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Progress in retinal sheet transplantation

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Abstract

The aim of retinal transplantation is to prevent blindness and to restore eyesight, i.e. to rescue photoreceptors or to replace damaged photoreceptors with the hope of re-establishing neural circuitry. A promising experimental paradigm is the sub-retinal transplantation of sheets of fetal retina, with or without its attached retinal pigment epithelium (RPE), into recipient rats with retinal degeneration. Sheets of fetal retina have already developed their primordial circuitry. Such transplants can develop lamination resembling a normal retina dependent on the presence of healthy RPE either from the host or from the graft. In several retinal degeneration models, transplants have been shown to restore visually evoked responses in an area of the superior colliculus corresponding to the placement of the transplant in the retina. The functional effect of transplants may be due to transplant/host connectivity and/or rescue of host photoreceptors.

In summary, sheets of fetal retina can morphologically repair an area of a degenerated retina, and there is evidence to suggest that transplants form synaptic connections with the host and restore visual responses in rats with retinal degeneration.
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Contents

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1. Introduction

When retinal transplantation studies began in the 1980s, many researchers had the unrealistic expectation that a cure for retinal blindness could be quickly achieved. Since this objective could not be met in a short period of time, the interest of funding agencies for this field has waned, and has further handicapped efforts to make this research effort succeed. This review article will discuss the progress that has been made since a previous review (Aramant and Seiler, 2002b) and will clarify the potential of retinal transplantation for the treatment of retinal degenerative disease.

In many diseases of the outer retina, the inner part of the neural retina can still remain relatively functional over a certain time period (Santos et al., 1997; Humayun et al., 1999; Johnson et al., 2003) (for review, see Pacione et al., 2003). In a study of pigmented Royal College of Surgeons (RCS) rat retina up to the age of 515 days, no abnormalities in synaptic counts and in ganglion cell characteristics were found by electron microscopy (Eisenfeld et al., 1984). However, this initial study was contradicted by subsequent studies showing that changes in ganglion cells occur about 3 months after photoreceptor loss in the RCS rat, due to the abnormal ingrowth of blood vessels from the choroid (Caldwell et al., 1989; Villegas-Perez et al., 1996; Wang et al., 2003). Similar changes occur in the *rd* mouse retina (Liu et al., 2000; Wang et al., 2000; Liu et al., 2001). Remodeling of the inner retina is a major secondary effect of outer retinal degenerations (Jones et al., 2003; Marc et al., 2003). It is thought that this process occurs due to denervation of the inner retinal neurons, and subsequent attempts by these neurons to find new synaptic input. This process involves cell death; rewiring, i.e. the formation of new circuits to replace lost innervation; and cell migration (Marc et al., 2003).

1.1. Therapies for retinal degeneration

It is well established that transplantation of retinal pigment epithelium (RPE) cells (review: Lund et al., 2001b), iris pigment epithelium (Rezai et al., 1997; Abe et al., 1999, 2000a; Schraermeyer et al., 2000) and Schwann cells (Lawrence et al., 2000; Keegan et al., 2003) can rescue existing photoreceptors in a degenerating RCS rat retina. This approach, apart from replacing dysfunctional RPE cells, is in large part based on the delivery of trophic factors by transplanted cells, as similar effects have been achieved by delivery of trophic factors alone to the eye (review: Chaum, 2003), which act on photoreceptors through Mueller cells (Wahlin et al., 2000, 2001). Delaying the loss of photoreceptors, through use of trophic factors, would slow down remodeling of the inner retina.

However, can anything slow down inner retinal remodeling in later stages of retinal degeneration when all rod photoreceptors have been lost? Several groups have transplanted retinal tissue to replace lost photoreceptor cells (review: Aramant and Seiler, 2002b). It has been shown that transplants of rods can slow the degeneration of cones (Silverman et al., 1993; Mohand-Said et al., 1997, 2000). This cone-preserving activity is due to a diffusible factor, specifically produced by rods (Mohand-Said et al., 1998), which has been characterized biochemically and found to be distinct from known trophic factors (Fintz et al., 2003). Another group has also observed a cone-rescue effect with retinal sheet transplants in *rd* mice (Arai et al., in press).

This review is focused on the approach of replacing lost or damaged cells, specifically by transplantation of neuroblastic retinal progenitor cells as sheets. This is based on the hypothesis that a degenerated retina can be repaired and vision might be restored if the diseased photoreceptors can be replaced with new functional cells which can make appropriate connections with the remaining functional part of the retina. The benefit of additional trophic effects of such transplants on the host retina must be considered.

As many retinal diseases affect both photoreceptors and RPE (Pacione et al., 2003), both cell types need to be replaced. However, this possibility is rarely discussed. One group has developed a unique approach: transplantation of sheets of fetal retina together with its RPE (Aramant et al., 1999; Aramant and Seiler, 2002c) to meet this obvious requirement (see Section 4).

The immunology of neural retinal and RPE transplants has been discussed in previous reviews (Aramant and Seiler, 2002b; Lund et al., 2003) and will not be discussed here.

1.2. History of retinal transplantation

In 1959, Royo and Quay reported transplantation of fetal eyes to the anterior chamber of rat eyes (Royo and Quay, 1959). Different retinal transplantation approaches were taken up again in the 1980s by transplantation of RPE (Gouras et al., 1983, 1984), and, in 1986, the first retina-to-retina transplant paper was published (Turner and Blair, 1986). Human retinal cells were first transplanted to a retinal lesion site in rats in 1990 (Aramant et al., 1990). A more detailed review is given in Aramant and Seiler (2002b).

The earliest transplantation experiments used small retinal pieces (aggregates) or dissociated cells, which develop rosettes because of the disruption of the donor tissue (Turner and Blair, 1986; Aramant et al., 1988; Del Cerro et al., 1989; Aramant and Seiler, 1991). After 8 months of cryopreservation, transplants of embryonic retina could survive and develop retinal layers (Aramant and Seiler, 1991). An important observation of this study was that transplants appeared to develop better lamination in the subretinal than in the epiretinal space (Aramant and Seiler, 1991).

After initial experiments of transplanting dissociated adult rods to RCS rats (Gouras et al., 1991a,c) or *rd* mice (Gouras et al., 1991b, 1992), Gouras developed the microaggregate procedure that consisted of cutting the neonatal retina into pieces that were small enough to pass through an injection needle without further disruption. This way, some of the transplanted pieces were randomly placed with the right polarity and could develop photoreceptor outer segments towards the *rd* mouse host RPE (Gouras et al., 1994; Gouras and Tanabe, 2003). The method was later used for transplants in the cat (Ivert et al., 1998).

Mechanically dissociated postnatal (P7-9) mouse retinal cells transplanted to 6–8-week-old *rd* mice could develop relatively large areas of photoreceptors in contact with the host RPE and with synaptic layers at the transplant/host interface (Kwan et al., 1999). Transplanted mice showed a return to a normal light/dark preference at 2 weeks after transplantation. The development of a synaptic circuitry between transplant and host was suggested by the authors.

The synaptic integration between retinal microaggregate transplants, expressing *Escherichea coli* β-galactosidase in rod photoreceptors, and an *rd* host retina, expressing *E. coli* β-galactosidase in rod bipolar cells has been further investigated ultrastructurally (Gouras and Tanabe, 2003). They found close membrane-to-membrane contact between transplant rods and host bipolar cells, but no evidence for synapse formation. Glial processes formed extensions between transplant and host.

The first demonstration that fetal retinal sheets grafted to normal and damaged retina could develop into laminated transplants was presented in 1995 (Aramant and Seiler, 1995) (see Sections 1.1.5 and 3).

