Cell Research (2010) 20:728-731.

www.nature.com/cr

LETTER TO THE EDITOR

Adjunctive MSCs enhance myelin formation by xenogenic oligodendrocyte precursors transplanted in the retina

Cell Research (2010) **20**:728-731. doi:10.1038/cr.2010.63; pulished online 4 May 2010

Dear Editor,

We examined myelin formation by oligodendrocytes co-transplanted with immunosuppressive mesenchymal stem cells (MSCs). Oligodendrocyte precursor cells (OPCs) were grafted into the mouse retina, and graft survival and maturation was determined with or without adjunctive MSCs. Green fluorescent protein (GFP)-labeled MSCs were present at 2 but not 6 weeks post transplant, suggesting an absence of trophic support in the retina microenvironment, and we found no evidence for their trans-differentiation into myelin-forming cells. OPCs in contrast survived, migrated parallel to the retinal axons and generated myelin-forming oligodendrocytes throughout the retinal layer. Co-transplantation of MSCs increased the frequency of OPC engraftment by 2-fold and increased the extent of myelin formation by 3-fold, independent of the number of MSCs co-grafted. Thus, adjunctive MSCs can enhance graft-derived myelin formation and may improve cell therapeutic approaches for remyelination therapy.

MSCs have therapeutic potential in three fields of stem cell regenerative therapy. First, they are multipotential and represent a resource for exogenous cell replacement [1]. Second, MSCs can suppress both T-cell proliferation and cytokine production [2] and they have therapeutic potential for immunosuppression for autoimmune disease [3] and also to prevent graft rejection and graft versus host disease. Third, they secrete trophic factors that, in principle, could stimulate endogenous cells for self-repair. MSCs isolated from many tissues (bone marrow, muscle, adipose, placenta, umbilical cord) can be purified, amplified *in vitro* and cryopreserved for patient-specific autologous and heterologous cell therapies. The immunosuppressive ability of MSCs also suggests a potential role as adjunctive carriers for cell transplants. Here we examined their ability to enhance myelin formation by xenogenic oligodendrocytes (OLs) in the retina. OLs are destroyed after tissue insult such as spinal cord injury and autoimmune-mediated multiple sclerosis, and myelin pathology is followed by irreversible neuronal degeneration [4]. In pre-clinical studies, exogenous OLs obtained from brain tissue or derived from embryonic stem cells (ESCs) *in vitro* can promote both myelin repair [5] and functional recovery [6]. Thus, OL transplants have potential for clinical therapeutic benefits, provided that they can be protected from immune rejection.

OLs extend multiple processes (Figure 1A), each of which forms a myelin sheath upon contact with neuronal axons in the central nervous system. Since OLs are post-mitotic and non-migratory, the pivotal cell for myelin transplants [5] is the mitotic and motile OPC (Figure 1B). The OPCs isolated from neonatal rat brain were expanded with mitogens [5] and grafted into the retinal layer of adult mice [7]. The retina provides several advantages for assessing myelin transplants [8]. The retinal layer is accessible, it provides myelin-competent axonal substrates and the entire retinal layer can be examined for quantitative assessment of graft survival. Since endogenous OPCs are not present in the eye, retinal axons do not require demyelination to provide a target. As a result, grafted cells do not modulate or compete with endogenous OPCs, and all myelin-forming cells present are derived from exogenous cells. Finally, we grafted wild-type cells into myelin basic protein (MBP)-null shiverer mice, which allowed the unequivocal identification of transplant-derived MBP^+ OLs.

We examined the short-term survival and migration of OPCs labeled with the lipophylic membrane dye PHK26, and for longer term studies we used OPCs transfected with a transgene encoding a cell surface-anchored green fluorescent protein (eGFP, Figure 1B). OPCs were identified up to 6 weeks post transplant, the longest time point in this study. At 24 h they were identified at and adjacent to the site of engraftment at the distal edge of the retinas. At 4 days there was radial migration parallel to axons tracking toward the optic disc in all retinas observed (Figure 1B). Consistent with this, the majority of MBP^+ cells in grafted retinas were found proximal to the optic disc at the center of the retina and distal from the site of injection. When OPCs were injected alone we observed MBP-positive myelin formation in 43% (3/7) of

