Editorial

Stem Cell INVESTIGATION

hESC-derived photoreceptors survive and integrate better in immunodeficient retina

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Retinal degenerative diseases resulting in photoreceptor death, such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP) lead to incurable vision loss in millions of patients worldwide. When photoreceptors are lost, the resulting visual deficit is permanent. Currently, there are no effective therapies for these diseases except to delay degeneration in early disease stages. A potential approach in regaining vision for more advanced disease is to replace the degenerated photoreceptors. Particularly, this is a viable strategy for AMD as it has been shown that ganglion cells can survive in a degenerate retina even when there is severe underlying photoreceptor loss (1). Finding a suitable source of transplantable cells to replace the dying host tissue is the main challenge.

There are two different strategies to replace photoreceptors: (I) transplantation of retinal progenitor sheets (fetal retina or stem-cell derived) (2-5). Fetal retinal sheet transplants have resulted in long-term visual improvements in different animal models of retinal degeneration (4,6-8) and in patients (9), and have shown to integrate and synaptically connect with a degenerated retina (6,8). (II) The second approach is injection of dissociated photoreceptor precursors (10-15). Studies have shown that a small percentage of subretinally injected photoreceptor precursor cells can integrate in the photoreceptors layer, form synaptic terminals (10,15) and outer segments (14,15). However, this requires the presence of an outer nuclear layer in the host retina; transplanted photoreceptor progenitors do not develop proper morphology in recipients with severe loss of photoreceptors (10,11,16). Therefore, most studies have been performed in transgenic mutants where the photoreceptors remain viable although non-functional, such as transgenic knockouts of rhodopsin, CRX or rod transducin (10,11,15). In such very specific models, several groups have shown some vision improvement with transplantation of photoreceptor precursors (11,13,15); however most experiments have been short-term (mostly 6, up to 12 weeks). Even when transplanted within the same species, dissociated photoreceptor precursor transplants disappear over time due to a slow rejection process (12). This is not the case with fetal retinal sheet transplants (2).

The study of Zhu *et al.* injected dissociated retinal cells derived from human pluripotent stem cells which has been done previously in few studies (11,17-20). Testing of human cells in animals requires immunosuppression. Although the retina has a relative immune privilege (21) this does not extend to xenografts. In spite of the so-called "immune privilege" of the eye, xenografts require immunosuppression to survive (21,22). In addition, retinal degeneration causes activation of microglia and macrophages. Therefore, this study of Zhu *et al.* developed an immunodeficient mouse (lacking IL2r-gamma) for transplantation of hESC-derived photoreceptor precursors (23), in a cross with retinal degenerate Crx –/– mice, a model of Leber's congenital amaurosis (LCA). Crx –/– mice have non-functional photoreceptors which degenerate very slowly over a long

time frame. This means there was still an outer nuclear layer present at the time of transplantation in their study which is different from severe retinal degeneration.

The title of the Zhu *et al.* paper is misleading: the animals were actually not immunosuppressed, but they were genetically manipulated to be immunodeficient and did not receive immunosuppressive drugs. The side effects of immunosuppressive drugs are difficult to balance against any visual benefits, in contrast to the benefits of immunosuppression for organ replacement (24).

The donor tissue in the Zhu *et al.* study contained mixed hESC-derived retinal cells (3 months of differentiation) not purified photoreceptor precursors (25). The cells were labeled with a lentivirus expressing enhanced green fluorescent protein (EGFP) under an ubiquitous promoter that labels all cells (11), This virus labeled 60–70% of all donor cells. GFP label was restricted to cells of human origin as confirmed by co-staining with human nuclear or cytoplasmic markers.