1.3. Label of donor tissue for transplantation

Researchers have used nuclear markers, such as ^{3}H-thymidine (Gouras et al., 1991c) or bromo-deoxyuridine (Seiler and Aramant, 1995) to label dividing cells in the donor tissue before transplantation. Similar to other nuclear markers, such as the y-chromosome (to transplant male tissue into a female recipients) (Wang et al., 2004), these methods have the disadvantage that cell processes of transplanted cells cannot be followed. Thus, transgenic animals were used that expressed marker molecules in the cytoplasm. For example, Gouras et al. (Gouras and Tanabe, 2003) transplanted donor tissue expressing X-gal in rod photoreceptors into recipients expressing X-gal in rod bipolar cells. The GFP mouse (Okabe et al., 1997) has become very popular for mouse studies of retinal transplantation (e.g., Arai et al., in press; Sakaguchi et al., 2003). Many groups have transfected donor cells with vectors carrying GFP (Lai et al., 1999; Hansen et al., 2003). However, this method likely does not work with tissue sheets because the virus will only infect cells on the surface and not penetrate into the tissue.

Recently, transgenic rats expressing human placental alkaline phosphatase (hPAP) in the cytoplasm of all cells (Kisseberth et al., 1999; Mujtaba et al., 2002) have been used as donor tissue. Before transplantation, hPAP-expressing fetuses can easily be selected by a histochemical stain. Such donor tissue provides a reliable cytoplasmic marker, outlining cells and their processes

to show the integration of retinal transplants in a degenerated host retina (Aramant and Seiler, 2002a). Using histochemistry or immunohistochemistry, hPAP-labeled donor cells can unequivocally be identified in the host eye.

1.4. Transplants of stem/progenitor cells

There is still confusion about the term "stem cell transplantation". The word "stem cell" is very popular, but should be restricted to cells early in development that have the capacity to differentiate into different cell types and different tissues.

Transplantation of multipotential retinal progenitor cells (reviewed in Ginis and Rao, 2003; Sakaguchi et al., 2003; Klassen et al., 2004) is not the main subject of this review. However, since there is considerable recent interest in the transplantation of stem/progenitor cells, some of the important studies in this field will be mentioned. In the mid-1990s, studies showed that it was possible to isolate progenitor cells from the adult brain by treatment with epidermal growth factor (EGF) and/or fibroblast growth factor (FGF) (Gage et al., 1995) and to use transfected cells for the production of growth factors in the brain (Martinez-Serrano et al., 1995). Neural progenitor cells were also isolated from the developing brain and transplanted to different models of neurodegenerative disease (Rosario et al., 1997; Svendsen et al., 1997). Later, adult hippocampus-derived progenitor cells were transplanted to the eye/retina of retinal degeneration models (Takahashi et al., 1998; Young et al., 2000). Brain-derived progenitor cells integrated very well into developing or into injured/diseased retina (Van Hoffelen et al., 2003); however, they did not integrate well into normal un-injured retina (Kurimoto et al., 2001; Chacko et al., 2003). Brain-derived progenitor cells integrated in the host retinal layers and differentiated their morphologies to look like retinal cells, but they did not express specific retinal cell markers (Takahashi et al., 1998; Chacko et al., 2000; Young et al., 2000). However, when transplanted to an immature developing retina, brain-derived progenitor cells develop more similarities with retinal neurons (Sakaguchi et al., 2003; Van Hoffelen et al., 2003).

What about retinal progenitor cells, in contrast to brain-derived cells? Retinal stem cells can be isolated from the ciliary margin of the adult retina (Tropepe et al., 2000), or from fetal retina (Chacko et al., 2000; Lu et al., 2002; Yang et al., 2002a,b; Qiu et al., 2004). As with brain progenitor cells, the degree of integration and migration into the host retina depends on the age or the disease/injury status of the recipient retina. Although transplanted progenitor cells can express opsin (Chacko et al., 2000), their differentiation potential appears to be limited to a glial lineage after transplantation to an adult host with retinal degeneration (Yang et al., 2002a). However, preliminary results indicate retinal progenitor cells that have been maintained in defined culture conditions (Qiu et al., 2004) develop transplants containing mostly opsin expressing cells with a good integration in young recipients with slow and fast retinal degeneration (Mui et al., 2004). It will be interesting to see whether they can differentiate fully as functional retinal cells, synaptically connect to other cells of the transplant and host retina, and have a clear beneficial effect on a host with retinal degeneration.

A recent review "Stem cells and retinal repair" gives a good overview of the studies with brain-derived stem cells, however, mentions little about transplantation of retinal progenitor cells and only speculates about hopeful uses and performance of stem cells to repair a retina (Klassen et al., 2004). In spite of the very promising characteristics and high expectations of stem/progenitor cells, the results so far indicate that there is a long way to go before transplantation of dissociated progenitor cells may be clinically applicable.

1.5. Transplants of retinal sheets

Silverman isolated photoreceptor sheets from adult and postnatal day 8 rat retinas by vibratome sectioning retinal wholemounts, which were then transplanted to the subretinal space (Silverman and Hughes, 1989; Silverman et al., 1992). However, it was important to include part of the inner nuclear layer (with Mueller cells) in the sheets to avoid rosette formation (Silverman et al., 1991). Silverman's procedure was later refined by using an excimer laser to isolate human cadaver photoreceptors (Kaplan et al., 1997; Huang et al., 1998; Tezel and Kaplan, 1998; Berger et al., 2003).

Silverman's method was modified to transplant full-thickness fetal retina to normal rabbits (Ghosh et al., 1998, 1999b,c; Ghosh and Ehinger, 2000), pigs (Ghosh and Arner, 2002), and cats (Bragadottir and Narfstrom, 2003). The donor tissue was rolled up in a cannula. The method required a second retinotomy and the induction of a local retinal detachment in which the donor tissue could be unrolled. If placed in the right polarity, such transplants can develop almost normal lamination. However, the polarity of the donor tissue cannot entirely be controlled with such an approach (Ghosh et al., 1999d), and the bleb formation and the required extra retinotomy may cause additional trauma to the donor tissue and the host retina. Another problem with this technique is the lack of integration between transplant and host retina, as seen in transplants performed in the pig and cat.

Since there are no suitable retinal degeneration models in the rabbit, the relevance of this transplant model for retinal degeneration is limited. In addition, the rabbit host retina overlying the transplant degenerates after longer survival times (Ghosh et al., 1999b, c)

because the rabbit retina is avascular except for the central horizontal streak; and its main nourishment comes from the choroid.

In contrast to transplants in the rabbit, transplants to the vascularized pig (Ghosh and Arner, 2002; Ghosh et al., 2004) or cat retina (Bragadottir and Narfstrom, 2003) do not form any contacts with the host retina, except for a few areas where host photoreceptors are missing. This can be either due to the age of the donor tissue (neonatal vs. fetal) or the presence of photoreceptors in the host retina. On the other hand, the subretinal transplant does not cause degeneration of the inner retina as in the rabbit since the host retina is vascularized (Ghosh and Arner, 2002; Bragadottir and Narfstrom, 2003; Ghosh et al., 2004).

Aramant and Seiler have developed a different approach with a specially designed instrument and procedure to transplant fragile fetal retinal neuroblastic progenitor cells as a sheet into the subretinal space (review in Aramant and Seiler, 2002b). In adult rat recipient models of retinal degeneration, an area of a damaged retina can be "repaired" by a sheet of fetal retina with or without RPE. Details of these models and their results are further described below.

2. Retinal degeneration models used for transplantation

Different models of photoreceptor degeneration have been used to test experimentally whether retinal transplants can restore vision and/or delay retinal degeneration.