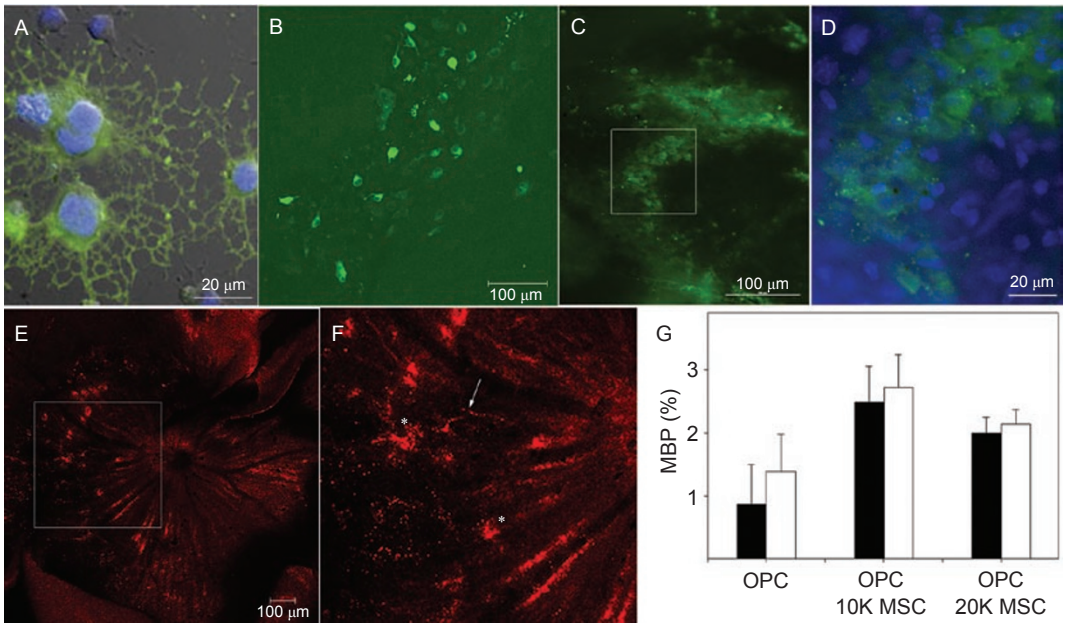

Figure 1 Adjunctive MSCs enhance MBP$^+$ transplants. **(A)** Galactocerebroside$^+$ OLs, generated *in vitro* from rat brain OPCs. **(B)** OPCs expressing a membrane anchored eGFP at 4 days post transplant *in vivo*. **(C**, **D)** Low- and high-magnification whole mount retinal preparations showing MSCs expressing a cytoplasmic GFP at 14 days *in vivo*. **(E)** Retinal preparations showing extensive MBP staining in 5-week graft recipients. **(F)** High magnification of the region boxed in panel **(E)** shows OL soma (asterisks) with multiple thin processes that terminate at myelin internodes (arrow). The optic nerve is center right. Scale bars represent 20 microns (panels **A**, **D**) and 100 microns (panels **B**, **C**, **E**). **(G)** Extent of MBP staining (% total area) at 2 weeks (solid bars) and 6 weeks (open bars) after transplanting OPCs plus the indicated number of MSCs. Methods: MSCs were isolated from C57BL/6-EGFP mouse bone marrow and expanded as described [2]. OPCs were isolated from newborn *Sprague Dawley* rat brain [5] and expanded in R1236 media [10]. For detection, OPCs were labeled by transfection of a membrane-anchored GFP reporter (construct details are available upon request). Cells were transplanted [7] into MBP-null C57Bl/*shiverer*, and aliquots from the injection needle were cultured to verify cell viability. All animal studies were approved by the RWJMS Institutional Animal Care and Use Committee. Antibodies for immunochemistry were as described [5] with intact retinas whole mounted and viewed with a Zeiss LSM510 confocal. The extent of MBP$^+$ myelin was determined using Nikon NIS Elements (vr 3.0) software. Statistical analysis (mean ± standard error) was performed using SigmaStat, with data collected from retinas harvested from two independent experiments with OPCs or MSCs only, and three independent experiments with OPCs plus MSCs.

the recipients. No MBP was observed in sham and non-transplant controls. When MSCs were injected alone, labeled cells were evident at 2 weeks post transplant (Figure 1C) and we did not detect these cells at later time points. The MSCs were identified by a cytoplasmic fluorescent protein (Figure 1D) and all such cells showed stereotypic MSC morphology. We found no examples of trans-differentiation of these cells into OL lineage, either by morphology or by anti-MBP immunochemistry.

