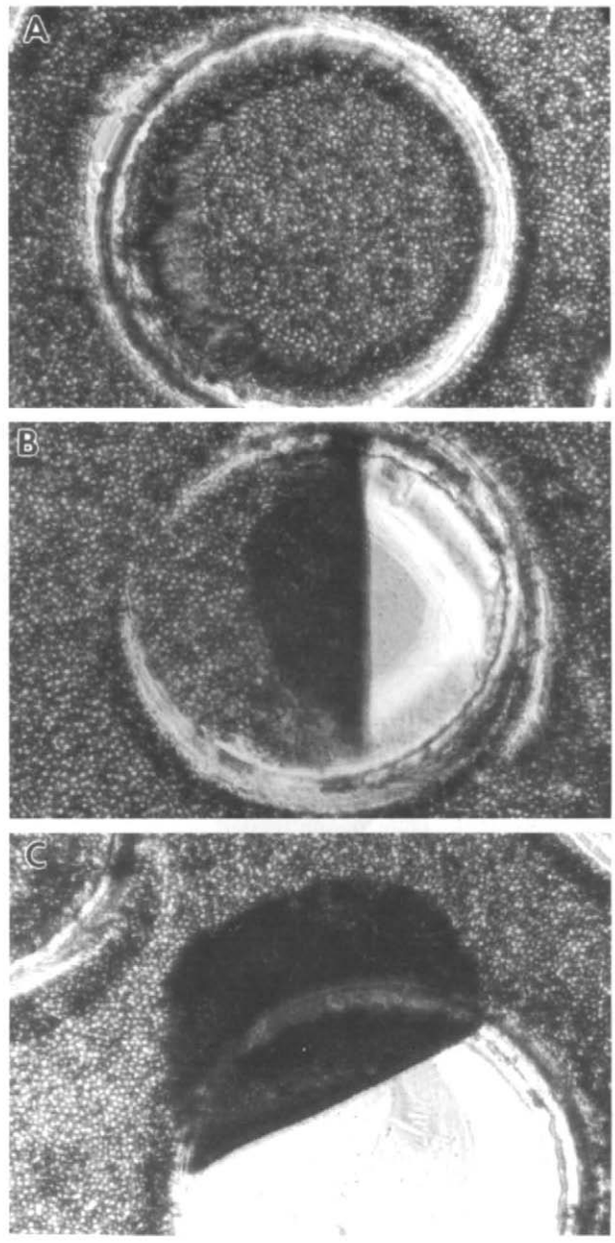

FIGURE 2. Human fetal RPE in culture. A trephine has cut a circular patch 0.6 mm in diameter (A). This patch can be gently rolled off its attachments to the culture plate (B) and (C) before being sucked into a transplant pipette.

however, retinal function over the transplant had diminished and fixation had become eccentric. During this time leakage of fluorescein slowly developed around and over the transplant (Algvere *et al.*, 1994). Only one patient did not have leakage but this was the only patient in which the transplant was placed adjacent to and not directly at the fovea. This patient has maintained almost the same visual acuity at 1 yr after transplantation as she had just prior to surgery. The fact that chronic leakage has been typical in these transplants suggests that they are encountering chronic rejection.

In prior animal experiments we found no rejection of RPE xenografts of cultured fetal human RPE transplants to monkey retina at 2–3 months after surgery (Sheng *et al.*, 1994). We have since extended these experiments to test 21 human RPE transplants in eight monkeys, 16 transplants in the perimacular region and five directly in the fovea (Gouras *et al.*, 1995; Berglin *et al.*, in preparation). After 6 months we examined each transplant histologically, performing quasi-serial sectioning. We found evidence of rejection in only 19% (3/16) of the perimacular transplants but 60% (3/5) of the foveal transplants. We concluded that RPE transplants, even xenografts, are not invariably rejected but those in the fovea appear to be more prone to rejection. We also

observed that all the rejected transplants showed leakage but non-rejected ones did not. Therefore the chronic leakage manifest in four of the five human RPE allografts, all in the fovea, could represent rejection. However, it was somewhat surprising that most monkey xenografts did not reject whereas most human allografts did. We hypothesized that the presence of an intact blood/retinal barrier was important because in the human subjects it had been compromised by both neovascularization and the surgery whereas in the monkey it was not.

Patch transplants in "dry" ARMD

In order to examine this experimentally we performed RPE transplantation in patients with advanced but "dry" forms of ARMD. In these patients no neovascular membrane exists and therefore the transplant could be introduced into the subretinal space with the blood/retinal barrier relatively undisturbed. In order to further minimize possible host/graft rejection we also reduced the size of the transplant.

In four of these patients we used a circular RPE patch transplant, 0.6 mm in diameter. By using circular patches there would be less corners to curl up to jeopardize the monolayer's flatness. With a circular transplant we would know its precise geometry visible in the fundus if it did not fold and deviations might provide clues to how folding occurs.

