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# **NEURORETINAL XENOTRANSPLANTATION TO IMMUNOCOMPETENT HOSTS IN A DISCORDANT SPECIES COMBINATION**

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Short title: Long-Term Retinal Xenografts

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## **LIST OF ABBREVIATIONS**

GFAP	Glial Fibrillary Acidic Protein
MHC	Major Histocompatibility Complex
ONL	Outer Nuclear Layer
PKC	Protein Kinase C
RPE	Retinal Pigment Epithelium

## **ABSTRACT**

In spite of its immune privileged state, xenotransplantation within the CNS is associated with rapid graft destruction in immunocompetent hosts. Efforts to enhance graft survival have mostly focused on host immune response, whereas relatively little attention has been paid to donor tissue characteristics. In the present paper, we explore long-term survival of xenogeneic full-thickness neuroretinal transplants in immunocompetent hosts and investigate the significance of tissue integrity in relation to graft survival.

Adult rabbits receiving no immunosuppression were used as hosts and fetal Sprague-Dowley rat neuroretina as donors. Using vitreoretinal surgical techniques, rabbits received either a full thickness or a fragmented neuroretinal graft to the subretinal space of one eye. Eyes receiving full-thickness grafts were examined morphologically after 91 days and fragmented grafts after 7-14 days.

Surviving full thickness grafts were found in 6 out of 8 eyes, 4 of which displayed the normal laminated appearance. Major Histocompatibility Complex (MHC) up-regulation in surviving grafts was minimal and they contained a well organized photoreceptor layer, Protein Kinase C (PKC) labeled rod bipolar cells, parvalbumin labeled AII amacrine cells and glial fibrillary acidic protein (GFAP) labeled Müller cells. Fragmented grafts (n=6) were all destroyed or showed severe signs of rejection. A mass of inflammatory cells derived from the choroid was evident in these specimens, and no labeling of retina-specific cells were seen.

We conclude that full-thickness rat neuroretina can survive for several months after subretinal transplantation to the subretinal space of immunocompetent rabbits, while fragmented counterparts are rapidly rejected. Surviving full-thickness grafts can develop many of the normal retinal morphological characteristics, indicating a thriving relationship between the initially

immature donor tissue and its foreign host. Our results strongly indicates that donor tissue integrity is a crucial factor for graft survival in CNS xenotransplantation.

**Keywords:** Immunogenicity; Development; Photoreceptor; Rabbit; Rat; Rejection

The concept of cross-species tissue transplantation has intrigued medical science for decades by its prospect of alleviating the constantly growing need for donor tissue (Reemtsma 1964; Kemp, 1996). The xenotransplantation paradigm is, however, severely hampered by the fact that cross-species grafts are invariably rejected in immunocompetent hosts. Although somewhat protracted, rejection is also the fate of xenografts to immunologically privileged sites such as the brain and anterior chamber of the eye (Larsson et al., 2000; Ross et al., 1993).

Retinal transplantation to the subretinal space of the eye, a site which has been found to enjoy a somewhat limited immune privilege, has been explored in the laboratory for more than two decades (Turner and Blair 1986; Jiang et al., 1993). The most common experiment involves a relatively simple surgical procedure with transplantation of mechanically disrupted tissue fragments or enzymatically dissolved cell suspensions. Successful survival of fragmented retinal xenogeneic transplants has been reported in immunosuppressed hosts. In immunocompetent counterparts, however, these types of grafts are invariably rejected within 1 month (Ehinger et al., 1991; Aramant and Seiler, 1994; DiLoreto et al., 1996). Transplantation of the retina as an intact sheet is a more recent development in the field, requiring a more advanced surgical method, but with the advantage of keeping the delicate architecture of the retina intact (Ghosh et al., 1998, Seiler and Aramant., 1998). In contrast to fragmented counterparts, allogeneic neuroretinal full-thickness grafts appear to survive indefinitely in the subretinal space where they attract very little, if any, attention from the host immune system (Ghosh et al., 1999, Seiler and Aramant, 1998). Studies on donor tissue characteristics in relation to graft survival include donor-age, inclusion of antigen presenting cells and blood vessels, but as yet, tissue-integrity has not been extensively explored (Aramant et al., 1988; Marion et al., 1990). We have previously reported that allogeneic fragmented grafts (rabbit-to-

rabbit) induce up-regulation of major histocompatibility complex (MHC) class I and II molecules in the graft and host while their full-thickness counterparts do not show such up-regulation (Ghosh et al., 2000). We have further reported that full-thickness donor tissue, at least in the short-term, can survive xenogeneic transplantation between discordant species (rat-to-rabbit) without immunosuppression (Rauer and Ghosh, 2001).

Given the importance of donor tissue availability in a future clinical setting, we now have expanded our earlier findings regarding retinal xenotransplantation to explore: 1) the long-term survival of full-thickness neuroretinal xenografts in an immunocompetent host, and 2) the donor tissue integrity in relation to graft survival by comparing the fate of full-thickness and fragmented xenografts.

