Syngeneic Schwann Cell Transplantation Preserves Vision in RCS Rat without Immunosuppression

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Purpose. To evaluate the efficacy of immunologically compatible Schwann cells transplanted without immunosuppression in the RCS rat retina to preserve vision.

Methods. Syngeneic (dystrophic RCS) Schwann cells harvested from sciatic nerves were cultured and transplanted into one eye of dystrophic RCS rats at an early stage of retinal degeneration. Allogeneic (Long-Evans) Schwann cells and unoperated eyes served as controls. Vision through transplanted and unoperated eyes was then quantified using two visual behavior tasks, one measuring the spatial frequency and contrast sensitivity thresholds of the optokinetic response (OKR) and the other measuring grating acuity in a perception task.

Results. Spatial frequency thresholds measured through syngeneically transplanted eyes maintained near normal spatial frequency sensitivity for approximately 30 weeks, whereas thresholds through control eyes deteriorated to less than 20% of normal over the same period. Contrast sensitivity was preserved through syngeneically transplanted eyes better than through allogeneic and unoperated eyes, at all spatial frequencies. Grating acuity measured through syngeneically transplanted eyes was maintained at approximately 60% of normal, whereas acuity of allogeneically transplanted eyes was significantly lower at approximately 40% of normal.

Conclusions. The ability of immunoprivileged Schwann cell transplants to preserve vision in RCS rats indicates that transplantation of syngeneic Schwann cells holds promise as a preventive treatment for retinal degenerative disease. (*Invest Ophthalmol Vis Sci.* 2007;48:1906–1912) DOI:10.1167/iovs.06-1117

Retinal degenerative disease (RDD) is a leading cause of vision loss and blindness. One approach to treating RDDs is retinal cell transplantation, which, when undertaken early in the course of degeneration in an animal model of RDD,[1] can limit the loss of vision.[2] McGill et al.[2] reported that human retinal pigment epithelial cell line (ARPE19) and human Schwann cell transplants delayed vision loss, and complementary studies have shown that both cell types preserve photoreceptors,[3–7] electrophysiological responses,[3,6,7] and the duration of optokinetic tracking.[3,4,6,7] Collectively, these studies support retinal cell transplantation as a treatment for RDD.

A major problem for translating these studies to clinical practice is that the donor cells were derived from cell lines (ARPE19)[8] or were harvested from xenogeneic stock (human Schwann cells),[9] leaving them vulnerable to immune system rejection. The specific need for immunosuppression in cell transplantation studies has been demonstrated by showing that allogeneic cell grafts were rejected by the host immune system when transplanted into rat[10] or mouse[11] eyes. As a consequence, these studies used immunosuppressants to maintain the viability of transplanted cells. Although immunosuppression is routine with major organ transplants, it is not desirable, particularly in the elderly population in whom RDDs are most prevalent.

Syngeneic transplantation, in which harvested and transplanted cells are genetically identical with the host, would in principle not require immunosuppression because the transplanted cells should not be recognized by the host immune system as foreign. Larsson et al.[10] confirmed that cells of syngeneic origin were not rejected when transplanted into rat eyes. Therefore, a possible approach in the RCS rat[12] would be to harvest biopsied RPE and to culture, genetically repair, and return the cells to the rat retina. However, this procedure is not practical because of the small size of the rat eye. Removal of all incompetent RPE would be nearly impossible and would require multiple surgeries in an already compromised retina, likely increasing retinal cell loss.

Schwann cells provide a promising source of cells for syngeneic transplantation because they can be harvested with relative ease from adult donors. In addition, they can survive and proliferate in culture, and they limit vision loss in RCS rats,[2] possibly through a paracrine effect.[13,14] In the present study, we evaluated a model of syngeneic Schwann cell transplantation by harvesting Schwann cells from dystrophic RCS rats and transplanting them into dystrophic RCS rats. Because the RCS rat is highly inbred, cells transplanted within strain should not be recognized as foreign by the host immune system; however, allogeneically transplanted cells should be more vulnerable to rejection.[10] Jiang et al.[11] supported this assertion by showing that in mice, at 12 and 35 days after injection, syngeneic transplants thrived; however, allogeneic transplants appeared healthy at 12 days after injection, but by 35 days after injection the allogeneic grafts were significantly smaller, though not completely eradicated. The goal of the present study was to test whether immunoprivileged (syngeneic) Schwann cells preserve vision in the RCS better than nonprivileged (allogeneic) cells, without the use of immune system suppression, providing a model of autologous transplantation for the treatment of RDD.