2.1. Light damage

Silverman and Del Cerro used light damaged albino rats as recipients for retinal transplants (Silverman and Hughes, 1989; Del Cerro et al., 1990, 1991). Their light damage procedures consisted in exposing albino rats to continuous white light for 2–4 weeks at 1900 lux (Silverman and Hughes, 1989) or 4–5 weeks at 3000–3500 lux (Del Cerro et al., 1990, 1991) with the light coming from the top of the cage. Such an intense exposure will inevitably also harm the RPE cells, as can be seen in (Del Cerro et al., 1995).

Another light damage model was developed by exposing albino rats for only 2–4 days to blue light of moderate intensity (680–1290 lux) surrounding the cage. This leads to apoptosis of almost all photoreceptors and reduces average ERG a- and b-wave amplitudes by about 98% while keeping the RPE intact, (Seiler et al., 2000). However, photoreceptors in the retinal periphery could not be completely eliminated. This light damage model was used for the first experiments with fetal intact-sheet transplants (Seiler and Aramant, 1998; Seiler et al., 1999b).

2.2. Inherited models of retinal degeneration

2.2.1. rd mice

A well-known retinal degeneration model, the *rd* mouse, has a defect in the β-phosphodiesterase gene (Bowes et al., 1990). Rod photoreceptors start to degenerate at postnatal day 11 before outer segments can form (Blanks et al., 1974; Carter-Dawson et al., 1978; Bowes et al., 1988; Kuo et al., 1989). Retinal remodeling, i.e. changes in the inner retina, start occurring soon after rod photoreceptors degeneration (Ogilvie et al., 1997; Peng et al., 2000; Strettoi and Pignatelli, 2000; Strettoi et al., 2003). Transplanted photoreceptors are easily identifiable because the retina does not have any rods left at the age of 3 weeks. Microaggregate retinal transplants (Gouras et al., 1992, 1994; Radner et al., 2001; Gouras and Tanabe, 2003), mechanically dissociated retinal cells (Kwan et al., 1999), photoreceptor sheets (Mohand-Said et al., 1997) and fetal retinal sheets (Arai et al., in press) have been transplanted to *rd* mice. Only when the mice were transplanted at an early age, an effect on rescuing visual responses was seen because of the rapid retinal degeneration (Kwan et al., 1999; Radner et al., 2001).

2.2.2. RCS rat—prominent photoreceptor rescue effect by RPE transplants and sham surgery

In the RCS rat, a well-established model of RPE dysfunction with subsequent photoreceptor degeneration (D'Cruz et al., 2000), RPE cells are unable to phagocytose rod outer segments. The accumulating debris in the subretinal space leads to photoreceptor death. Changes in the inner retina (Fletcher and Kalloniatis, 1996; Hanitzsch et al., 1998) and the ganglion cell layer (Villegas-Perez et al., 1996, 1998) occur in later stages, due to photoreceptor loss and choroidal neovascularization (Jones et al., 2003).

At the age of 3–4 weeks, before complete loss of the outer nuclear layer, transplantation of RPE can rescue photoreceptors (Li and Turner, 1988; Lopez et al., 1989; Li et al., 1990). The analysis of the transplantation results is made more complicated by the sham surgery effect on promoting photoreceptor recovery and survival, indicating that lack of trophic factors causes photoreceptor apoptosis in the RCS rat. Sham surgery has a transient rescue effect (Silverman and Hughes, 1990), which is due to the upregulation of trophic factors after injury (Humphrey et al., 1997). RCS rat photoreceptors can be rescued by bFGF treatment (Faktorovich et al., 1990). Transplantation of other cell types producing trophic factors, such as embryonic stem (ES) cells (Schraermeyer et al., 2001), and Schwann cells (Lawrence et al., 2000) which can be transfected to produce additional trophic factors (Lawrence et al., 2004), also have a rescue effect.

Photoreceptor rescue can be demonstrated by electrophysiological (Yamamoto et al., 1993; Jiang and Hamasaki, 1994; Sauvé et al., 1998; Girman et al., 2003) and behavioral tests (Little et al., 1998; Coffey et al., 2002). Iris pigment epithelial cells (IPE), which can easily be isolated from the recipient, have also been shown to delay photoreceptor degeneration (Rezai et al., 1997; Schraermeyer et al., 1999). However, the effect of IPE transplants is not different from sham surgery (Schraermeyer et al., 1999), and IPE transplants even have an effect when grafted to the choroid (Schraermeyer et al., 2000).

Adult RCS rats have seldom been used as recipients of neural retinal transplants (Gouras et al., 1991c; Aramant et al., 1999). Aramant et al. transplanted sheets of fetal retina together with its RPE into RCS rats at the age of 1.3 to 2.1 months (Aramant et al., 1999). At this late time point, transplantation of dissociated RPE cells has no rescue effect on photoreceptors. On the other hand, sheet co-transplants of retina with RPE can restore visual responses in the superior colliculus up to 6.8 months after transplantation (Woch et al., 2001) (see Section 5). Although there was a sham surgery effect, the visual responses in sham surgery rats were significantly different from those of transplanted rats.

2.2.3. Transgenic rodent models

There are many transgenic mouse models of retinal degeneration available. They have been used in different attempts to rescue photoreceptors by trophic factors (LaVail et al., 1998) and gene therapy (review: Adler et al., 1999; Pacione et al., 2003), and some have been used for transplantation of neural precursor cells (Pressmar et al., 2001).

La Vail et al. have created eight different lines of transgenic rats, carrying either the P23 H or the S334ter rhodopsin mutation with different rates of photoreceptor degeneration (Nishikawa et al., 1997; Steinberg et al., 1997; Liu et al., 1999). Few of these lines have been characterized (An et al., 2002; Jones et al., 2003; Sagdullaev et al., 2003; Thomas et al., 2004b). The rats were produced by Chrysalis DNX, Princeton, NJ. Some of these rat lines have been used for testing of various strategies of photoreceptor rescue (Liang et al., 2001; McGee Sanftner et al., 2001). Two of these lines (S334ter lines 3 and 5) are routinely used for transplantation of fetal retinal sheets (Sagdullaev et al., 2003; Thomas et al., 2004b).

2.2.4. Larger animal models

Monkeys have been used as recipients for transplants of RPE (Gouras et al., 1984; Sheng et al., 1995; Murata et al., 2000) and IPE cells (Abe et al., 2000a), but not as recipients of retinal transplants.

Small pieces of 3–5 day old kitten retina were transplanted to the subretinal space of Abyssinian cats (Ivert et al., 1998). These transplants developed rosettes. This cat model was later also used for transplantation of full-thickness retinal sheets (Bragadottir and Narfstrom, 2003). The developed transgenic pig retinal degeneration model (Li et al., 1998) has recently been used for transplantation of full-thickness retinal transplants (Ghosh et al., 2004).

3. Morphological repair of damaged retinas by fetal retinal sheets

3.1. Introduction

The advantages of retinal sheet transplantation and the detailed method are discussed in (Aramant and Seiler, 2002b). The fetal retinal donor tissue is freshly isolated (not placed in tissue culture) and dissected as a sheet before transplantation. The instrument consists of a hand piece and a flexible silicon nozzle, which can be made with different sizes and curvatures depending on the size of the animal's eye, and on whether a transscleral or a transvitreal approach is used. The tissue is loaded in the nozzle tip and is locked in place. Transplantation places the donor tissue (no injection) with a very small amount of medium to the eye, thus requiring only a minimal bleb that causes minimal trauma.

For transplantation into rodents, the approach is transscleral via a small incision behind the pars plana. In larger eyes (such as cats and humans), a standard transvitreal approach with vitrectomy and retinotomy is used (Radtke et al., 1999, 2002, 2004). After insertion of the instrument into the subretinal space, the nozzle is retracted over a fixed mandrel (non-movable plunger), which exposes the tissue and places it in the target area. Thus, the tissue is not pushed or injected into the site. The placement is entirely controlled by the surgeon.