## **EXPERIMENTAL PROCEDURES**

### **Donor tissue**

Sprague-Dawley rats of age E19-20 (19-20 days after conception) were used as donors. At this stage, the retina consists of an undifferentiated neuroblastic cell layer and a multi-layered ganglion cell layer (Fig. 1). After decapitation, both eyes were enucleated. The cornea, lens and vitreous were removed with micro-scissors. The neuroretina was dissected from the underlying retinal pigment epithelium (RPE) with micro forceps, trimmed to a 2-3 mm full-thickness sheet, and put in 4°C Ames' solution. Transplantation of the tissue was performed within 360 min. The neuroretina was either transplanted as a full-thickness sheet (n=8), or as a suspension of tissue fragments (n=6, see below). One neuroretina was used for each host eye.

### **Host animals**

Pigmented mixed strain adult rabbits, aged 4 months, from a local breeder, were used as hosts. Eight animals were used for full-thickness grafts, and 6 for the fragment transplants.

### **Surgical procedures**

#### *Preoperative preparations*

Thirty min. prior to surgery, 10% phenylephrine and 1% cyclopentolate was instilled in the right eye of the host rabbit. The rabbit was anesthetized with one part xylazine (Rompun®, 20 mg/ml, Bayer) and three parts ketamine (Ketogan®, 50 mg/ml) to 1 ml/kg intramuscularly in the thigh. Just before surgery 0.5% tetracaine eye drops were instilled in the eye.

#### *Operation*

The surgical protocol for the full-thickness transplantation has been reported earlier (Rauer and Ghosh, 2001). Briefly, a core vitrectomy including posterior vitreous detachment was per-



formed using an Alcon Accurus® vitrectomy machine. A Zeiss surgical microscope equipped with a BIOM® lens was used for visualization of the fundus. After the vitrectomy, a retractable polyethylene capillary inside a slightly bent metal cannula was inserted through the sclera into the vitreous cavity under visual guidance. A retinotomy was made approximately 2-3 mm inferior to the optic disc with the tip of the capillary, and a retinal detachment bleb formed by infusing Ames' solution into the subretinal space with an attached syringe. A second, smaller retinotomy was made in the bleb periphery. The full-thickness graft, oriented with the outer retina down, was drawn into a glass tube, and the tube inserted into the vitreous cavity through the 10 o'clock sclerotomy. Using an attached syringe, the transplant was injected into the bleb through the larger retinotomy, while the excess Ames' solution escaped out of the smaller retinotomy. The conjunctiva and sclerotomies were then sutured. Gentamicin (2,5 mg) and betamethasone (1 mg) were injected subconjunctivally, and a chloramphenicol ointment (Chloromycetin®, Pfizer AB, Sweden) was instilled. The animals were observed closely until awakening.

The surgical protocol for the fragment transplantation has been reported earlier (Bergström et al., 1992). Briefly, a 0.5 mm wide sclerotomy was made 2 mm behind the limbus at 10 o'clock. A contact lens was put on the cornea, with methyl-cellulose (Methocel®, Novartis Sverige AB, Täby, Sweden) used as contact medium. The same instrument used for bleb-formation in full-thickness transplantation (above) was used to fragment the donor tissue by drawing in the neuroretinal sheet together with 5 µl Ames' solution into the thin plastic tube. The instrument was then inserted through the 10 o'clock sclerotomy under visual guidance. The cannula was advanced through the vitreous and a retinotomy was made approximately 2-3 mm inferiorly of the optic disc. The fragment transplant solution was injected forming a detachment bleb after which the injection cannula was removed from the eye.

### *Postoperative management and follow up*

No postoperative treatment was given. During the first postoperative week, the fundus of all operated eyes was inspected using a binocular indirect ophthalmoscope. Animals receiving full-thickness transplants were terminated after 91 days and those receiving fragmented transplants after 7 (n=3) or 14 (n=3) days. All proceedings and animal treatment were in accordance with the guidelines and requirements of the Government Committee on Animal Experimentation at Lund University and were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 or the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, or the European Communities Council Directive of 24 November 1986 (86/609/EEC).

### **Tissue preparation**

The rabbits were sacrificed with 5 ml sodium pentobarbital (60 mg/kg) intravenously. The operated eye was enucleated, a cut made through the sclera in the pars plana region, and the eyes put in 0.1 M Sørensen's phosphate buffer at pH 7.4 containing 4% paraformaldehyde for 10-15 min. The cornea, lens and vitreous were then removed, and the posterior part of the eye again put in the aforementioned paraformaldehyde buffer for 4 h. Tissue specimens 2-3 mm wide including the transplant and parts of the medullary rays and optic nerve were dissected out. After an initial washing in Sørensen's 0.1 M phosphate buffer at pH 7.4 and repeated washings in Sørensen's buffer with sucrose added in increasing concentrations (10% - 25%), the specimens were embedded in Yazulla (30% egg albumen and 3% gelatine in water). They were sectioned on a cryostat at 12  $\mu$ m and mounted on glass slides. Every tenth slide was stained with hematoxylin and eosin.

