The Emerging Role of Biological Sex in Cell Therapy for Spinal Cord Injury

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ABSTRACT: Neural progenitor cell (NPC) transplantation is a promising potential therapy for replacing spinal cord neurons and glial cells following spinal cord injury (SCI). Despite the rapid advancement of NPC transplantation to SCI clinical trials, we still lack understanding of fundamental biology underlying how NPC grafts interact with the injured host nervous system. Our recent study demonstrated a potent effect of biological sex mismatch between donor and host on graft immune rejection. Here we discuss the implications of this study in the context of clinical trials for SCI, and important topics for future research in SCI cell transplantation.

KEYWORDS: Neural progenitor cell transplantation, graft rejection, sex mismatch, clinical trials

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COMMENT ON: Pitonak et al. (2022) Effects of biological sex mismatch on neural progenitor cell transplantation for spinal cord injury in mice. *Nature Communications* 13(1):5380. PMID: 36104357.

Scientists have worked for nearly half a century to explore the potential of transplanted neural stem cells and neural progenitor cells (NPCs) to replace lost neural tissue following spinal cord injury (SCI).[1-3] Decades of intensive research have illuminated the ability of transplanted NPCs to differentiate into neurons and macroglia, extend graft-derived axons for long distances throughout the host nervous system, support ingrowth of regenerating host axons, and foster the formation of electrophysiological relays. The overarching goal of this field of research is to translate NPC transplantation to the clinical setting where, with luck, the transplanted cells might aid functional recovery for individuals living with SCI. Despite a generally positive outlook from many researchers in the field about the promise of NPC transplantation, most reports of functional recovery are modest. Other studies report negative data or failure to replicate positive findings.[4-6] This raises the question, *"Why is functional recovery variable following NPC transplantation into sites of SCI?"* What are the biological factors that might contribute to variability in the therapeutic efficacy of grafts between animals, across labs, or even within the same lab? Identifying and controlling such sources of variability in graft biology, which contribute to variability in functional outcomes, will be instrumental in the development of human cell grafts that impart robust and reproducible efficacy in the clinic.

Pitonak et al[7] begins to illuminate such sources of variability in cell grafting approaches by highlighting the importance of biological sex. In this study, we utilized a model of "mouse-to-mouse" grafting in which adult C57BL/6 mice received donor cells obtained from embryos of the same syngeneic strain. To isolate the role of sex, we transplanted spinal cord NPCs derived from either male or female (or both male and female) mouse embryos into sites of SCI in male and female mice. Through this work, we showed that female host animals that received male donor cells exhibited hypervascularization, increased perivascular cell density and heightened infiltration of T cells compared with other experimental groups, demonstrating a cellular inflammatory response in sex-mismatched female recipients, but not males.[7]

This work raises several key questions with relevance to ongoing and future clinical applications. The most pertinent of these questions could be, *"Might this happen in humans, too?"* The large body of clinical data on human organ transplantation suggests that this may very well be the case. Human organ transplantation has been successfully performed for over 70 years, since the first successful transplantation of a human kidney in 1950. A number of large retrospective studies have since revealed that sex matching is an important consideration for transplantation, as female recipients of male donor organs – including kidneys, corneas, hearts, lungs, and liver – routinely exhibit the worst chances of graft survival and the highest chances of adverse outcomes.[8-14] This has been attributed to the presence of H-Y antigens, minor histocompatibility antigens expressed by genes on the Y chromosome, in male tissue.[15] These H-Y antigens promote B-cell and T-cell responses, resulting in elevated levels of H-Y-specific antibodies in the female patient, and constitute a major risk factor for graft rejection following transplantation of male grafts into female recipients.[15] While Pitonak et al did not investigate the molecular mechanisms of male NPC graft immune rejection by female mice, we are continuing to investigate the role of H-Y alloimmunity in ongoing preclinical work.

Will biological sex mismatch between donor cells and recipients represent a new clinical hurdle in the successful translation of human cell therapies for SCI? If so, how can we

monitor and/or mediate this effect? As NPC transplantation clinical trials for SCI gain traction,[16,17] these are questions of high clinical relevance. A notable ongoing SCI clinical trial pioneered by Okano and colleagues will evaluate the super-donor integration-free human induced pluripotent stem cell line, YZWJs513, which is derived from a male donor.[17] A future clinical trial is currently being planned by the Tuszynski group (personal communications), and will utilize NPCs derived from H9 human embryonic stem cells, which are genetically female.[18] Both of these studies will be allografting studies, meaning that the donor cells are not genetically identical to the recipient. Because of this, immunosuppression will already be required and indeed is included in the Japanese study design,[17] as is the case with any clinical organ transplantation. However, the planned immunosuppression is transient. It will therefore be important to document any sex-dependent differences in outcomes of male versus female patients who receive either sex-matched or sex-mismatched transplants. Perhaps future studies can include biomarker assessments to uncover any sex-dependent differences in the cellular and/or molecular inflammatory response experienced over time following cell transplantation.

Future preclinical work is also needed to understand how the early immune rejection response observed in Pitonak et al evolves over time. Our study focused on the subacute-to-early chronic phase of SCI, as grafts were examined at only 4 weeks post-transplantation. Hence, we still do not understand how this immune response might be affecting important outcomes such as graft survival and functional recovery in the long term. We did not monitor functional (motor or sensory) behavioral outcomes in our study, but long-term behavioral studies are planned. It will also be important to address the potential of transient immunosuppression to promote graft survival, integration, and immunocompatibility in sex-mismatched groups. Experimental organ transplantation studies—many focusing on kidney transplantation—have shown that there are differences in the acute versus chronic immune response,[19-22] so it is possible that outcomes could be worsened several months down the line. Inflammation within the spinal cord, at worst, could aggravate negative symptoms such as pain or spasticity. Moreover, immune rejection could potentially impact graft *efficacy*. The goal of NPC transplantation is to promote novel host/graft synaptic connections that can mediate the recovery of neurological function.[1,2] If grafts are attacked by the host immune system, the establishment and/or maintenance of synapses between graft and host would likely be eroded.