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Materials and Methods

Animals

Thirty pigmented dystrophic RCS rats (*rdy*$^-$, *p*$^+$), six nondystrophic RCS rats (*rdy*$^+$, *p*$^+$), and six Long-Evans rats were used in this study. Animals were housed in rooms with a 12-hour dark/12-hour light cycle, a constant temperature of 22°C, and food and water available ad libitum. Animals were housed and handled with the authorization of the Institutional Animal Care and Use Committee (IACUC) of the University of Utah and the University of Lethbridge Animal Care Committee, which approve only those procedures that are in accordance with National Institutes of Health (NIH) and the Canadian Council on Animal Care (CCAC) guidelines, respectively. All procedures used in this study conform to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Donor Cells

Sciatic nerves were dissected and transferred to Leibowitz L15 medium. After removal of contaminating tissue, the nerves were cut into 100-μm pieces in a McIlwain tissue chopper and digested in a collagenase/trypsin mixture for 90 minutes at 37°C. The digestion was stopped with Dulbecco modified Eagle medium plus 10% fetal calf serum (DMEMF). After cells were centrifuged at 1000 rpm for 5 minutes, they were resuspended in medium and titrated through a glass capillary and a 25-gauge needle. Cells were plated onto poly L-lysine–coated 35-mm dishes in DMEMF, plus glutamine, pyruvate, and penicillin-streptomycin and incubated at 37°C 5% CO_2. After 24 hours, the medium was changed to remove unattached cells and debris. One week later, cells were removed from the dish with trypsin-EDTA and suspended in DMEM without FCS. This suspension was penned by incubating at 37°C in a 100-mm dish previously coated with rabbit anti–rat IgG. Cells were ready for transplantation within 24 to 48 hours. After counting the number of contaminating fibroblasts (5%–7%), cells were removed from the dish. Because Schwann cells were less adherent to the dish than fibroblasts and were morphologically distinct, it was possible to reduce further the number of contaminating cells by selecting only those cells that moved in suspension rapidly. Once most cells were lifted, the medium was added to stop the reaction. Syngeneic Schwann cells were derived from dystrophic RCS rats, and allogeneic Schwann cells were derived from Long-Evans rats.

Transplantation

Transplantations were performed at the University of Utah. A modification of the procedure used by Keegan et al.[15] was used in this experiment. Briefly, host rats (dystrophic RCS; $n = 18$) were anesthetized with a ketamine/xylazine mixture and received monocular subretinal transplants of syngeneic Schwann cells (1×10^4 cells in 2 μL medium) or allogeneic Schwann cells (1×10^4 cells in 2 μL medium) at postnatal day (P)21. Grafts were introduced transsclerally into the subretinal space in the dorsotemporal quadrant of the right eye with a glass micropipette (outer diameter, approximately 150 μm; inner diameter, approximately 75 μm) attached to a 10-μL syringe (Hamilton, Reno, NV).

Behavioral Assessment of Vision

Previously, we used a visual perception task (Visual Water Task[16] [VWT]) to measure grating acuity in dystrophic RCS rats after the transplantation of ARPE19 and human Schwann cells.[2] Although grating thresholds in rats measured in the VWT are probably the measure of vision most comparable to clinical assessments of acuity, the task requires a lengthy training and testing period for each threshold (on the order of weeks for each eye), limiting the number of thresholds that can be measured over the course of retinal degeneration in RCS rats. The repeated measurement of contrast sensitivity in dystrophic RCS rats is even more problematic when using the VWT because significant retinal degeneration occurs in the time (months) it takes to measure a full-contrast sensitivity curve. Recently, we developed a virtual optokinetic system[17,18] (VOS), that allows for rapid, repeated measurements of spatial frequency and contrast sensitivity thresholds of the optokinetic response (OKR). With the VOS, spatial frequency thresholds and contrast sensitivity curves can be generated through each eye on a daily basis, enabling the longitudinal measurement of visual function in rats with retinal degeneration. In the present study, we took advantage of the strengths of each of these tasks to evaluate, over an extended period, the effects of transplanted retinal Schwann cell on vision. First, we quantified spatial frequency and contrast sensitivity thresholds of adult nondystrophic RCS rats and unoperated dystrophic RCS rats from approximately 4 weeks of age until thresholds could no longer be measured as a background for comparison with RCS rats that have received transplants. To evaluate the effects of syngeneic and allogeneic Schwann cell transplants on preserving vision in the dystrophic RCS rat, we measured spatial frequency and contrast sensitivity of the optokinetic response through each eye weekly from 10 to 35 weeks of age. We separately measured grating acuity in the VWT binocularly at 16 weeks and monocularly at 24 and 28 weeks.