3.2. Difficulties/requirements

However, the percentage of transplants developing a lamination similar to normal retina is only about 25–33% in rats, the rest will develop rosettes (Seiler and Aramant, 1998). Sometimes, transplants with photoreceptor rosettes can have parallel inner retinal layers. The percentage is still lower in *rd* mice (Arai et al., in press) because the tiny mouse eyes are especially challenging. There are several reasons for this. Because of the transscleral approach used in rodents, the surgeon cannot see where the tissue is placed. Thus, damage to RPE or Bruch's can easily happen if the instrument is inserted in the wrong angle. Damage to RPE, Bruch's

membrane or the donor tissue usually results in rosette formation and prevents the maintenance of outer segments. Transplants accidentally placed into the vitreous will also form rosettes, but can sometimes be well organized with the exception that the photoreceptor outer segments degenerate.

When the recipient's photoreceptor degeneration has advanced too far with choroidal neovascularization and with tight adherence of the retina to the RPE, no laminated transplants can be achieved because force has to be used to try to detach the host retina, which will inevitably cause major host tissue disruption.

4. Transplantation of sheets of retina together with its RPE—a necessary and unmatched approach

4.1. Why cografts?

Transplantation of RPE only, whether dissociated or sheets, seems to have many problems. A variety of methods have been used to mimic a monolayer of RPE cells by culturing them on various matrices, but they all appear to have failed. Even when transplanted as freshly dissected gelatin-embedded sheets, RPE cells do not stay as monolayers with junctional complexes, but migrate into the host retina and/or form cell clumps already after 7 days (Wang et al., 2004). This is similar to what has been observed in clinical trials with AMD patients (Del Priore et al., 2001). In addition, there are problems with rejection when allogeneic RPE transplants are used (Algvere et al., 1999).

The transplantation of either RPE, stem cells or photoreceptors alone, or rescuing strategies with growth factors or gene therapy cannot restore vision if photoreceptors and RPE have degenerated, as is the case in many outer retinal diseases. After transplantation of neural retinal sheets without RPE, transplant photoreceptors will develop fully and maintain outer segments only if supported by healthy RPE (Aramant and Seiler, 2002b). In a degenerating host retina, the RPE cells may have lost their ability to support the development of the fetal neural retinal sheet transplant. However, transplanting retina together with its RPE will compensate for this because a beneficial reciprocal relationship between the two donor tissues can be maintained, including the nourishment of the photoreceptors from the choroid. In addition, the RPE will produce growth factors and interact with the retinal transplant. The transplanted RPE will also profit from the developing neural retinal transplant.

4.2. Difficulties/requirements

The dissection procedure has been described in detail elsewhere and is based on dispase treatment of the fetal eyeballs (Aramant et al., 1999; Aramant and Seiler, 2002c).

Compared to transplantation of fetal retina only, the cografting of fetal retina with its RPE poses an additional difficulty: the fetal RPE can easily loosen from the retina during removal of the choroid because the photoreceptor outer segments have not yet developed. This is discussed in (Aramant and Seiler, 2002b).

According to these studies, transplants require healthy RPE (either from the host or the donor) to develop normal appearing photoreceptor outer segments. However, after grafting of retina-only to RCS rats, some transplants still maintained shorter outer segments in contact with the host RPE after 5–7 months (Aramant and Seiler, 2002b).

An interesting observation: the host RPE disappears in many cases, so that the donor RPE settles directly on Bruch's membrane. Alternatively, in few cases, donor RPE can be seen on top of the host RPE, with the development of a second basal lamina separating the two tissues. This did not negatively influence the development of transplant photoreceptors.

4.3. Cografts of human fetal retina with RPE

To obtain data for design of pilot clinical trials, a study was performed to investigate whether cografts of human fetal retina and RPE to nude rats could develop and maintain their cytoarchitecture after long survival times (Aramant and Seiler, 2002c). 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 with junctional complexes between the cells, which supported the development of the retinal sheet with a normal lamination, including photoreceptor inner and outer segments (see Fig. 1). As the recipient rats 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. These results suggested that transplanted sheets of fetal retina with RPE could develop a normal morphology; and that this would also be feasible in human patients.

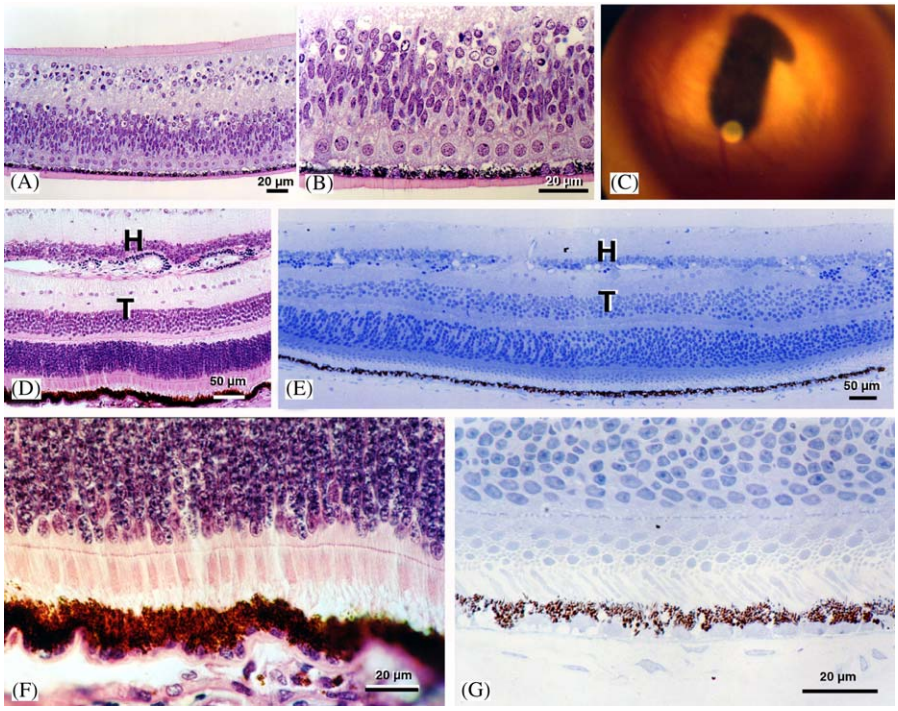

Fig. 1. Donor tissue and morphology of laminated co-grafts of human fetal retina to athymic nude rats. (A,B) Fetal human retina, 13 weeks gestation, with RPE. (C) Pigmented transplant in the back of an albino rat eye, 3 months after surgery. This transplant showed a good lamination when analyzed at 8.9 months after surgery (see 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. Taken from Fig. 1 of Aramant and Seiler, 2002: Transplanted sheets of human retina and retinal pigment epithelium develop normally in nude rats, Exp. Eye Res. 75(2), 115–125; © 2002 Elsevier; with permission.

5. Functional effects

5.1. Light/dark shift of phototransduction proteins in transplant photoreceptors

Do the photoreceptors in retinal transplants respond to light, and in what way?

Photoreceptors are responsible for transforming light into electrical signals—the phototransduction process. Some of the proteins involved in this process, such as rod-α transducin and S-antigen (rod arrestin), migrate in the photoreceptor cell depending on the light/dark cycle (Whelan and McGinnis, 1988). This translocation depends on the cytoskeleton (McGinnis et al., 2002). In *rds* mice with slow retinal degeneration that do not form outer segments, this light/dark depending translocation does not occur—S-antigen stays in the inner segments (Nir and Agarwal, 1991). The translocation of S-antigen is independent from transducin signaling (Mendez et al., 2003).