For co-transplant studies, OPCs (25 000 cells) were mixed with MSCs (5 000, 10 000 or 20 000 cells), co-injected into the retina, and then examined at 2-6 weeks for MBP-positive OL differentiation. In short-term (2 week) recipients, MSCs were in clusters that did not align with retinal axons (Figure 1C) and they did not co-localize with the OPCs. With co-transplants we observed MBP-positive myelin in 92% (11/12) recipients. In all cases the myelin was aligned with retinal axons projecting toward the optic nerve, and in most cases MBP-positive OLs were again located close to the nerve (Figure 1E). The MBP-positive cells had a highly branched morphology with processes forming T-intersections at myelin sheaths (Figure 1F). The extent of MBP-positive retina was determined by quantitative fluorescence (Figure 1G). With OPCs only we observed myelin at both time points, and there was a 1.6-fold increase in myelin at 6 weeks post transplant. With OPCs plus MSCs we observed 2-3-fold higher levels of myelin at all time points examined, independent of the number of adjunctive MSCs (Figure 1G). There was a slight decrease with higher numbers of MSCs, although the levels were higher than observed with OPCs only. Together these results demonstrate that

adjunctive MSCs can enhance the extent of MBP-positive myelin-forming oligodendrocytes generated from exogenous OPCs, and indicate that adjunctive MSCs are an attractive strategy to improve cell therapeutic approaches for remyelination therapy.

Our results eliminate two potential mechanisms for MSC-enhanced myelin engraftment. We find no evidence for a direct contribution by MSC trans-differentiation into OLs, and in the retinal model there are no endogenous cells for the MSCs to stimulate. Thus, adjunctive MSCs either directly stimulate the exogenous OPCs, or modify the host environment to indirectly stimulate exogenous OPCs. While we cannot distinguish between these alternatives, our results may favor the latter, since the MSCs appear to be only transiently stable in this and many other graft delivery models. The ability of MSCs to suppress T-cell proliferation and cytokine production has received considerable attention. In pre-clinical models they prevent rejection of allogeneic skin grafts [2], autoimmune encephalomyelitis [3] and collagen-induced arthritis. The use of MSCs in clinical applications is quickly expanding to clinical trials for bone marrow transplantation to suppress graft versus host disease and as a treatment for osteogenesis imperfecta, Crohn's and glycogen storage diseases. In addition to their role in immune suppression, MSCs also represent a potential autologous resource for mesenchymal cell replacement therapy including adipocytes, osteoblasts and chondrocytes [1]. They have also been reported to generate neurons and glia, although reports of trans-differentiation have generated considerable controversy [9]. It remains possible that trans-differentiation requires a longer time frame than the studies here, although to date we have not been successful in deriving OPCs from MSCs *in vitro* [10]. This is in sharp contrast to the myelin-forming ability of transplanted brain glia, where stem-derived allografts and xenografts promote myelin repair, and as little as 7% cell replacement is sufficient for functional recovery [6]. Thus, cell replacement remains a viable strategy to treat demyelinating disease, although cell replacement without addressing the underlying disease would only 'feed the fire' and transplant therapy also requires combined immune suppression. Our study suggests that adjunctive MSCs may provide a beneficial environment to enhance myelin graft repair.

There remains a need to identify a suitable OPC population for myelin cell replacement therapy. Adult brain OPCs are not accessible, and fetal derived allografts are an ethically challenging resource. At present the least problematic resource are OPCs derived *in vitro* from pluripotential stem cells, and considerable efforts have been made to generate OPCs from both mouse and human ESCs. We have established an efficient method of sequential culture conditions for generating OPCs from murine ESCs [10], and our studies suggest that the timing of OPC differentiation *in vitro* can reflect the normal developmental program *in vivo*. However, our analysis also suggests that sequential differentiation does not eliminate all teratogenic cells in these cultures and ESC-derived cell types must be purified in order to generate a safe graft population. Since ESC-derived allografts require a significant human leukocyte antigen match to suppress rejection, it will be necessary to expand the available pool of ESC lines to service large patient populations. The evolving field of somatic cell reprogramming may resolve this need, as the generation of autologous induced pluripotential stem cells has opened up the possibility of cryopreserving patient-specific cells for tailored cell replacement therapy. Combining these cells with adjunctive MSCs can provide a novel strategy for effective cell replacement therapy in regenerative medicine.

Acknowledgments

Monoclonal antibodies from the Developmental Studies Hybridoma Bank were developed with the support of the NICHD and maintained by the University of Iowa, Department of Biological Sciences. We thank the reviewers for their critical insight and apologize for the limited referencing due to format restrictions. This work was supported by grants from the New Jersey Commission on Science and Technology to RMcK (07-2042-014-85, 08-01-00) and YS (07-2042-014-91), and the National Institutes of Health to YS (AI057596, DE19413, DE019932). AA was supported by the RWJMS summer research fellowship program.

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