Virtual Optokinetic System. The VOS apparatus consists of four computer monitors positioned around a square testing arena.[17] An unrestrained rat is placed on a platform in the center of the arena, and a sine wave grating drawn on a virtual cylinder is projected on the monitors in 3D coordinate space (OptoMotry; CerebralMechanics). A video camera provides real-time video feedback from above, and the position of the head on each frame is used to continually center the hub of the cylinder at the rat's viewing position. On each trial the cylinder is rotated at a constant speed (12 deg/sec), and the experimenter judges whether the rat makes tracking movements with its head and neck to follow the drifting grating. Spatial frequency threshold—the point at which animals no longer tracked—was obtained by incrementally increasing the spatial frequency of the grating at 100% contrast. Contrast sensitivity thresholds were measured at up to eight different spatial frequencies by systematically decreasing the contrast until no tracking was observed. Thresholds through each eye were measured separately by reversing the rotation of the cylinder.[18] Experimenters were masked to previously recorded thresholds and treatment conditions whenever possible, and thresholds were regularly confirmed by more than one observer.

Visual Water Task. The VWT is a visual perception task previously described in detail.[1,2,16,19] Briefly, a trapezoidal pool with a midline divider at the wide end is filled with water to submerge a hidden escape platform. Two computer monitors face into the wide end of the pool. The platform is always placed directly below the monitor displaying the positive (grating) stimulus, and nothing is placed below the monitor displaying the negative (gray) stimulus of the same luminance. The left/right positions of the stimuli are alternated randomly over trials (Vista; CerebralMechanics).

Training and testing procedures were the same as described elsewhere,[1,2] except animals were tested with both eyes open at 16 weeks of age and through each eye separately at 24 and 28 weeks. Monocular testing required placement of a small occluder over one eye during each trial that was removed immediately after the trial. In the event the occluder became dislodged, that trial was disregarded and a new trial was run. When animals were no longer able to distinguish between a single grating cycle and a gray screen in the unoperated eye, the stimuli were changed to a black screen (negative) and a white screen (positive), which together subtended approximately 0.015 cyc/deg. Animals were tested in blocks of trials at progressively incrementing spatial frequencies until they could no longer distinguish between the stimuli at a minimum of 7/10 correct. Accuracy for a given frequency was measured in blocks of 10 trials when near threshold and shorter blocks at the low spatial frequencies, thereby minimizing the number of trials far from threshold. Acuity was calculated as the 70% correct point on a best-fit curve of the data. All testing was performed early in