To produce circular RPE patch transplants we constructed a set of trephines that could cut out circular areas from 0.5 to 1.0 mm in diameter. We chose 0.6 mm diameters because these produced small enough patches that folded minimally when sucked into the transplant pipette and also had a relatively high ratio of untouched to trephined cells along its edge. Figure 2 shows a circular patch of cultured human fetal RPE trephined and rolled off a culture plate before being sucked into a transplant pipette (Lavid *et al.*, 1995).

We have transplanted such patches into four patients with advanced but "dry" ARMD. In order to further minimize possible rejection, we placed the transplants at the edge of the macula, away from the fovea. At 6 months after transplantation only one of four transplants shows any evidence of leakage. Because most of these transplants show no leakage and all support visual function through the adjacent photoreceptors, for now 6 months after surgery, we are concluding that the presence of an intact blood/retinal barrier tends to prevent the development of leakage from and probably rejection of these transplants.

We were not satisfied, however, for several reasons. The circular transplants are partially folded, appearing elliptical rather than circular. Although this does not cause the contiguous receptors to degenerate, it is less than optimal. In animal experimentation, such photoreceptors abutting a multilayered transplant are not as long as they are next to an unfolded one. We have measured the lengths of outer and inner segments and the width of the outer nuclear layer and compared them to the width of adjacent RPE transplants in monkeys. The thicker the transplant becomes due to folding, the more reduced is the width of the overlying photoreceptor layer although many photoreceptors, rods and cones, survive over multi-layered transplants (Berglin *et al.*, in preparation).

In addition, we have not covered the foveal area which we believe to be the most critical to save from the destruction of ARMD. We would like to cover the fovea but this must be performed as gently as possible because we know from monkey experiments that the foveal area appears to be more vulnerable to rejection.

Cell suspension RPE transplants in "dry" ARMD

In order to cover the foveal atraumatically with healthy RPE we are using concentrated suspensions of partially dissociated RPE cells. The dissociation leaves small groups of RPE cells, 10–15 together among smaller groups of individual cells.

We used this method before (Gouras *et al.*, 1986; Lopez & Gouras, 1987) but have improved tailoring the size of the detachment to the volume of the cell suspension. This produces a more complete coverage as the cells are captured by the reattaching neural retina. This method allows a smaller orifice in the transplant pipette, about 0.05 mm, compared to 0.2–0.3 mm for transplanting a complete RPE patch. The retinotomy is smaller and self-seals, preventing any cells from efflux-ing into the vitreal chamber. The smaller size and the self-sealing action of the retinotomy are advantages that facilitate RPE transplantation in the submacular space.

We have performed such RPE cell suspension transplants on four patients with "dry" forms of ARMD. The cell suspension spreads across the subretinal space, including the fovea. These patients, now 3 months after surgery, have the same vision as they had before surgery and have no evidence of leakage even though the fovea has been included in the transplant area.

So far we have performed RPE transplantation in 13 patients, five with "wet" and eight with "dry" forms of ARMD. Most of the former slowly develop chronic leakage over the transplant; most of the latter do not. Therefore, we believe that rejection seldom occurs with RPE allografts to the subretinal space if the blood/retinal barrier is intact. If there is no chronic leakage visual function is maintained over the transplant, implying that the transplants are functioning. Whether such function can arrest or reverse the progression of ARMD remains to be seen.

The best analogy is the RCS rat model. If one transplants a suspension of healthy RPE cells into the subretinal space of the RCS rat (Reppucci *et al.*, 1988) before the photoreceptors have degenerated, this degeneration can be prevented (Li & Turner, 1988; Lopez *et al.*, 1989; Gouras *et al.*, 1989) essentially for the life of the animal (LaVail *et al.*, 1992; Yamamoto *et al.*, 1993). A similar strategy may help in ARMD if the RPE cell is the major cause of the disease.

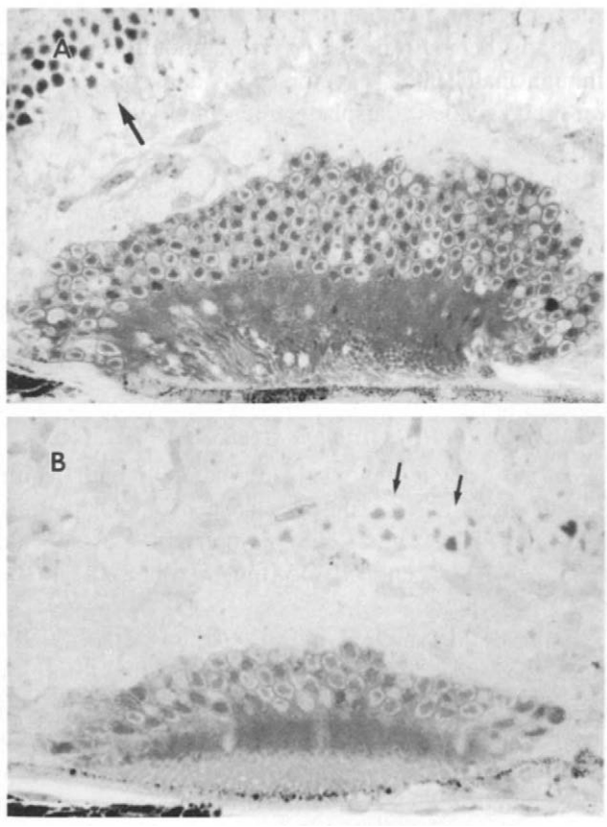

FIGURE 3. Transgenic LacZ expressing (shaded) photoreceptors that have been transplanted into the subretinal space of normal mice 4 (A) and 6 (B) months previously. The arrows identify nuclei of host photoreceptor cells.