In the first part of the study, the cells were injected into the subretinal space of mice with a normal retina, either wildtype or IL2r-gamma knockout, without immunosuppression. Very few cells integrated into the outer nuclear layer of normal mice, whereas robust integration was observed in IL2r-gamma knockout mice. This integration represented 2.5–4% of the injected cells. In the wildtype retina, transplantation caused an upregulation of lymphocytes, T-cells, dendritic cells and activation of microglia/macrophages. In contrast, host retinas of IL2r-gamma knockout mice contained more CD3+ cells, but CD4 and CD8a expression (T-cell marker) was almost completely eliminated. Also absent were CD49b natural killer cells, and CD11c dendritic cells. CD68 and F4/80 (marker for activated microglia/macrophages were significantly reduced. Thus, knockout of IL2r-gamma caused a suppression of the normally occurring immune response. GFP-cells that were integrated in the host outer nuclear layer expressed mature photoreceptor and synaptic markers. To sum up the first part: due to the reduced immune response, transplanted hESC-derived retinal cells survived and integrated better in the IL2r-gamma mice than in mice with normal immune system.

In the second part of the study, hESC-derived retinal cells were transplanted to the Crx –/– model of LCA, either with normal immune system or IL2r-gamma knockouts. At 3 months, few cells survived in Crx –/– mice with a normal immune system, but there was significantly higher integration in IL2r-gamma knockout animals. However, only about 20% of the integration rate (4,000 *vs.* 20,000 cells) was seen compared with IL2r-gamma knockout mice with normal retina. Transplanted cells could still be detected at 9 months post-transplantation which is a significantly longer survival time tested than in previous studies with non-immunodeficient animals.

Improvement of vision in transplanted animals was done by testing for pupillary responses. This test consists of illuminating the transplanted eye, and recording pupillary constriction from the non-transplanted eye. This test has been used in previous studies of photoreceptor precursor transplants (10,16). At 3 and 9 months post-transplant, a partial restoration of pupillary responses was seen in Crx –/– mice that were also IL2r-gamma knockouts, but not in transplanted Crx –/– with an intact immune system. However, the intensity of the pupillary reflex does not correlate with the number of photorecptors cells (26).

Transplanted Crx –/– mice with IL2r-gamma knockout also had very small detectable B-waves in ERG recordings that were absent in sham controls. However, the responses were very small and close to the noise level, with a small number of animals tested.

Another indication of visual function restoration was the demonstration that the immediate early genes *c-fos* and *Arc* were upregulated in visual brain centers after intense light exposure, in transplanted Crx –/–, IL2r-gamma knockout mice. The authors did not show a comparative panel of mice with normal retina. Dark-adapted anesthetized mice were exposed for 2 h with a light intensity of 10,000 lux, followed by sacrifice after 2 h. However, this light exposure was much more intense than in other publications investigating the upregulation of *c-fos* expression by light exposure. E.g., Barnard *et al.* (27) exposed mice to 15 min of fluorescent white light of 33 $\mu W/cm^2$, which would approximately correspond to 225 lux. It would have been interesting if they had seen an effect of the transplant under physiological light intensity conditions.

Recently, several laboratories have demonstrated that recipient photoreceptors incorporate GFP label from transplanted photoreceptor precursor cells that were injected into the subretinal space. This means that GFP label alone is insufficient to tell whether donor cells really integrated into the host retina (28-30). However, Zhu *et al.* showed that GFP-labeled cells stain for human specific markers and do not co-express a mouse-specific MHC class I marker which clearly determined that they were all of human origin. Thus, there was no cytoplasmic transfer in this study.

In summary, the study of Zhu *et al.* shows that using an immunodeficient mouse model significantly improves the integration and survival of transplanted retinal cells. One word of caution: Zhu *et al.* argued that immunosuppression may have enhanced transplant integration in the study of Shirai *et al.* (5), but this study already used cyclosporine A as immunosuppressant. The study by Zhu *et al.* confirms that immunodeficient animal models are better for transplant survival and integration than models that need immunosuppression by drugs because of the side effects of immunosuppressant drugs (24). It is unclear however, how this would translate to future clinical trials. In a previous clinical trial with fetal retina-RPE sheet transplants to patients with RP and AMD, no immunosuppression was used, and transplants survived for many years (9).

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Footnote

Conflicts of Interest: The author has a proprietary interest in the implantation instrument and method to transplant retinal sheets (Ocular Transplantation LLC).

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