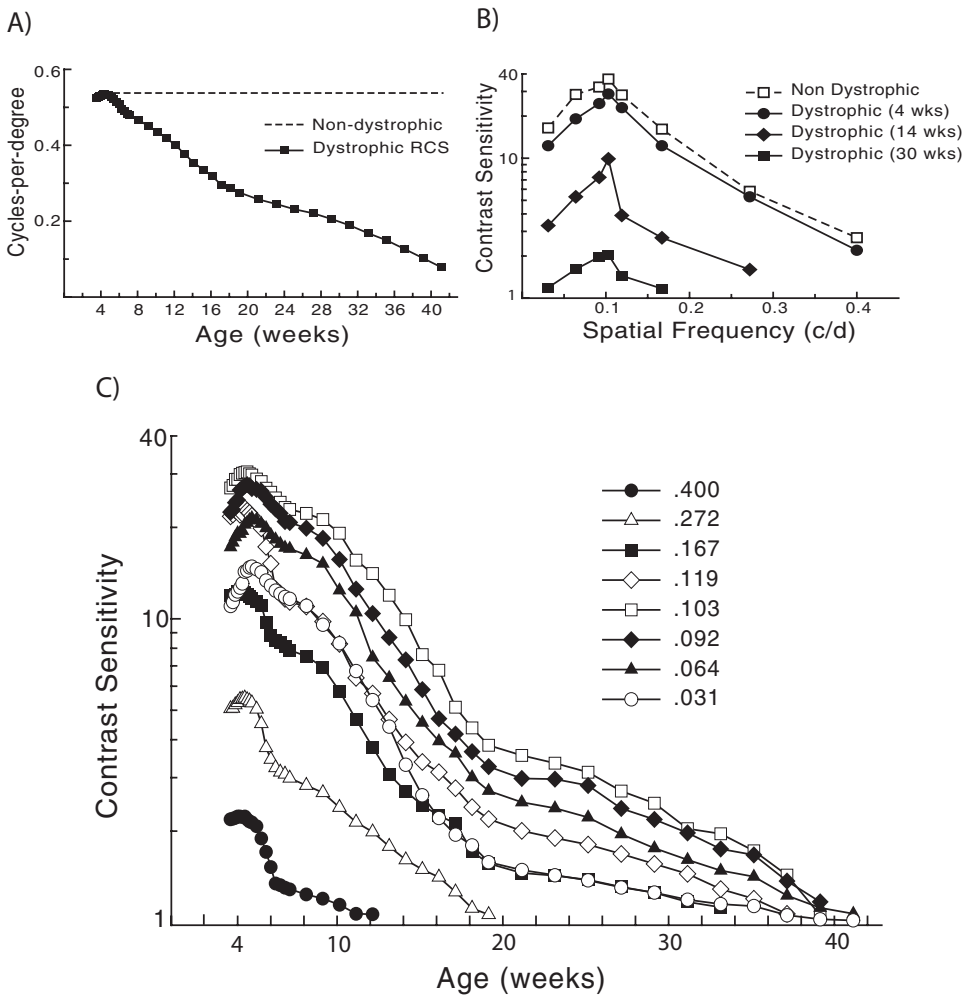

FIGURE 1. (**A**) Spatial frequency thresholds measured in nondystrophic and dystrophic RCS rats. Nondystrophic thresholds remained unchanged at 0.530 cyc/deg, whereas dystrophic RCS rats thresholds were normal at an early age but degenerated to near unmeasurable levels at 41 weeks. (**B**) Contrast sensitivity function of nondystrophic rats and dystrophic RCS rats at 4, 14, and 30 weeks of age. These curves showed a loss of sensitivity at all spatial frequencies tested as the dystrophic rats aged. (**C**) Contrast sensitivity at each spatial frequency plotted with time. Sensitivity to contrast at each spatial frequency declined progressively from approximately 4 weeks of age, completely losing sensitivity to some spatial frequencies with age.

the light phase of the circadian cycle. Thresholds through each eye were obtained in sequence.

Statistical Analysis

Data for OKR contrast sensitivity and spatial frequency thresholds and visual acuity thresholds are reported as group means. Differences between groups were analyzed by repeated-measures ANOVA and Fisher post hoc analysis, with $P < 0.05$ as the level of significance.

RESULTS

Characterization of RCS Rat Visual Function in VOS

We characterized the visual function of RCS rats in the VOS before evaluating the effects of cell transplantation to determine whether the nondystrophic RCS rats exhibited abnormal OKR, to compare visual thresholds measured in the VOS with those measured in the VWT, and to provide a baseline measure of visual function against which visual thresholds from dystrophic animals, with and without transplants, could be compared.

Spatial frequency and contrast sensitivity thresholds of adult nondystrophic RCS rats were comparable with those of normal Long-Evans rats measured previously in the VOS[18] (Figs. 1A, 1B). Dystrophic RCS rats were tested from 4 to 41 weeks of age, during which time spatial frequency threshold decreased from 0.53 cyc/deg to less than 0.08 cyc/deg (Fig. 1A). Contrast sensitivity was measured weekly and was near normal at 4 weeks of age but degenerated rapidly thereafter (Figs. 1B, 1C). Spatial frequency and contrast threshold sensitivities declined rapidly until the 19th week, after which they declined more slowly (Figs. 1A, 1C).

Effects of Transplantation (VOS)

Spatial Frequency Thresholds. Spatial frequency thresholds measured through unoperated eyes and through eyes with allogeneic Schwann cell transplants were near 0.40 cyc/deg at 10 weeks and declined to approximately 0.1 cyc/deg by 35 weeks of age (Fig. 2A). This deterioration in sensitivity followed the same trend as in the background dystrophic controls (Fig. 1A). There was no significant difference between allogeneic Schwann cell–transplanted eyes and unoperated eyes ($F_{(2,372)} = 0.30$; $P = 0.9704$; data not shown).

In contrast, spatial frequency thresholds measured through eyes that received syngeneic Schwann cell transplants were near normal (90%–95%) at all ages tested (10–35 weeks; Fig. 2A). Compared with unoperated and allogeneically transplanted eyes, syngeneically transplanted eyes were significantly better at all ages ($F_{(3,384)} = 5253.181$; $P < 0.001$). The most graphic example of this difference is at 30 weeks of age: spatial frequency thresholds were near unmeasurable levels through unoperated eyes, whereas syngeneically transplanted eyes were near normal at approximately 0.500 cyc/deg.