### *Immunohistochemistry*

For immunohistochemical labeling, the following primary antibodies were used: Anti-major histocompatibility complex I and II (MHC, directed against rabbit, monoclonal, diluted 1:100, Mab 73.2 and Mab 45-3, Spring Valley Laboratories, Woodbine, Md., USA); Anti-parvalbumin (monoclonal, diluted 1:1000, MAB1572, Chemicon International, Temecula, Ca, USA); Anti-glial fibrillary acidic protein (GFAP, monoclonal, diluted 1:200 MAB3402, Chemicon International, Temecula, Ca, USA). Anti-protein kinase C (PKC, monoclonal, diluted 1:200, K01107M, Nordic Biosite AB, Täby, Sweden). Sections were thawed for 30 min before rinsing for 15 min in phosphate-buffered saline (PBS) and 0.25% Triton X-100 at 7.2 pH. Primary antibodies were diluted with PBS, 0.25% Triton X-100 and 1% bovine serum albumin and incubated for 18-20 h at +4°C. After rinsing for 5 min x 3 in PBS and 0.25% Triton X-100, the sections were incubated with a secondary antibody (fluorescein isothiocyanate (FITC) Sigma Chemical Company or Texas-Red, Jackson ImmunoResearch, USA) for 45 min in darkness in a 1:80 dilution with PBS, 0.25% Triton X-100 and 1% BSA. After another rinse in PBS and 0.25% Triton X-100 for 15 min x 2, they were mounted in anti-fading mounting medium.

Photographs of all specimens were obtained with a digital camera system (Olympus, Tokyo, Japan). No digital image manipulation was performed on photographs from immunolabeled sections. Photographs of hematoxylin and eosin stained sections were adjusted for brightness.

### **Control experiments**

All immunohistochemical labeling was performed on sections from unoperated adult rabbit and rat eyes. MHC class I and II labeling was also performed on fetal E20 rat retina. Negative

controls were obtained by performing the complete labeling procedure without the primary antibodies.

To establish the immunocompetence of the host animals, 4 separate adult rabbits from the same local breeder received 2 x 2 cm skin transplants from each other. The animals were examined daily for 7 days after which they were terminated and the grafts examined histologically.

## RESULTS

For a complete overview of the results, see Table 1.

### Control experiments

All skin grafts were macroscopically rejected after 7 days. On microscopic examination they displayed an extensive infiltration of inflammatory cells in the subcutaneous layer, with corresponding MHC class I and class II labeling.

Unoperated adult rat and rabbit eyes displayed GFAP labeling in astrocytes, and weakly labeled Müller cell inner end-feet. PKC labeled rod bipolar cells and parvalbumin labeled AII amacrine cells were seen in adult retinas as previously described in the literature. No MHC class I or class II labeled structures were seen in either adult or fetal retinas. The choroid of adult rabbit specimens displayed singular MHC class I and class II labeled cells as previously reported (Ghosh et al., 2000). There was no GFAP, Parvalbumin, PKC, MHC class I or class II labeling in the negative controls.

### Full-thickness transplants

#### *Macroscopic findings*

On postoperative examination, the fundus could be clearly visualized in 6 out of 8 eyes with the full-thickness transplant in place in the subretinal space. One eye displayed a mild cataract and another a moderate vitreous hemorrhage. At dissection, 91 days postoperatively, all retinas were attached, and 7 of the 8 eyes displayed a clear vitreous without any macroscopic signs of hemorrhage or inflammation. One eye contained whitish vitreous opacities. Grafts were found in the subretinal space in 6 eyes.

### *Hematoxylin and eosin staining*

In 6 of the 8 eyes receiving a full-thickness graft, the transplant was found in the subretinal space in hematoxylin and eosin stained sections. Four of the transplants in their major part displayed a laminated morphology measuring 0.6 - 4.0 mm in vertical length (Fig. 2A). The outer nuclear layer (ONL) contained 4-6 rows of photoreceptors with inner segments (Fig. 2B and C). Only a few short outer segments were seen. The inner layers of the grafts were thinner than normal and in all 4 cases appeared to have fused with the overlying host retina in which no outer layers remained. Inner layers of the host straddling the graft displayed varying degrees of degeneration and pigmented cells were often found within these layers. Outside the transplant area, the host retina appeared normal. The host RPE was mostly continuous, but in some sections displayed disruption in the transplant area. There was no sign of inflammation in the choroid. In 2 of the 8 eyes, degenerated grafts in direct contact with the host choroid through large defects in the RPE were found. The remaining 2 eyes contained no graft. In these eyes, the transplant area consisted of an amorphous fibrotic cell mass with a severely degenerated host retina and disrupted RPE.

### *Immunohistochemistry*

No MHC class I labeled cells were found in the graft or host retina in the 4 eyes with laminated grafts (Fig. 2D). In 1 of the 4 specimens, a small cluster of MHC class II labeled cells were found at the border between graft and host retina and also the host RPE in the transplant area displayed enhanced labeling (Fig. 2E). In all other areas, and in the remaining 3 eyes, no MHC class II labeling was found. There was no sign of increased MHC class I or class II expression in the choroid of these specimens as compared with in normal rabbit eyes. One of the 2 eyes with degenerated grafts displayed an accumulation of MHC class I and class II labeled cells in the choroid bordering the transplant area, and a few labeled cells were also present

within the graft. In the other degenerated graft, no such labeling was found. In the eyes without any grafted tissue, there was no increase of MHC class I or II labeling compared with normal rabbit retina.