Seiler et al., 1999b, showed that this translocation also occurs in sheet transplant photoreceptors. Light-damaged albino rat eyes with retinal transplants were fixed in light or dark. In light adapted transplants, rod inner segments predominantly stain for rod α-transducin (Fig. 2). In dark-adapted animals, transducin shifted to the outer segments. S-antigen (rod arrestin) showed a reversed distribution to transducin. Rhodopsin did not change as was expected. Photoreceptors of transplanted retinal sheets show a normal shift of signal transduction proteins, thus apparently can respond to light similar to normal photoreceptors (Seiler et al., 1999b).

5.2. Transplant effect on visual behavior

The "startle reflex" used by Del Cerro (Del Cerro et al., 1991) showed that aggregate retinal transplants had some small effect on vision in light-damaged rats.

The pupillary light reflex, which has been used to test retinal transplants placed into the brain (Klassen and Lund, 1990; Radel et al., 1992, 1995), or the eye (Silverman et al., 1992) or to test the effect of RPE transplants in the RCS rat (Klassen et al., 2001), is apparently not correlated to the number of functional photoreceptors (Kovalevsky et al., 1995), can even be present in mice with severe retinal degeneration, and is mediated by melanopsin containing ganglion cells (Semo et al., 2003). Thus it is unreliable as a test for retinal transplants. The details of these and other tests are discussed in previous reviews (Lund et al., 2001b; Aramant and Seiler, 2002b).

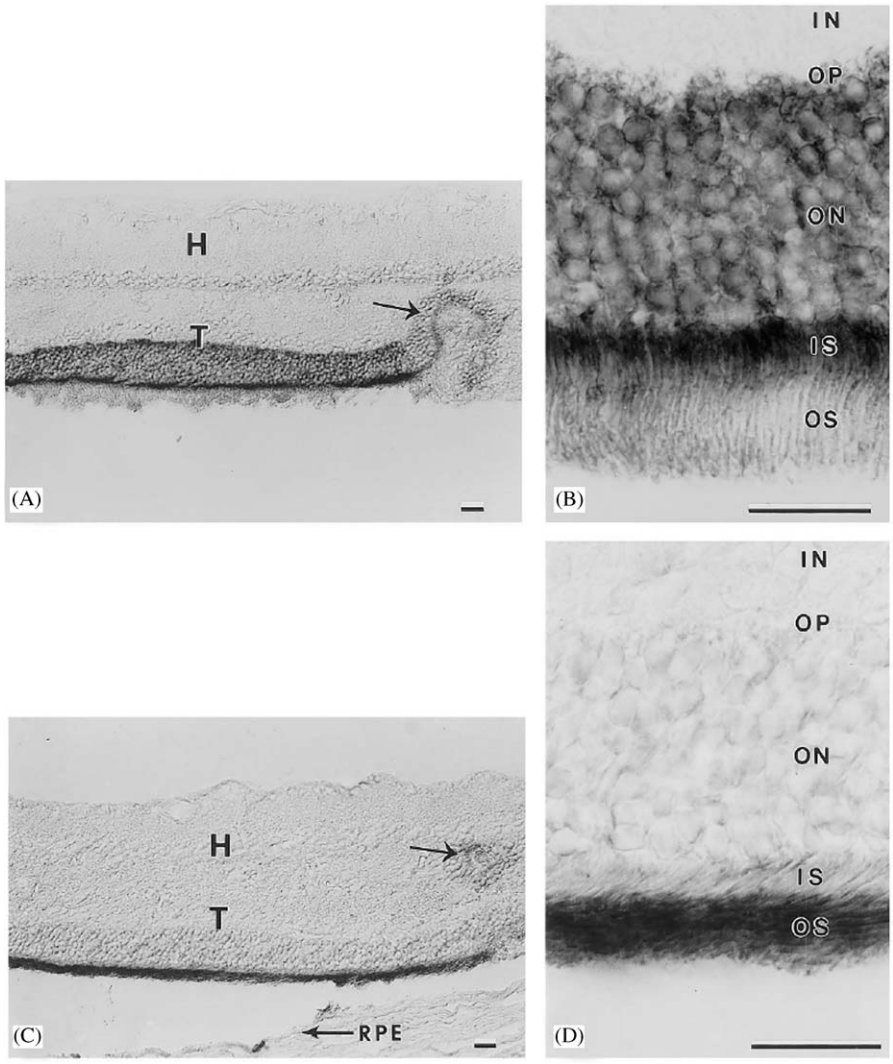

Fig. 2. Rod α-Transducin in light- and dark-adapted rat retinal transplants to light-damaged rats. (A) Transplant perfusion-fixed in light. The transplant has developed a parallel layer of photoreceptors which is clearly immunoreactive for a-transducin in the inner segments and in the outer nuclear layer. The curled up photoreceptor layer (rosette) in the edge of the transplant shows only weak transducin immunoreactivity. There is no a-transducin staining in the overlying light-damaged host retina. The host RPE are not seen in the frame of the picture because of a processing artifact. E17 donor, 147 days after surgery. (B) Another light-adapted transplant with main immunoreactivity for transducin in the inner segments. E19 donor, 61 days after surgery. (C) Transplant fixed at the end of dark cycle. Transducin has shifted to the outer segments of the parallel organized photoreceptors. The outer segments of photoreceptors in a rosette (arrow) do not show any staining. E18 donor, 71 days after surgery. (D) Enlargement of (C). All figures are oriented with the photoreceptor layer towards the bottom of the micrograph. Paraffin sections, 8 μm. Bars = 20 μm. Labels used: H, host; IN, inner nuclear layer; IS, inner segments of photoreceptors; ON, outer nuclear layer; OP, outer plexiform layer; OS, outer segments of photoreceptors; RPE, retinal pigment epithelium; T, transplant. Taken from Fig. 1 of Seiler et al. (1999): Photoreceptor function of retinal transplants implicated by light-dark shift of S-antigen and rod transducin, Vision Res. 39, 2589–2596; copyright Elsevier; with permission.

More recently, tests of visual acuity, specifically optokinetic head tracking, have been used to assess visual function in pigmented rats, specifically to study different retinal degeneration models (Hetherington et al., 2000; Lund et al., 2001b; Thaung et al., 2002) and photoreceptor rescue after RPE or Schwann cell transplantation (Lund et al., 2001a,b; Coffey et al., 2002). A modified design has been developed recently to test each eye independently. Using this design, it was shown that retinal sheet transplants to S334ter-3 rats with rapid retinal degeneration delayed the deterioration of the optokinetic response (Thomas et al., 2004a).

Another test of visual acuity, the Visual Water Task (Prusky et al., 2000), has been used recently to quantify vision loss (McGill et al., 2004) and to study the effect of RPE transplants (Prusky et al., 2003) in RCS rats. However, it has not yet been used to study the effect of retinal transplants.

5.3. Visual function of transplants shown by electrophysiology

In 3 of 10 *rd* mice that had received retinal aggregate transplants at the age of 13 days, ganglion cell responses could be recorded over the transplant whereas no responses were found in sham surgeries, or in mice that had received transplants at the age of 8 weeks (Radner et al., 2001). This indicated that the functional effect of transplants depends on the stage of degeneration of the host retina. However, ganglion cell responses to light can also be found in non-transplanted homozygous albino S334-ter line 3 rats, even after severe retinal degeneration (An et al., 2002). Thus, it is not clear whether ganglion cell responses really represent functional vision. Those light responses could be due to melanopsin containing host ganglion cells (Semo et al., 2003).

Silverman recorded visually evoked potentials (VEPs) in light-damaged rats with photoreceptor sheet transplants (Silverman et al., 1992). Unfortunately, his results were not independently confirmed by others.