Contrast Sensitivity. At 30 weeks of age, unoperated eyes and eyes that received allogeneic transplants exhibited a distinctly abnormal contrast sensitivity curve, with only the lowest spatial frequencies measurable (Fig. 2B, solid circle). Syn-

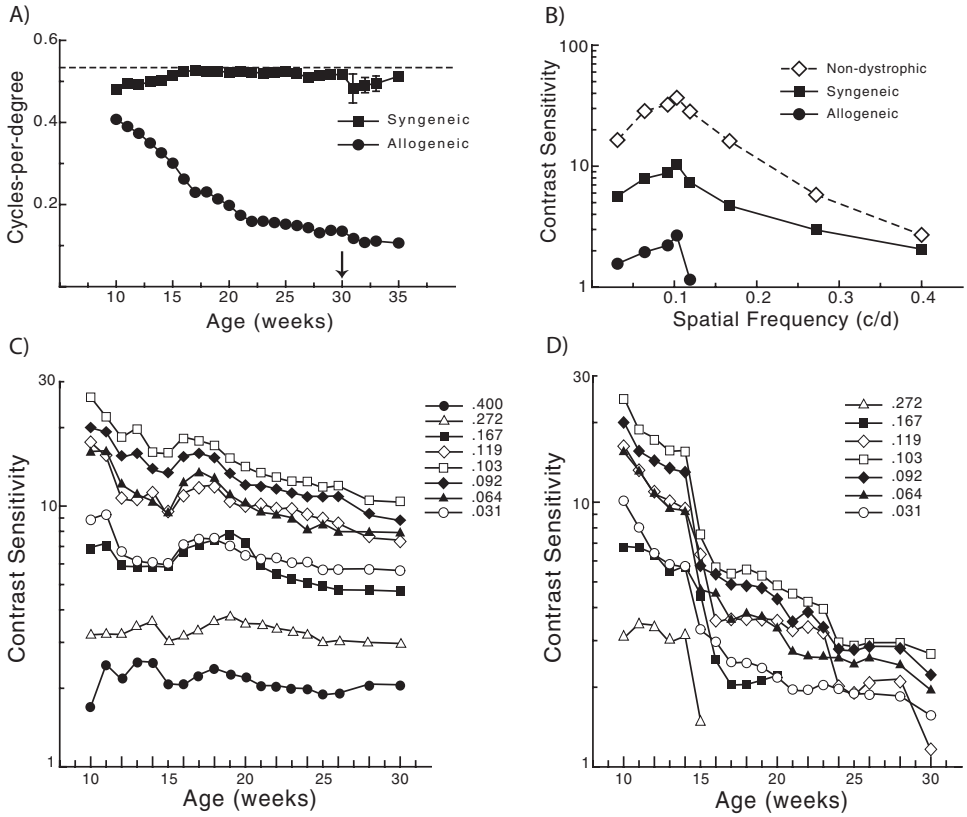

FIGURE 2. (**A**) Spatial frequency thresholds measured through dystrophic RCS rat eyes with monocular syngeneic or allogeneic Schwann cell transplants. Unoperated eyes served as controls. Syngeneically transplanted eyes had thresholds near normal (nondystrophic) levels for up to 35 weeks of age. Allogeneically transplanted eyes were no different from unoperated controls. *Dotted line*: nondystrophic RCS rats. *Arrow*: time point from which Figure 2B was constructed. (**B**) Contrast sensitivity function of nondystrophic, syngeneically transplanted, and allogeneically transplanted eyes at 30 weeks of age. Allogeneic contrast sensitivity function was severely impaired, and no measurable values were evident at the higher spatial frequencies. Syngeneic contrast sensitivity function was significantly better than allogeneic function and was comparable to that of dystrophic RCS rats at 14 weeks of age; however, values did not reflect normal (nondystrophic) values. (**C**) Contrast sensitivity profile of animals after syngeneic transplantation. Although a small decline occurred with most spatial frequencies, all spatial frequencies remained intact, and the highest two spatial frequencies were least affected by the course of degeneration. (**D**) Contrast sensitivity profiles of animals after allogeneic transplantation. All spatial frequency contrast sensitivity values were severely impaired by 15 weeks of age and continued to decline thereafter.

geneically transplanted eyes, on the other hand, displayed a typical inverted U–shaped contrast sensitivity curve, though the sensitivity was significantly lower ($F_{(1,7)} = 11{,}433.883$; $P < 0.001$) than nondystrophic eyes, with the largest differences at the low- and middle-range spatial frequencies (Fig. 2B). Contrast sensitivity in the eye of a dystrophic RCS rat at 14 weeks (Fig. 1B) is similar to that of a syngeneically transplanted eye at 0.1 cyc/deg and lower; however, spatial frequencies greater than 0.1 cyc/deg are preserved approximately the same as in a dystrophic RCS rat eye at 10 weeks (data not shown). In addition, at 14 weeks, contrast sensitivity at 0.4 cyc/deg is immeasurable in the background dystrophic controls, but the syngeneically transplanted eyes retained sensitivity near the levels recorded in nondystrophic animals (Fig. 2B).