All surviving grafts displayed GFAP labeling in their major part (Fig. 2F and G). The labeled fibers were arranged vertically throughout the outer part of the grafts corresponding well to Müller cells. Occasionally, labeled fibers extended outwards and horizontally expanding into the subretinal space, but in most cases, the outer part of the fibers resembled normal Müller cell outer end-feet (Fig. 2G). In the inner part of the graft, Müller cell fibers were more randomly organized and often extended into the remaining host retina. No glial barrier was seen between the two entities. In the host retina straddling the graft, moderately labeled, vertically arranged Müller cells were seen. The fibers did not form a glial barrier towards the graft. GFAP labeling was also present at some distance from the transplant area, but was absent in the peripheral retina.

AII amacrine cells labeled by anti-parvalbumin antibodies were present in all surviving grafts (Fig. 2H). In some areas, labeled cells were randomly arranged in the inner part of the graft, occasionally with processes extending towards the host retina. In others, labeled cells were more organized, with one row of cells extending processes ending in the inner plexiform layer of the graft. The host retina straddling the graft in some areas displayed normally arranged parvalbumin labeled AII amacrine cells whereas in other areas, very few labeled cells remained.

PKC labeled rod bipolar cells were present in all laminated grafts although in lesser number than in the normal adult rat retina. The cells were located at the outer border of the inner nuclear layer and extended axons ending in indistinct structures interpreted as growth cones at the graft-host interface (Fig. 2I). In the host retina straddling the graft, PKC labeled rod bipo-

lar cells in some areas were absent, and in others were found to be normally arranged in almost normal number.

## **Fragmented transplants**

### *Macroscopic findings*

On postoperative examination, the fundus was visualized in 6 out of 6 eyes. The graft, identified as a grey mass in the subretinal space was seen in 4 cases, but was considerably smaller than preoperatively. At dissection, 7 (n=3) or 14 (n=3) days postoperatively, a small graft was seen in the subretinal space in 1 out of 6 eyes. In the remaining specimens, the transplant area displayed choroidal and RPE atrophy. There were no signs of inflammation in the vitreous, retina or choroid.

### *Hematoxylin and eosin staining*

In one of the 6 eyes receiving fragmented tissue, a small graft in the form of poorly developed rosettes could be seen 7 days after transplantation (Figure 3A). In the remaining eyes, no grafted cells could be identified, and the transplant area was infiltrated with a diffuse mass of mononuclear and polymorphonuclear cells derived from the choroid through large gaps in the host RPE (Figure 3B). Inflammation was more pronounced in eyes examined 14 days postoperatively as compared with 7 days. The outer layers of the host retina in the transplant area were absent and inner layers showed varying degrees of degeneration.

### *Immunohistochemistry*

The eye in which a small graft had been found displayed no up-regulation of MHC class I labeling, but MHC class II labeled cells were evident in the choroid as well as in the host retina and choroid (Fig. 3C and E). In the remaining 5 eyes, derived from 7 (n=2) and 14 (n=3) days, the majority of inflammatory cells in the transplant area displayed MHC class I and class II labeling (Fig. 3D and F).



In all 6 eyes, there was a massive GFAP up-regulation in Müller cells in the host retina in the transplant area. An ingrowth of host retinal Müller cells into the transplant was evident in the 7-day specimen containing a small graft in which also the transplant displayed minimal labeling (Fig. 3G). In 14-day specimens, in-growing GFAP labeled Müller cell fibers had formed an encapsulating glial barrier at the border towards the inflammatory cell mass in which no labeling could be found.

As in eyes receiving full-thickness grafts, the host retina straddling the transplant area displayed a varying amount of parvalbumin and PKC labeled cells. No labeling was found in the small remaining 7-day graft or any of the other cell masses in the transplant area.

## DISCUSSION

We have shown that full-thickness neuroretinal xenografts can survive for several months after subretinal transplantation to an immunocompetent host while fragmented counterparts are rapidly rejected. The surviving full-thickness grafts develop many of the normal retinal morphological characteristics indicating a thriving relationship between the initially immature donor tissue and its foreign host.

The literature regarding rat-to-rabbit transplantation is to our knowledge non-existing except for our own previous study of short-term retinal transplants (Rauer and Ghosh, 2001). In xenograft nomenclature, the terms concordant and discordant are often used to reflect the phylogenetical distance between involved species. Rabbits and rats belong to different orders just like the well described discordant mouse-to-rabbit model (Rodentia-to-Lagomorpha) which indicates that rat-to-rabbit xenografting should also be considered discordant (Zhang et al., 2000).