As scientists in the field of SCI research, our common goal is to help patients suffering from SCI to achieve better quality of life through therapeutic interventions. Decades of failed trials and inconclusive results highlight the major gaps in knowledge that remain about SCI pathophysiology as well as treatment mechanisms. For NPC transplantation therapy, we must learn more about the fundamental biology that underlies therapeutic efficacy, and the sources of variability that challenge reproducibility in outcomes. Gaining a deeper foundational knowledge in this way is the best approach to expedite the development of robust, reproducible treatments for SCI patients.

Author Contributions

J.N.D. and A.T. wrote the manuscript.

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REFERENCES

1. Tucker A, Aceves M, Butts JC, Dulin JN. Spinal Interneurons and Cell Transplantation. In: Zholudeva LV, Lane MA, eds. *Spinal Interneurons: Plasticity After Spinal Cord Injury*. Academic Press; 2022;381-422, Ch. 15.
2. Fischer I, Dulin JN, Lane MA. Transplanting neural progenitor cells to restore connectivity after spinal cord injury. *Nat Rev Neurosci*. 2020;21:366-383.
3. Assinck P, Duncan GJ, Hilton BJ, Plemel JR, Tetzlaff W. Cell transplantation therapy for spinal cord injury. *Nat Neurosci*. 2017;20:637-647.
4. Anderson AJ, Piltti KM, Hooshmand MJ, Nishi RA, Cummings BJ. Preclinical efficacy failure of human CNS-Derived stem cells for use in the pathway study of Cervical Spinal cord Injury. *Stem Cell Reports*. 2017;8:249-263.
5. Sharp KG, Yee KM, Steward O. A re-assessment of long distance growth and connectivity of neural stem cells after severe spinal cord injury. *Exp Neurol*. 2014;257:186-204.
6. Yousefifard M, Rahimi-Movaghar V, Nasirinezhad F, et al. Neural stem/progenitor cell transplantation for spinal cord injury treatment; a systematic review and meta-analysis. *Neuroscience*. 2016;322:377-397.
7. Pitonak M, Aceves M, Kumar PA, et al. Effects of biological sex mismatch on neural progenitor cell transplantation for spinal cord injury in mice. *Nat Commun*. 2022;13:5380.
8. Graňák K, Kováčiková L, Skálová P, et al. Kidney Transplantation and "Sex Mismatch": A 10-Year single-Center analysis. *Ann Transplant*. 2020;25:e921117.
9. Böhringer D, Spierings E, Enczmann J, et al. Matching of the minor histocompatibility antigen HLA-A1/H-Y may improve prognosis in corneal transplantation. *Transplantation*. 2006;82:1037-1041.
10. Kawauchi M, Gundry SR, de Begona JA, et al. Male donor into female recipient increases the risk of pediatric heart allograft rejection. *Ann Thorac Surg*. 1993;55:716-718.
11. Prendergast TW, Furukawa S, Beyer AJ 3rd, Browne BJ, Eisen HJ, Jeevanandam V. The role of gender in heart transplantation. *Ann Thorac Surg*. 1998;65:88-94.
12. Candinas D, Gunson BK, Nightingale P, Hubscher S, McMaster P, Neuberger JM. Sex mismatch as a risk factor for chronic rejection of liver allografts. *Lancet*. 1995;346:1117-1121.
13. Roberts DH, Wain JC, Chang Y, Ginns LC. Donor-recipient gender mismatch in lung transplantation: impact on obliterative bronchiolitis and survival. *J Heart Lung Transplant*. 2004;23:1252-1259.
14. Gratwohl A, Döhler B, Stern M, Opelz G. H-Y as a minor histocompatibility antigen in kidney transplantation: a retrospective cohort study. *Lancet*. 2008;372:49-53.
15. Popli R, Sahaf B, Nakasone H, Lee JY, Miklos DB. Clinical impact of H-Y alloimmunity. *Immunol Res*. 2014;58:249-258.
16. Tsuji O, Sugai K, Yamaguchi R, et al. Concise Review: Laying the groundwork for a first-in-human study of an induced pluripotent stem cell-based intervention for spinal cord injury. *Stem Cells*. 2019;37:6-13.
17. Sugai K, Sumida M, Shofuda T, et al. First-in-human clinical trial of transplantation of iPSC-derived NS/PCs in subacute complete spinal cord injury: Study protocol. *Regen Therapy*. 2021;18:321-333.
18. hPSCreg.eu. *WAe009-A (WA09, H9)*, 2022. https://hpscreg.eu/cell-line/WAe009-A.
19. Ingulli E. Mechanism of cellular rejection in transplantation. *Pediatr Nephrol*. 2010;25:61-74.
20. Becker LE, Morath C, Suesal C. Immune mechanisms of acute and chronic rejection. *Clin Biochem*. 2016;49:320-323.
21. LaRosa DF, Rahman AH, Turka LA. The innate immune system in allograft rejection and tolerance. *J Immunol*. 2007;178:7503-7509.
22. Libby P, Pober JS. Chronic rejection. *Immunity*. 2001;14:387-397.