The contrast sensitivity profile of each spatial frequency measured longitudinally through syngeneic and allogeneically transplanted eyes is shown in Figures 2C and 2D. Syngeneically transplanted eyes exhibited a profile in which each spatial frequency remained intact until at least 30 weeks of age. Contrast sensitivity at low and middle spatial frequencies in the syngeneically transplanted eyes was variable and showed an overall decline during the testing period (Fig. 2C). Allogeneically transplanted eyes exhibited a profile in which the high spatial frequencies (0.4 cyc/deg) were unmeasurable at 10 weeks or became immeasurable early in testing (0.27 cyc/deg). Allogeneically transplanted eyes also showed a dramatic decline in contrast sensitivity at all spatial frequencies around 15 weeks of age that declined until some spatial frequencies reached immeasurable levels (Fig. 2D). Although the preservation of contrast sensitivity in the syngeneic Schwann cell–transplanted eyes was not complete, as it seemingly was with the spatial frequency threshold, it was significantly better than in the allogeneically transplanted eyes.

Effects of Transplantation (VWT)

Training. All rats readily learned to associate swimming to the platform with escape from water. On average, approximately 200 trials (7 days of training) were required for animals to reach 90% accuracy over 40 trials, and there were no obvious behavioral differences in this ability. Animals performed the task as well for monocular testing as for binocular testing.

Testing. Visual acuity of syngeneic and allogeneically transplanted animals was measured binocularly at 16 weeks of age. Mean acuities were 0.62 cyc/deg from animals with syngeneic Schwann cell transplants and 0.52 cyc/deg from animals with

allogeneic transplants. When we retested at 24 and 28 weeks, we measured the acuity thresholds from each eye separately. Eyes that received syngeneic transplants had mean acuity of 0.61 cyc/deg (24 weeks) and 0.63 cyc/deg (28 weeks), approximately the same values as measured earlier with both eyes open. Allogeneically transplanted eyes had acuities of 0.49 cyc/deg (24 weeks) and 0.42 cyc/deg (28 weeks), slightly lower than when measured binocularly, possibly because of the effects of age on the degenerating retina. Unoperated eyes from syngeneically transplanted animals had mean acuities of 0.15 at 24 and 28 weeks, whereas unoperated eyes from allogeneically transplanted animals had mean acuities of 0.24 (24 weeks) and 0.2 cyc/deg (28 weeks). ANOVA confirmed that syngeneically transplanted eyes had significantly higher thresholds than allogeneically transplanted eyes ($F_{(1,21)} = 9.673$; $P = 0.0053$). When compared with unoperated eyes at 24 and 28 weeks, the allogeneically transplanted eyes were significantly better than unoperated controls ($P < 0.001$), and syngeneically transplanted eyes were better than all other groups ($P = 0.0067$). These results also confirm that when making discriminations binocularly in the VWT, rats use the eye with the higher visual acuity.

Discussion

Results of this study show that syngeneic Schwann cell transplantation into the dystrophic RCS rat retina preserves OKR sensitivity and visual acuity and did so without suppression of the host immune system. All animals performed competently in the tasks, indicating that only thresholds and not general behavioral proficiency changed over time or with treatment condition. The ability to measure vision through each eye independently in each task allowed for the effects of transplantation, in a within-animal design, to be evaluated directly. In addition, the measurement of optokinetic and perceptual forms of vision facilitated a more thorough evaluation of the effects of the transplantation.

This study is the first longitudinal description of OKR spatial frequency and contrast sensitivity thresholds in the RCS rat. Previously,[1] we evaluated the spatial vision of the RCS rat in a visual perception task[16] to provide a background for cell transplantation studies. We found that nondystrophic animals had acuity and contrast sensitivity typical of a pigmented laboratory rat, and the dystrophic strain showed a progressive decline in visual acuity from near normal in juvenile animals to immeasurable levels in most animals by 11 months of age. Those results indicated that the mutation in the *MERtk* gene[12] was responsible for the loss of vision in dystrophic RCS rats and that no significant visual abnormalities were present in the background strain. Results of the present study, using measurements of OKR, confirm this conclusion by showing that the nondystrophic RCS rats had normal OKR sensitivity and that dystrophic animals degenerated from normal values at 4 weeks of age to no response by 41 weeks. Although spatial frequency thresholds in the two tasks differed in animals with normal vision (VWT, approximately 1.0 cyc/deg; VOS, approximately 0.53 cyc/deg), after approximately 12 weeks of age in dystrophic RCS rats, thresholds measured in both tasks were essentially the same (compare Fig. 2 in McGill et al.[1] with Fig. 1A here). This indicates that in the late stages of visual degeneration in the RCS rat, using the VOS is most practicable because the task requires no reinforcement training and multiple thresholds can be measured on a daily basis.