From this perspective, the survival and development of xenogeneic full-thickness grafts appears even more puzzling. One factor that must be considered is the potential immune privilege of the subretinal space. Xenogeneic transplantation of fragmented tissue to the brain, a site enjoying partial immune privilege, has been extensively explored, and rejection mechanisms have been well characterized. In the brain, fragmented grafts escape hyperacute rejection and are instead rejected by comparably slow T-cell mediated mechanisms (Pollack et al., 1990; Larsson et al., 2000). The anterior chamber of the eye is another example of an immune privileged site which is manifested by a significantly longer survival time of corneal grafts compared with heart graft in discordant xenogeneic transplantation (Kaplan et al., 1975; Ross et al., 1993). The subretinal space also enjoys immune privilege but the extent of this phenomenon in the transplant situation has been questioned (Jiang et al., 1993; Zhang et al.,

1998). A subretinal injection of fluid and/or tissue constitutes a potential threat to this protected environment since damage to the retinal pigment epithelium (RPE) disrupts the outer blood-retinal barrier (Lopez et al., 1995). As a consequence, in the transplant situation, a subretinal graft may be exposed to immune cells in the non-immune privileged choroid (Yang et al., 1997; Ghosh et. al, 2000). The surgical procedure for fragment transplantation involves one small sclerotomy and a single subretinal injection whereas the protocol for full-thickness transplantation requires three sclerotomies, vitrectomy, subretinal injection, a second retinotomy, and finally actual transplantation into the subretinal space. We have previously reported that full-thickness xenogeneic grafts can up-regulate MHC class I and II in 15-34 day specimens, and if the RPE is extensively damaged, rejection of the transplant by invasion of inflammatory cells from the choroid ensues (Rauer and Ghosh, 2001). However, based on our present and previous work, we can now conclude that even though the surgical procedure for full-thickness transplants is more traumatic than the one used for fragmented counterparts, the RPE heals well and grafts survive in at least half the cases. RPE wound healing is dependent on proper (photoreceptor-to-RPE) neuroretinal contact, and the presence of an intact donor retina in the subretinal space appears to induce a superior wound healing compared with when disorganized fragmented tissue is used (Ozaki et al., 1997; Ghosh et. al, 2000).

Allogeneic fragmented retinal grafts to the subretinal space, and even to the choroid, survive well initially, but subsequently show signs of chronic rejection (Bergström, 1994; Ghosh et. al., 2000; Seiler et. al., 1990). In contrast, xenogeneic fragmented retinal grafts are completely rejected in immunocompetent hosts within one month (DiLoreto et. al., 1996; Warfvinge et al., 2006). By examining 1-2 week specimens, we were able to capture fragmented grafts in fulminate rejection which confirms that rejection of xenogeneic fragmented grafts is elicited rapidly. Fragmented grafts can, however, survive for extended periods in Cyclospor-

ine treated or athymic hosts, indicating a T-cell mediated rejection mechanism comparable to the brain (Aramant and Seiler, 1994, Ehinger et. al., 1991; DiLoreto et. al., 1996). Our data indicates that the actual mechanism of rejection is related to the mechanical trauma involved in the fragmentation process. Disruption of neuronal tissue elicits a multitude of events including the release of cytokines and cell death, but also MHC class I and II up-regulation within the cells (Emgård et al., 2002; O'Malley and MacLeish, 1993). Rejection of allogeneic as well as xenogeneic neural grafts, is closely coupled with up-regulation of MHC molecules in the graft which in turn triggers T-cell infiltration (Rao et. al., 1989; Litchfield et al., 1997). Interestingly, MHC class I and II up-regulation has been found in allogeneic rat-to-rat fragmented grafts, but not in syngeneic counterparts (Larsson 1999). Further, we have previously found MHC up-regulation in allogeneic fragmented transplanted cells, but not in full-thickness counterparts (Ghosh et al. 2000). Taken together our present results and previous studies suggest that retinal elements in the donor tissue up-regulate MHC molecules in response to fragmentation trauma, and that the ensuing inflammation is a rejection caused by the immunological disparity between donor and host.

In contrast to the fate of fragmented grafts, full-thickness counterparts appear to induce very little attention from the host immune system. In allogeneic pig and rabbit models, we have repeatedly shown that immature as well as adult full-thickness retinal transplants can survive for extended periods in immunocompetent hosts without any sign of rejection (Ghosh et al., 1999; Wassélius et al., 2001; Ghosh et al., 2007). Long-term survival of immature full-thickness grafts has also been reported in a rat-to-rat model (Seiler and Aramant, 1998). Thus, the main conclusion of past and present data support the hypothesis that the integrity of the donor tissue is of utmost importance for the survival of neuroretinal transplants. When kept intact, such tissue can apparently survive even discordant xenotransplantation to immuno-

competent hosts for several months and develop many of the normal components necessary for retinal function. These results may broaden our understanding of xenotransplantation and will hopefully help to alleviate shortage of donor tissue in a future clinical setting.