It is well known that different retinal areas are represented topographically in the rodent superior colliculus (SC) (Siminoff et al., 1966). Recording of visual responses in the SC showed loss of light sensitivity in the RCS rat (Sauvé et al., 2001) and demonstrated the photoreceptor rescue after transplantation of RPE or Schwann cells to 3 to 4 week old RCS rats (Sauvé et al., 1998; Lawrence et al., 2000; Sauvé et al., 2004). Lund's group has also shown transplant-specific effects in the visual cortex (Girman et al., 2003).

Sheets of retina with RPE transplanted to 5–7-week-old RCS rats can restore visual responses in an area of the SC that topographically corresponds to the placement of the transplant in the retina (Woch et al., 2001). Transplant responses had a longer latency than normal controls. The amplitudes of transplant responses were significantly different from the faint responses recorded in some sham surgeries, and more similar to normal controls. Similar results were observed in S334ter, line 3 transgenic rats (Sagdullaev et al., 2003) (Figs. 3 and 4). In this rapid degeneration model, no responses were observed with sham surgery. Recently, transplanted rats have been investigated in a slow degeneration model, the S334-ter line 5 (Thomas et al., 2004b). Since the transplant was placed into an area of the retina that develops a scotoma before other retinal regions, transplant-derived responses could be identified in the corresponding caudal SC. These responses were specific for "good", well-laminated transplants; no specific responses were seen with disorganized transplants and with sham surgery.

However, in fetal retinal sheet transplants to *rd* mice, no correlation between transplant organization and visual responses in the SC could be seen. The visual responses were rather related to a rescue effect on cones in the host retina as indicated by recoverin staining (Arai et al., in press). Thus, transplants may have different effects depending on the retinal degeneration model.

5.4. Mechanism of transplant effect?

Are these visual responses in the SC in the rat models of retinal degeneration due to synaptic transmission of signals from transplant photoreceptors to the host retina, and/or are they due to a simple trophic effect of the transplant on host cones? Cones persist in the host retina for a long time after all rods are lost.

Diffusible trophic factors produced by rods can prevent cone degeneration in *rd* mice (Mohand-Said et al., 1998; Fintz et al., 2003). There are also reports about rescue effects on rod photoreceptors by RPE transplants (Li and Turner, 1988; Lopez et al., 1989), growth factors (review: Chaum, 2003), and even sham surgery (Silverman and Hughes, 1990; Wen et al., 1995; Humphrey et al., 1997; Schraermeyer et al., 1999).

However, there are several arguments why a rescue effect cannot be the only cause for the transplant effect on visual responses in rat degeneration models.

Based on S-antigen immunoreactivity, no qualitative difference was observed in the number of remaining blue cones near the transplant site and other areas of the host retina (Woch et al., 2001; Sagdullaev et al., 2003; Thomas et al., 2004b). This is however in contrast to what was seen in retinal sheet transplants to *rd* mice (Arai et al., in press).

The visual responses in the caudal SC were observed only in a very small area of the SC precisely corresponding to the placement of the graft in the retina. If a trophic effect of the transplant on host photoreceptors was the cause, it should have an effect on a larger retinal area, as has been observed in *rd* mice with rod photoreceptor transplants (Mohand-Said et al., 1997).

Finally, in the S334-5 rat model with slow retinal degeneration, good quality visual responses were recorded only when the transplant maintained normal laminar morphology (Thomas et al., 2004b). This may be correlated to the observation that photoreceptors in laminated transplants stain prominently for rod α-transducin, whereas photoreceptors in rosettes show only very weak staining (Seiler et al., 1999b).

Thus, if there is a rescue effect in the rat models, it appears to be subtle. It is difficult to explain why the transplant effect appears to be only due to a rescue effect in *rd* mice. The main reason may be the fast rate of photoreceptor degeneration and the difficulty of sheet transplant surgery in the small mouse eye.

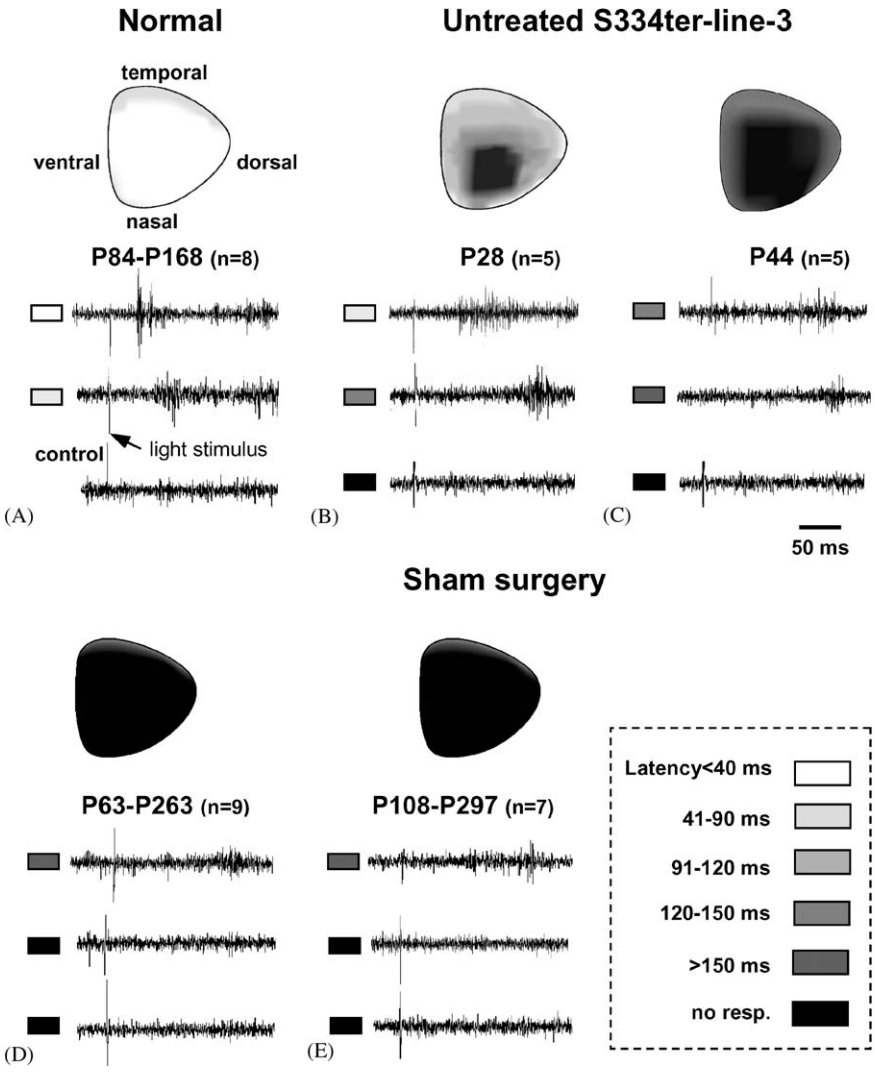

Fig. 3. Superior colliculus (SC) recordings. Spatiotemporal changes in the mean latency of visual responses in the SC of untreated S334ter-line-3 rats compared with normal and sham-surgery rats. Diagrams represent the mean visual latency of all responses in the contralateral SC in control rats: (A) normal, (B–D) untreated S334ter-line-3 rats at postnatal ages (P28, P44, and P63 or more), and (E) sham-surgery rats. Different gray-scale shading represents increasingly longer latencies and black represents no visual response. The legend indicates the range of latencies represented by shading. no resp. = no response. Traces below each diagram illustrate a representative response recorded from one location in one animal in that group. The control trace shows a single sweep that was recorded within a visually responsive area with the light stimulus triggered but the eye occluded. Taken from Fig. 2 of Sagdullaev et al., 2003: Retinal transplantation-induced recovery of retinotectal visual function in a rodent model of retinitis pigmentosa. Invest. Ophthalmol. Vis. Sci., 44(4), 1686–1695; copyright Invest. Ophthalmol. Vis. Sci.; with permission.