Another distinct advantage to using the VOS is that the task enables the repeated measurement of contrast sensitivity, which is not feasible with the VWT. The contrast sensitivity curve of adult nondystrophic RCS rats measured with the VOS displayed a typical inverted-U shape.[1,20,21] However, sensitivity is generally higher than that measured in the VWT, and peak sensitivity is approximately 0.1 cyc/deg, which is a lower spatial frequency than that obtained from the VWT (approximately 0.2 cyc/deg).[1,18] We did not measure contrast sensitivity in dystrophic RCS rats using the VWT because the length of time required to generate a curve precluded accurate measurements in animals with degenerating retinas. With the VOS in the present study, we found that contrast sensitivity of dystrophic RCS rats at 4 weeks of age was similar to that of adult nondystrophic rats (Fig. 1B), indicating that when the retina is relatively intact, it supports normal function. As the retina degenerated thereafter, however, contrast sensitivity decreased steadily at all spatial frequencies. Sensitivity at the two highest spatial frequencies tested (0.40 and 0.27 cyc/deg) degenerated to immeasurable levels quickest, paralleling the results of the loss of spatial frequency sensitivity (Fig. 1A). Lower spatial frequencies appeared to lose contrast sensitivity in two phases—an early, fast degenerating phase that lasted until approximately 20 weeks of age and a slower phase—possibly because the loss of retinal circuits in the RCS rat is not linear or that the visual system compensates for the loss of circuits nonlinearly.

The central hypothesis tested in this study was that immunoprivileged (syngeneic) Schwann cells, transplanted into the RCS retina without the aid of immunosuppression, would be superior to transplanting allogeneic cells for preserving vision. Indeed, we found that this was the case. Spatial frequency thresholds through syngeneic cell transplanted eyes were near normal until 35 weeks of age, which is the best preservation of visual function in RCS rats that has been reported. Function through allogeneic cell transplanted eyes, on the other hand, was far worse and no different from that of unoperated eyes (Fig. 2A). The difference between the effects of syngeneic and allogeneic transplants on contrast sensitivity, however, was not as dramatic (Fig. 2B). Sensitivity through syngeneically transplanted eyes at 30 weeks of age was better than that through allogeneically transplanted eyes, but it was not normal. Rather, it was comparable to that measured in 10- to 14-week-old unoperated dystrophic RCS rats, which was still markedly better than that measured through unoperated or allogeneic cell transplanted eyes. The difference between the two transplanted groups can be seen in more detail in Figures 2C and 2D in that sensitivity degenerated only gradually through eyes that received syngeneic Schwann cell transplants, whereas, a rapid loss of sensitivity was apparent after 14 weeks of age through allogeneically transplanted eyes.

Measures of grating acuity showed a profile of preservation (Fig. 3) similar to that observed with contrast sensitivity: grating acuity measured through eyes that received syngeneic transplants was preserved, though not completely (approximately 70% of threshold in unoperated animals). The level of preservation was still much better than that through allogeneically transplanted eyes (approximately 40% of unoperated animals), which in turn was better than in unoperated controls. Monocular testing in the VWT also confirmed that acuity through transplanted eyes had the highest thresholds. Given the near normal level of preservation of OKR sensitivity through syngeneically transplanted eyes and the significant loss of OKR sensitivity in the allogeneically transplanted eyes, we expected to see higher visual acuity in the animals with the syngeneic transplants and lower thresholds in those with the allogeneic transplants. Clearly, measures of spatial frequency thresholds in the VOS are not the same as measures of acuity in the VWT. Using the two tasks in the same study, however, provided comprehensive evidence that syngenically transplanted Schwann cells are superior to allogeneically transplanted cells, when no immunosuppressants are used.