## REFERENCES

1. Aramant R, Seiler M, Turner JE, 1988. Donor age influences on the success of retinal grafts to adult rat retina. *Invest Ophthalmol Vis Sci.* 29, 498-503.
2. Aramant RB, Seiler MJ, 1994. Human embryonic retinal cell transplants in athymic immunodeficient rat hosts. *Cell Transpl.* 3, 461-74.
3. Bergström A, Ehinger B, Wilke K, Zucker CL, Adolph AR, Aramant R, Seiler M, 1992. Transplantation of embryonic retina to the subretinal space in rabbits. *Exp Eye Res.* 55, 29-37.
4. Bergström A, 1994. Embryonic rabbit retinal transplants survive and differentiate in the choroid. *Exp Eye Res.* 59, 281-289.
5. DiLoreto D Jr, del Cerro C, del Cerro M, 1996. Cyclosporine treatment promotes survival of human fetal neural retina transplanted to the subretinal space of the light-damaged Fischer 344 rat. *Exp Neurology* 140, 37-42.
6. Ehinger B, Bergström A, Seiler M, Aramant RB, Zucker CL, Gustavii B, Adolph AR, 1991. Ultrastructure of human retinal cell transplants with long survival times in rats. *Exp Eye Res.* 53, 447-460.
7. Emgård M, Blomgren K, Brundin P, 2002. Characterisation of cell damage and death in embryonic mesencephalic tissue: a study on ultrastructure, vital stains and protease activity. *Neuroscience.* 115, 1177-87.
8. Ghosh F, Arnér K and Ehinger B, 1998. Transplant of full-thickness embryonic rabbit retina using pars plana vitrectomy. *Retina* 18, 136-142.
9. Ghosh F, Johansson K and Ehinger B, 1999. Long-term full-thickness embryonic rabbit retinal transplants. *Invest Ophthalmol Vis Sci.* 40, 133-140.
10. Ghosh F, Larsson J, Wilke K, 2000. MHC expression in fragment and full-thickness allogeneic embryonic retinal transplants. *Graefe's arch Clin Exp Ophtahalmol.* 238, 589-598.
11. Ghosh F, Engelsberg K, English RV, Petters RM, 2007. Long-Term Neuroretinal Full-Thickness Transplants in Severely Degenerated Rhodopsin Transgenic Pigs. *Graefe's Arch Clin Exp Ophtahalmol.* 245, 835-846. Epub 2006 Oct 27.
12. Jiang LQ, Jorquera M, Streilein JW, 1993. Subretinal space and vitreous cavity as immunologically privileged sites for retinal allografts. *Invest Ophthalmol Vis Sci.* 34, 3347-3354.
13. Kaplan HJ, Stevens TR, Streilein JW, 1975. Transplantation immunology of the anterior chamber of the eye. I. An intra-ocular graft-vs-host reaction (immunogenic anterior uveitis). *J Immunol.* 115, 800-804.
14. Kemp E, 1996 Xenotransplantation. *J Intern Med.* 239, 287-297.

15. Larsson J, Juliusson B, Holmdahl R, Ehinger B, 1999. MHC expression in syngeneic and allogeneic retinal cell transplants in the rat. *Graefe's arch Clin Exp Ophthalmol.* 237, 82-85.
16. Larsson LC, Widner H, 2000. Neural tissue xenografting. *Scand J Immunol.* 52, 249-56.
17. Litchfield TM, Whiteley SJ, Yee KT, Tyers P, Usherwood EJ, Nash AA, Lund RD, 1997. Characterisation of the immune response in a neural xenograft rejection paradigm. *J Neuroimmunol.* 73, 135-144.
18. Lopez PF, Yan Q, Kohen L, Rao NA, Spee C, Black J, Oganessian A, 1995. Retinal pigment epithelial wound healing in vivo. *Arch Ophthalmol.* 113, 1437-46.
19. Lund RD, Rao K, Kunz HW, Gill TJ 3rd, 1988. Instability of neural xenografts placed in neonatal rat brains. *Transplantation* 46, 216-23.
20. Marion DW, Pollack IF, Lund RD, 1990. Patterns of immune rejection of mouse neocortex transplanted into neonatal rat brain, and effects of host immunosuppression. *Brain Res.* 519, 133-143.
21. O'Malley MB, MacLeish PR 1993. Induction of class I major histocompatibility complex antigens on adult primate retinal neurons. *J Neuroimmunol.* 43, 45-57.
22. Ozaki S, Kita M, Yamana T, Negi A, Honda Y 1997. Influence of the sensory retina on healing of the rabbit retinal pigment epithelium. *Curr Eye Res.* 16, 349-58.
23. Pollack IF, Lund RD, Rao K, 1990. MHC antigen expression in spontaneous and induced rejection of neural xenografts. *Prog Brain Res.* 82, 129-140.
24. Rao K, Lund RD, Kunz HW, Gill TJ 3d, 1989. The role of MHC and non-MHC antigens in the rejection of intracerebral allogeneic neural grafts. *Transplantation* 48, 1018-1021.
25. Rauer O and Ghosh F, 2001. Survival of full-thickness retinal xenotransplants without immunosuppression. *Graefe's Arch Clin Exp Ophthalmol.* 239, 145-151.
26. Reemtsma K, McCracken BH, Schlegel JU, Pearl M, 1964. Heterotransplantation of the kidney, two clinical experiences. *Science* 14, 700-702.
27. Ross JR, Howell DN, Sanfilippo FP, 1993. Characteristics of corneal xenograft rejection in a discordant species combination. *Invest Ophthalmol Vis Sci.* 34, 2469-2476.
28. Seiler M, Aramant RB, Ehinger B, Adolph AR, 1990. Transplantation of embryonic retina to adult retina in rabbits. *Exp Eye Res.* 51, 225-228.
29. Seiler MJ, Aramant RB, 1998. Intact sheets of fetal retina transplanted to restore damaged rat retinas. *Invest Ophthalmol Vis Sci.* 39, 2121-31.
30. Turner JE and Blair JR, 1986. Newborn rat retinal cells transplanted into a retinal lesion site in adult host eyes. *Brain Res.* 391, 91-104.