The light stimulus for the SC recording studies consisted of a full-field stimulus with bright light of 1300 cd/m^2, which bleaches rods very fast. Each site in the superior colliculus is stimulated repeatedly (16 times), and responses are averaged. If there was a rod response in the first stimulus, it would be gone soon after. In each experiment, at least 30 sites are tested (each with a full-field stimulus). To clearly show that the visual responses originate in the transplant photoreceptors, the recording stimulus has now been modified to specifically identify rod responses (unpublished data). Rods are only found in the transplants, not in the host retina.

6. Connectivity

6.1. Morphological indications for transplant-host connectivity

Can the visual effect of transplants be due to synaptic connections between transplant and host? The ''integration'' of retinal sheet transplants varies, but it is usually better with transplants to recipients with more advanced retinal degeneration. This is likely due to the fact that the inner retinal neurons in a retina with photoreceptor degeneration are deprived of synaptic inputs from the photoreceptors. Retinal injury has been shown to induce

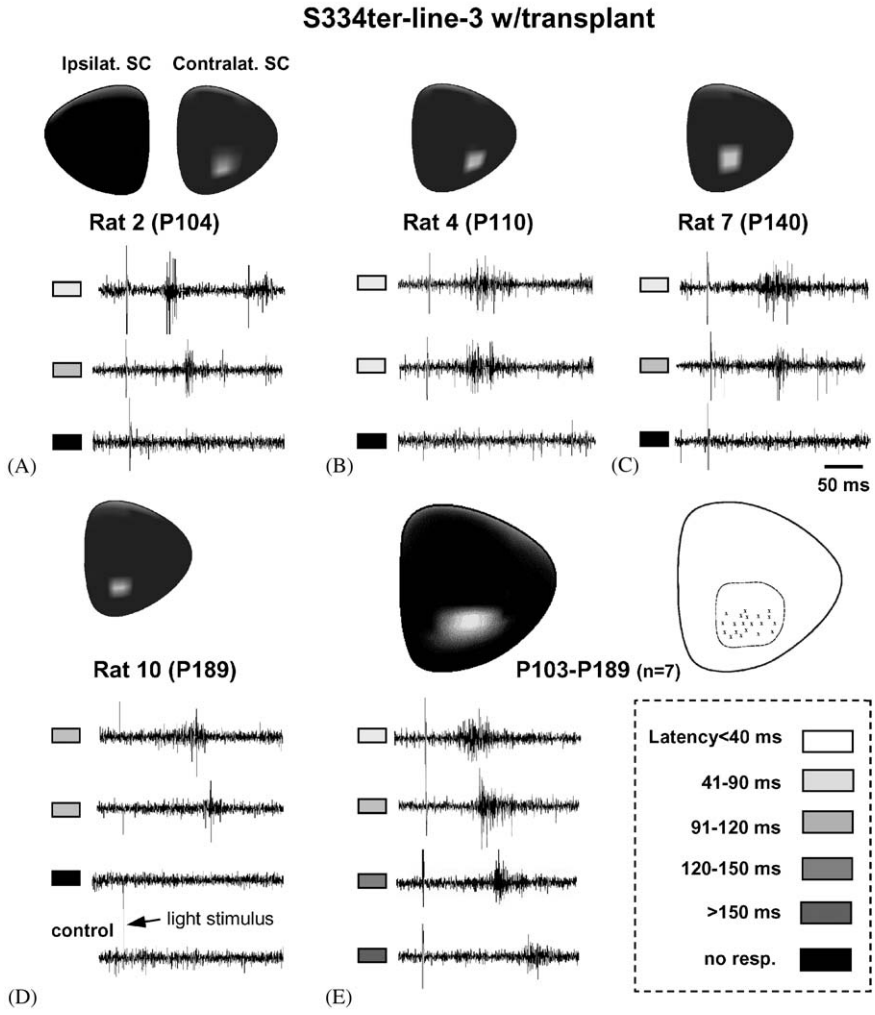

Fig. 4. Areas of visually evoked activity are found in the SC of S334- ter-line-3 rats with successful transplants. Different degrees of shading depict differences in the mean visual latencies on the diagrams of the contralateral SC of four individual rats with successful transplants of increasing postsurgical age (A–D). Enlarged diagrams show the average over all seven rats (E) and the individual locations of every visually responsive site recorded (F). Dashed line in (F) outlines the area of the SC from which no visual responses could be evoked in untreated rats at the age of transplantation (P28). In most rats, recordings alternated between the contralateral and ipsilateral SCs, shown in (A). The legend and other conventions are the same as in Fig. 3. Taken from Fig. 3 of Sagdullaev et al., 2003: Retinal transplantation-induced recovery of retinotectal visual function in a rodent model of retinitis pigmentosa. Invest. Ophthalmol. Vis. Sci., 44(4), 1686–1695; copyright Invest. Ophthalmol. Vis. Sci.; with permission.

upregulation of trophic substances (Cao et al., 1997, 2001). Photoreceptors in the host retina appear to present a barrier for transplant-host connectivity as was shown for aggregate transplants (Zhang et al., 2003a) and in a retinal explant culture system (Zhang et al., 2003b). It also appears to be related to reactive glial cells of the host retina since robust integration can be seen in retinal transplants to transgenic recipient mice lacking both glial fibrillary acidic protein (GFAP) and Vimentin (Vim) (Kinouchi et al., 2003). Dissociated enhanced green fluorescent protein (EGFP) expressing donor retina (P0–P30) was transplanted to wild-type mice, and transgenic mice lacking GFAP and/or Vimentin in their glial cells. The GFAP/Vim lacking mice contained normally appearing Mueller cells in their retinas and had normal ERGs. Transplants to GFAP/Vim mice showed extensive migration and neurite extension, in contrast to transplants to wild-type mice.

The neuroblastic progenitor retinal sheet transplant develops an inner nuclear layer with retinal interneurons, as shown by immunohistochemistry, just like the host retina. The neural circuitry between transplant and host retina would be different from a normal retina, e.g., a transplant bipolar cell may contact a host bipolar cell. Can this duplication of inner retinal neurons hinder appropriate connections between transplant and host, or can the host retina and brain adapt to obtain visual information from an abnormal circuitry?

In different transplant models, neuronal processes of transplant and host cells have been observed to cross the

transplant/host interface (Ghosh et al., 1999a; Seiler et al., 1999a; Zhang et al., 1999; Seiler et al., 2001; Zhang et al., 2003a). This indicates that synaptic connectivity is possible, although it appears to be limited. It has been shown in a tissue culture system that neuronal processes do not cross the interface between retinal explants if they are place on top of each other, but are readily observed if explants are placed side-by-side (Zhang et al., 2003b).

6.2. Synaptic connections between transplant and host shown by pseudorabies virus, an excellent state-of-the art tracing method

Synaptic connections between transplant and host retina are also indicated by trans-synaptic tracing from the host brain to the transplant (Seiler et al., 2003) (manuscript submitted) using an attenuated pseudorabies virus which is specifically transferred from one neuron to the next at synaptic contact points, and transported exclusively in the retrograde direction (Card, 1998).The use of the attenuated pseudorabies virus PRV Bartha is well established and studied since at least 1990 (Card et al., 1990) for tracing multisynaptic circuits in the brain. The specificity of the virus is due to its double-membrane envelope with specific glycoproteins, which can only be assembled in the Golgi apparatus of neurons (Card et al., 1993). The virus can only leave an infected cell and infect another one if it contains the complete envelope; virus capsids without envelope (''naked'' viruses) cannot leave a cell. Because the glycoproteins of the virus envelope also bind specifically to astrocytic membranes, glial cells that closely surround the synapse can be infected, in contrast to macrophages and microglia. However, since glial cells cannot produce infectious virus with an envelope, they help to prevent non-specific extracellular spread and contamination (Card et al., 1993). This is however confusing for many scientists that are unfamiliar with the virus properties. For a complete description of the mechanism involved with of this state-of-the-art technique, it is recommended to read the original articles. Transneuronal transfer of the virus has been shown to be dependent on the development of functional synapses (Rinaman et al., 2000), close contact between nerve cells is not sufficient for virus transfer. Injection of PRV Bartha into the SC labels neuronal cells in retinal sheet transplants (Seiler et al., 2003) (manuscript submitted).