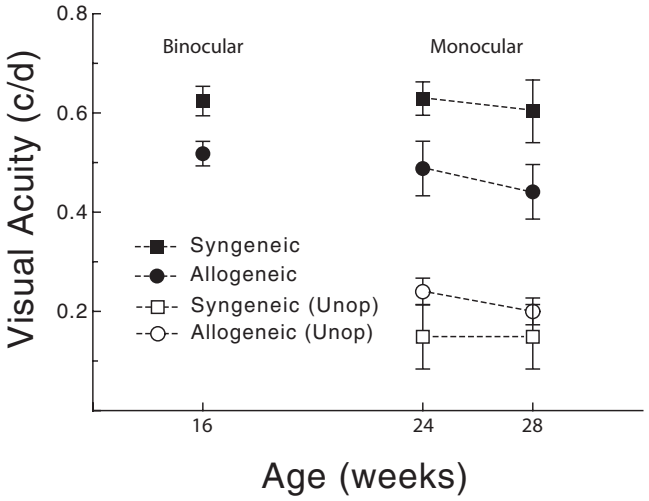

FIGURE 3. Grating acuity thresholds measured in eyes after syngeneic and Schwann cell transplantation and in unoperated eyes. Thresholds were measured in animals binocularly at 16 weeks and monocularly at 24 and 28 weeks of age. Animals that underwent allogeneic transplantation had higher acuity thresholds than unoperated controls, but those that underwent syngeneic transplantation had higher thresholds than all other groups.

The better preservation of visual function through syngeneically transplanted eyes might be explained by a differential response of the immune system to transplantation types. Jiang et al.[11] report that immune responses are mounted against transplanted allogeneic tissue, which will eventually destroy it, whereas the syngeneic tissue will survive. One study[22] that examined the expression of the major histocompatibility complex (MHC) after syngeneic and allogeneic transplantation in the rat retina (without immunosuppression) showed that though both types of transplants can survive, significant upregulation of MHC antigens occurs after allogeneic transplantation but little, if any, upregulation of antigens occurs after syngeneic transplantation. Collectively, these results are consistent with the explanation that our allogeneic transplants might have been compromised, which also may explain why allogeneically transplanted cells provide better preservation than no transplants. In addition, this may explain why we found relatively good preservation of contrast sensitivity only up to 14 weeks of age in allogeneically transplanted animals. Conversely, the modest preservation of visual acuity in animals with allogeneically transplanted Schwann cells may not be attributed to actions of the cells themselves but, rather, may be explained by a transient sham effect induced by retinal damage unavoidably caused by the transplantation surgery.[23,24]

Previously, we evaluated the effects of ARPE19 and human Schwann cells on preserving vision in the RCS rat. In that study, visual acuity was preserved for up to 7 months of age with the ARPE19 transplants, and up to 5 months of age with the human Schwann cells. Although the ARPE19 cell line may have an advantage because it is homologous to the defective host RPE, and therefore potentially capable of replacing a number of physiological functions, no evidence indicates that ARPE19 cells actually perform these functions, specifically the phagocytosis of photoreceptor outer segments. In addition, ARPE19 also has the drawback of not being syngeneic to the recipient and therefore requiring some level of immunosuppression (cyclosporine in McGill et al.[2]), again raising safety concerns that attend all cell lines. Although it has not been shown that Schwann cells phagocytose photoreceptor outer segments, they do appear to survive without pathologic manifestations in the subretinal space. In addition, Schwann cells have the advantage that they can be harvested from the peripheral nerve of a patient and introduced into the subretinal space of that same patient through autologous transplantation.

The process by which Schwann cells work to preserve visual function is unknown, but a likely candidate is through the release of trophic factors. Schwann cells have been shown to produce a number of growth factors, including bFGF, CNTF, and GDNF,[25,26] all of which have been shown to preserve photoreceptors in the degenerating retina.[4,14,27,28] Degenerating retinas are known to undergo atrophy and reorganization of inner retinal circuitry,[29–31] and such trophic support may enable retinal neurons after deafferentation to respond relatively normally to their remaining inputs.

Our behavioral tasks used visual stimuli that were well into photopic light levels (VOS, 45 cd/m^2; VWT, 36 cd/m^2), suggesting that clinically relevant visual function is measured and preserved with Schwann cell transplantation. Girman et al.[32] show, with the use of multiunit recordings from superior colliculus, that after cell transplantation in the RCS rat, cone threshold responses (which normally deteriorate over time) are spared, whereas rod function is diminished early and never recovers. If cone function is responsible for the beneficial effects of the transplants, then measures of outer nuclear layer thickness or photoreceptor nuclei may not be appropriate for determining treatment effects in rodent models. In the rat retina, cone photoreceptors compose approximately 5% of the entire photoreceptor population. In addition, estimates of photoreceptor survival cannot predict visual performance. McGill et al.[1] show that the amount of surviving photoreceptors in RCS rats is near normal early in life but that by 1 month of age, animals are already visually impaired (approximately 80% of normal visual acuity). Conversely, at late stages of retinal degeneration (6 months of age), when almost no photoreceptors remained, RCS rats still had approximately 30% of normal visual acuity.[1]

In conclusion, preserving vision through syngeneic Schwann cell transplantation in the RCS rat provides an incentive for future cellular-based experiments aimed at developing practical treatments for human RDD.

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