31. Warfvinge K, Kiilgaard JF, Klassen H, Zamiri P, Scherfig E, Streilein W, Prause JU, Young MJ, 2006. Retinal progenitor cell xenografts to the pig retina: immunological reactions. *Cell Transplant.* 15, 603-12.
32. Wassélius J and Ghosh F, 2001. Adult rabbit retinal transplants. *Invest Ophthalmol Vis Sci.* 42, 2632-2638.
33. Yang P, de Vos AF, Kijlstra A, 1997. Macrophages and MHC class II positive cells in the choroid during endotoxin induced uveitis. *Br J Ophtalmol.* 81, 396-401.
34. Zhang X, Bok D, 1998. Transplantation of retinal pigment epithelial cells and immune response in the subretinal space. *Invest Ophthalmol Vis Sci.* 39, 1021-1027.
35. Zhang Z, Bédard E, Luo Y, Wang H, Deng S, Kelvin D, Zhong R, 2000. Animal models in xenotransplantation. *Expert Opin Investig Drugs* 9, 2051-68.



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## LEGENDS

**Figure 1.** Embryonic day 20 (E20) rat retina. Hematoxylin and eosin staining. The retina consists of an undifferentiated neuroblastic layer (NBL) and a multi-layered ganglion cell layer (GCL). Scale bar = 50  $\mu\text{m}$ .

**Figure 2.** Fetal full-thickness rat-to-rabbit retinal transplants (T) 91 days postoperatively. **A-C** hematoxylin and eosin staining. **D - I** immunohistochemistry.

**A:** The full extent of the retinal transplant under the host (**H**) retina can be seen (between arrows). The transplant measures approximately 4.0 mm.

**B and C** (two different grafts): The transplant contains an outer nuclear layer (**ONL**) and a thin inner nuclear layer (**tINL**). There are no proper outer segments, instead the disrupted inner segment are in contact with the host retinal pigment epithelium (**RPE**). In **A** the RPE is continuous, while in **B**, gaps are evident. In the host retina overlying the transplant, outer layers are absent and the inner nuclear layer (**hINL**) is thinner than normal. Pigmented cells can be seen in the innermost part of the host retina (**C**, arrows).

**D:** MHC class I labeling: No labeling is seen. Yellow granules representing pigment deposits are present in the area between transplant and host and in the inner part of the host retina (arrows).

**E:** MHC class II labeling. A few structures in the area between transplant and host are labeled (arrow), as well as one segment of the RPE.

**F and G:** GFAP labeling. In **F**, Müller cells in the entire extent of the transplant and the straddling host retina are labeled. In **G** (detail), the outer part of the Müller cells in the transplant display a vertical organization, but in one area they extend fibers into the subretinal space (arrow). In the inner part of the graft, labeled fibers display a more random organization, but there is no glial membrane. Labeled Müller cells in the host are shorter than normal, but display the normal vertical organization.

**H:** Parvalbumin labeling. Labeled cells in the transplant are well organized and have formed a partial inner plexiform layer (**hIPL**).

**I:** PKC labeling. In the transplant, one row of moderately labeled rod bipolar cells are seen. The labeled cells have their perikarya in the outer part of the tINL and extend fibers towards the host, often ending in intensely labeled structures resembling growth cones (arrows). Numerous yellow pigment granules are present between transplant and host retina.

Scale bars **A** and **F** = 250  $\mu\text{m}$ ; **B - E**, **G - I** = 50  $\mu\text{m}$ .

**Figure 3.** Fetal fragmented rat-to-rabbit retinal transplants (T). The left column shows a specimen derived 7 days after transplantation which was the only surviving fragmented graft .

The right columns depicts a 14-day specimen. **A** and **B** hematoxylin and eosin staining. **C - H** immunohistochemistry.

**A** and **B**: Seven days after transplantation (**A**), a small remaining graft in the form of undeveloped rosettes can be seen under the host (**H**) retina. Minimal disruption of the retinal pigment epithelium (**RPE**) is present, but there is no apparent inflammation in the choroid. Fourteen days post transplantation, no graft is visible, and the entire transplantation area including the choroid is filled with inflammatory cells. The RPE is completely disrupted and only a few pigmented cells remain (arrows). The host (**H**) outer layers are missing and inner layers are degenerated in the the transplant area.

**C** and **D**: MHC class I labeling. There is no apparent up-regulation in the 7-day specimen (**C**) while after 14 days (**F**), massive labeling is present within the inflammatory mass.

**E** and **F**: MHC class II labeling. In the 7-day specimen (**E**), a small number of labeled cells can be seen in the RPE and overlying host retina (arrow). In the 14-day specimen (**F**), labeling is present throughout the inflammatory mass in the subretinal space, and also within cells in the host retina.

**G** and **H**: GFAP labeling. Seven and 14 days post transplantation, there is a massive GFAP up-regulation in Müller cells in the host retina straddling the small transplant. An ingrowth of host retinal Müller cells into the transplant is evident, and in the 14-day specimen, the host appears to have formed an encapsulating glial barrier at the border towards the inflammatory cell mass (**H**, arrows). The transplant itself contains a few GFAP labeled structures after 7 days, and no discernible labeling after 14 days.