Photoreceptor transplantation

Will it be possible to restore visual function to a macula that has lost it? This is a difficult question and its answer will depend on whether the responder is an optimist or a cynic. I shall give the optimistic response.

Neural retina, especially embryonic tissue, survives well when transplanted to a foreign host brain (Lund, 1991) or retina (Turner & Blair, 1986; del Cerro *et al.*, 1989; Silverman & Hughes, 1989; Aramant *et al.*, 1990; Ehinger *et al.*, 1991; Gouras *et al.*, 1991). These transplants are capable of delivering synaptically transmitted information about light stimulation to the brain (Lund, 1991). This has not yet been demonstrated unequivocally for intraretinal transplants but the results are promising. All attempts to transplant neural retina have led to rosette formation. Rosettes are balls of retinal cells within which outer segments form centripetally. These outer segments can develop reasonably well (Ehinger *et al.*, 1991) and mediate responses to light (Adolph *et al.*, 1994). These photoreceptors inevitably degenerate, however, because they are not in contact with the RPE layer and the formation of rosettes is also not conducive to effective visual function.

We have shown that if one uses relatively small aggregates of neonatal retina, and the orientation of the transplant to the RPE layer is correct which occurs about 50% of the time, healthy photoreceptors with normal outer segments will form in contiguity with the foreign host RPE without rosette formation. These photoreceptors will survive essentially for the life of the animal, a mouse (Gouras *et al.*, 1992,b). Figure 3 shows an example of transplants at 4(A) and 6(B) months after surgery. Cones as well as rods can survive transplantation.

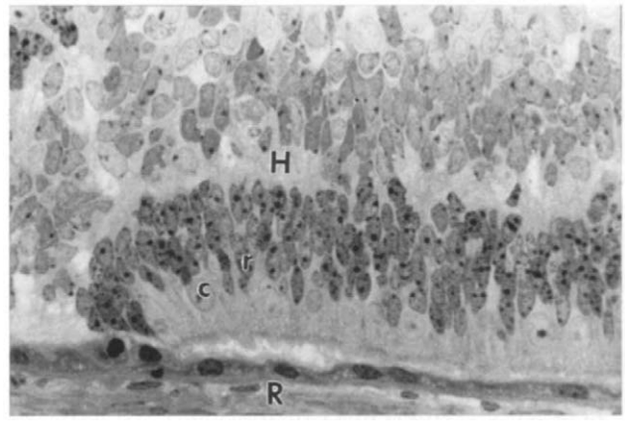

FIGURE 4. A transplant of fetal human neural retina to the subretinal space of an immunosuppressed rabbit retina at 1 week after surgery. The human transplant (H) shows both inner and outer nuclear layer, the latter contains both cones (c) and rods (r) which are in close contact with the rabbit (R) RPE layer.

For the human fovea, cones are the critical photoreceptors. The only way to learn how to transplant cones experimentally is to work with primates, preferably human fetal tissue, which has been opened up in the U.S.A. by the Clinton administration. It would be extremely difficult to do this using fetal monkey tissue. Figure 4 shows such a human fetal photoreceptor transplant properly oriented to the RPE layer of an immunosuppressed host rabbit, 1 month after surgery. This was an 18 week old human fetal specimen now one additional month in a foreign host retina. It shows numerous human cones as well as rods surviving well and oriented correctly. Very little outer segment material has yet developed but the appearance of these cones and rods is promising (Lin *et al.*, 1995). One has to temper this optimistic view of photoreceptor transplantation with the sober reality that it is an extraordinarily difficult feat. Many hurdles face the experimenter, gliosis, mechanical fragility of the tissue, cone alignment and synaptic precision that defies current microsurgical technology. It also defies the more cautious views of funding agencies so necessary to support such research. In addition, unjustified exaggeration gives false hopes to the blind. Nevertheless the concept is rational, perhaps like an Archimedean dream of moving the earth with a big enough stick. Here it is more like moving a cone if you had a small enough stick.

CONCLUSION

We have shown that it is possible to begin reconstructing the human macula from the ground up, i.e., from the RPE layer inward. The RPE layer is the most tractable

because it is non-neural and functions merely by close apposition with the photoreceptors. It is also possible to transplant photoreceptors by placing them in the proper orientation in the subretinal space, where they survive indefinitely. Therefore, the building blocks for reconstructing the outer retinal layers are viable and functional as transplants. Whether it will actually restore vision to a blind retina remains to be seen.

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