7. Clinical trials

Clinical trials of retinal transplantation have been motivated by the lack of available treatment to recover or prevent vision loss from retinitis pigmentosa (RP) and other diseases of the outer retina: (1) Oral vitamin A therapy can slow the rate of ERG loss in RP patients but does not improve lost vision (Sibulesky et al., 1999). (2) Gene and pharmacologic therapy are being developed (Acland et al., 2001; Narfstrom et al., 2003), but they are still not in use in clinical trials at this time, although a clinical trial of gene therapy in Leber's Congenital Amaurosis will probably be initiated soon (Dejneka et al., 2003; Ali, 2004). (3) Development and use of a visual prosthesis is actively being pursued in many centers but the visual potential of existing devices is not known (Rizzo et al., 2001; Chow et al., 2002; Margalit et al., 2002; Hetling and Baig-Silva, 2004).

7.1. RPE and IPE transplantation

Because it has been shown that RPE transplants can rescue photoreceptors in RCS rats, RPE cells have been transplanted to age related macular degeneration (ARMD) patients. These clinical trials have been reviewed previously (Aramant and Seiler, 2002b). In summary, there were problems with RPE allografts, which prevented any long-term beneficial effects. These problems were related to rejection, inflammation and/or changes in the RPE cells after tissue culture. Immunosuppressive treatment appeared to prevent graft failure (Del Priore et al., 2001; Kaplan et al., 2003). Two patients with RP that had received RPE transplants with immunosuppression experienced improved performance on visual field testing (Kaplan et al., 2003).

Autologous transplants of adult RPE cells (Binder et al., 2002) and IPE cells (Abe et al., 1999, 2000b; Thumann et al., 2000) have been performed to overcome the rejection problems with allogeneic RPE, mostly to patients with ''wet'' exudative ARMD. Subjective improvements in visual acuity were reported.

7.2. Neural retinal transplantation

Previous clinical trials with fetal retinal aggregate and adult photoreceptor sheet transplants have been described in (Aramant and Seiler, 2002b). Since then, one more study of 8 patients with adult photoreceptor sheet transplants has been published (Berger et al., 2003). These experiments have shown no clinical signs of rejection, but also no improvement in vision. However, there might have been subtle graft rejection not visible in clinical exams.

7.3. Transplantation of sheets of fetal retina together with its RPE

The team in Louisville, KY, with the surgeon Radtke is now the only group in the US that has obtained an Investigational New Drug Application (IND) number from the Food and Drug Administration (FDA) for clinical trials to transplant fetal retinal sheets with its

RPE to patients with RP or ARMD. Five RP patients that had only light perception received cografts of retina with RPE and were followed for one year (Radtke et al., 2002). No changes in vision occurred, but these trials demonstrated that the procedure is safe as no apparent rejection was observed, as would have been indicated by fluorescein leakage. Following these results, the FDA allowed the inclusion of patients with vision of 20/800 in the study. A RP patient that had 20/800 vision at the time of transplantation, improved her ETDRS visual acuity to 20/160 after one year (Radtke et al., 2004). However, no changes could be detected with multifocal ERG. The vision in the unoperated eye of 20/400 did not change. No clinical evidence of rejection was observed although the transplant sheet lost its pigmentation by 6 months.

One reason why no change with the multifocal ERG was detected with the improved patient may have been due to the use of an old type of monitor for stimulation, rather than the new more sensitive stimulus camera. The documentation of preoperative vision by different methods is extremely important to reliably judge any postoperative changes. Tests for accurate and reproducible measurement of vision at very low levels must be developed.

7.4. Basic research results that justify clinical trials with fetal retinal sheet transplants

What is unique with the procedure performed by Radtke et al.? What justifies these clinical trials? Based on extensive research with several different retinal degeneration models, there are many encouraging points being a summary of this review:

(1) Use of a documented gentle and unique technique of instrument and method. (2) The donor retinal neuroblastic and progenitor cells, transplanted as a sheet, already have a primordial circuitry established. (3) An area of a damaged retina can be reconstructed so that it morphologically resembles a normal retina. (4) The photoreceptors in the transplants appear to be able to function like normal photoreceptors to transform light into electrical signals as indicated by light/dark shift of phototransduction proteins. (5) Using a state of the art tracing method, little known but well developed during the last 15 years, there are indications of synaptic connections between transplant and host retina. (6) Recordings in the brain have documented that light sensitivity can be restored or preserved in an area of the brain that corresponds to the placement of the graft in the retina. (7) In a visual acuity test, using an optokinetic apparatus that can test each eye individually, transplanted retinal degeneration rats can preserve functional vision. (8) In many retinal diseases the patients need both new photoreceptors and retinal pigment epithelium. A fragile monolayer sheet of fetal retinal epithelium can gently be co-transplanted and after 10 months it can still stay as a monolayer sheet with an apparent healthy supporting function between the choroid and the photoreceptors. (9) Rodent experimental data (discussed in (Aramant and Seiler, 2002b) and clinically confirmed results show that the donor tissue is well tolerated in the subretinal space, can survive without immunosuppression and is safe to use in patients. (10) The transplants with this technique have unequivocally demonstrated to have a beneficial effect on several retinal degeneration models.

8. Future directions

There are many strategies for improvement to make retinal sheet transplants more effective. Transplanted fetal retinal sheets with and without their RPE, can restore visual responses in the superior colliculus and delay the deterioration of visual behavior in animal models of retinal degeneration. Both development of synaptic connections between transplant and host, and trophic effects of the transplant on the host retina contribute to this effect. To elucidate the mechanism, a combination of different methods needs to be used in the same animals to show functional synaptic connections: morphologically by tracing and electron microscopy, electrophysiologically and by behavioral tests. A reliable cytoplasmic label of the donor cells, now accessible and in use, will also be helpful to demonstrate synaptic connections.

The prospective transplant interconnections with the host retina need to be improved and enhanced, which is expected to occur in future experiments outlined below. Retinal interneurons in the transplant may interfere with the synaptic connectivity of transplant photoreceptors with the host retina. Elimination of the inner limiting membrane on the surface of the donor retina might lead to improvements in the connectivity between transplant and host. Connectivity could be supported by factors that reduce glial reactivity and trauma to the retina because the formation of glial scars is another barrier to integration. Treatment with trophic factors, or gene delivery into donor cells also needs to be explored. This requires carefully selected control experiments to account for the normal upregulation of trophic factors that is seen after injury (Cao et al., 2001).

Retinal transplantation, and approaches to develop retinal prostheses, remain presently the only potential treatments once photoreceptors are lost. Growth factor and gene therapy can only work to delay retinal diseases, not replace lost photoreceptors. In the future, one can conceive of stimulating stem cells in the adult eye to regenerate the lost photoreceptors, or to bioengineer a retina from stem cells.

In summary, given the progress that has been made in retinal transplantation research and the paucity of treatments on the horizon to restore vision to patients who are already blind from retinal degenerative disease, retinal transplantation research should be given the every opportunity to proceed with experiments to reach this critically important objective.

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