Scale bars = 100  $\mu$ m.

**Table 1:** Overview of surgical, postoperative and morphological data for each animal.

Figure 3

Figure 1

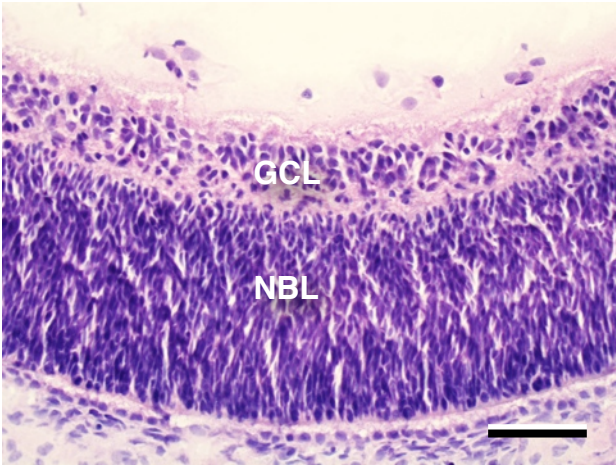


Figure 3  
Figure 2

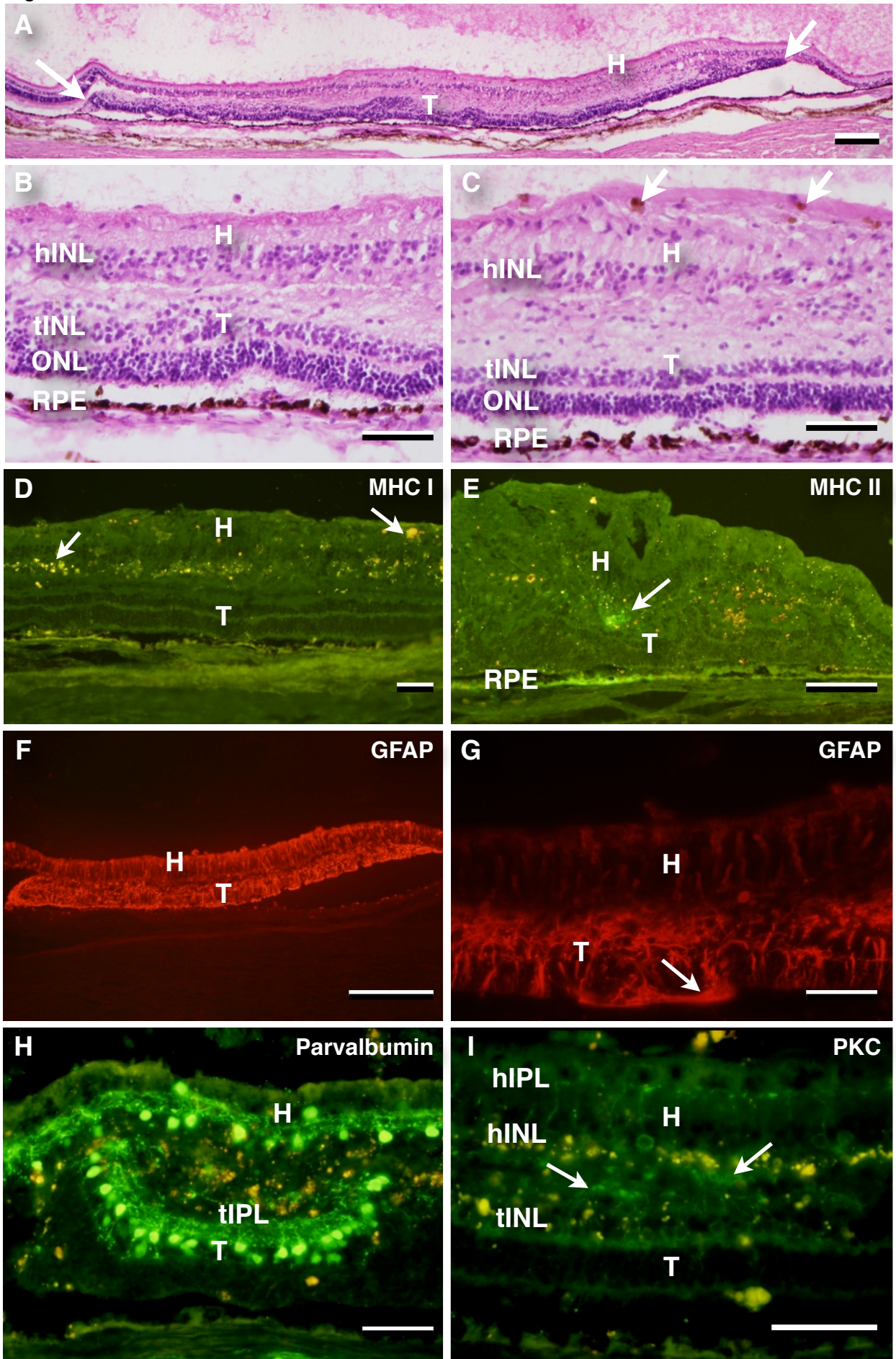




Figure 3

