Peripheral Monitoring of Immune Response to Intraspinal Stem Cell Therapy

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Introduction

- Clinical investigations of intraspinal stem cell therapies are underway for a range of neurological diseases, including ALS, SCI, and MS.
- Originally considered entirely immuno-privileged, the CNS is now considered relatively privileged and immunological reactions to exogenously transplanted cell grafts have been demonstrated in mammalian models.
-From these observations, immunosuppression regimens have been employed clinically. However, graft rejection remains a significant risk and an assay to non-invasively monitor the immune response to transplanted intraspinal cell grafts is essential.

- We hypothesize that graft-specific host antibodies generated after stem cell transplantation may be detected in the peripheral blood and could be used as a diagnostic marker of cellular graft rejection.

Methods

- Large Animal Model: Ten Göttingen minipigs received five thoracolumbar intraspinal microinjections of 100,000 donor human neural progenitor cells using a stereotactic platform. Five pigs received tacrolimus (0.0125 mg/kg BID IV) and five did not receive immunosuppression. Plasma was isolated from peripheral blood collected pre-transplant and serially post-transplant at day 7, 14, and 21.

- Clinical Trial: Plasma was collected from six patients with Amyotrophic Lateral Sclerosis enrolled in the Phase 1 trial at Emory (NCT01348451) receiving intraspinal microinjections of donor human spinal cord stem cells. The patients received tacrolimus (4 - 8 ng/mL oral BID), mycophenolate mofetil (1000 mg oral BID), and basiliximab (two doses, 20 mg IV) post-operatively. Tacrolimus and mycophenolate were given for the duration of the trial. Plasma was collected pre-operatively and at post-operative week 2, 4, and 8 from three patients with naïve transplants and three patients with repeat transplants.

- Flow Cytometry Cross Match: Donor human stem cells were co-incubated with collected antibody-containing plasma from the pigs or trial patients (acting as the "primary" antibody). The cells were washed and then incubated with a FITC-conjugated secondary antibody specific to either pig or human IgG, respectively. The cell samples were washed and then run on an LSRFortessa flow cytometer. Relative mean fluorescent intensity (MFI) was measured for post-operative time points and compared to pre-operative baseline to detect the generation of graft-specific antibodies in the plasma. All time points were done in triplicate.

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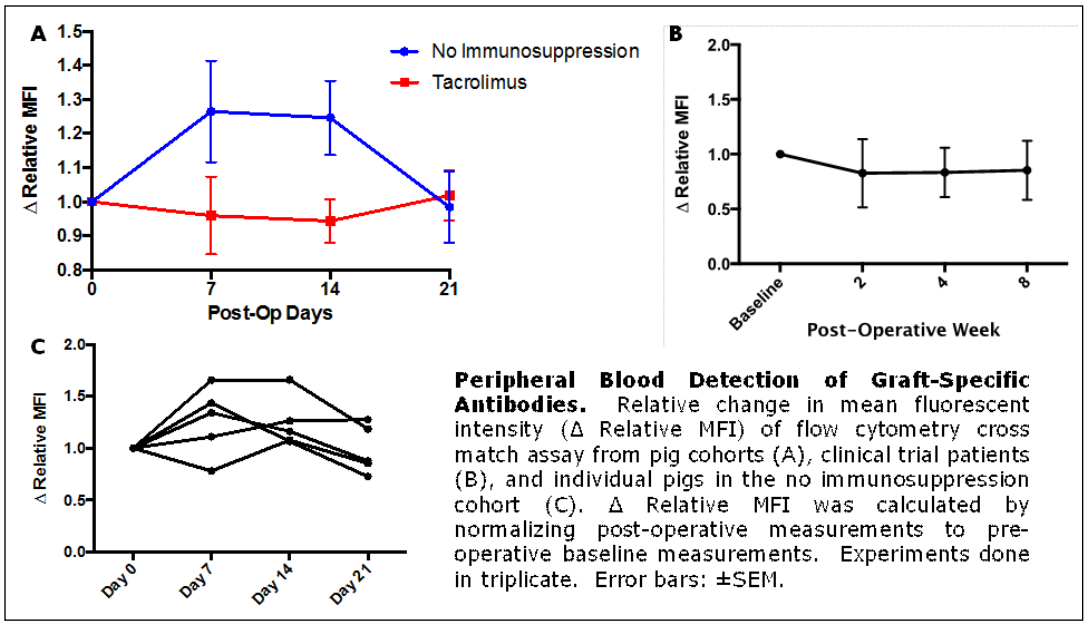

Peripheral Blood Detection of Graft-Specific Antibodies. Relative change in mean fluorescent intensity (Δ Relative MFI) of flow cytometry cross match assay from pig cohorts (A), clinical trial patients (B), and individual pigs in the no immunosuppression cohort (C). Δ Relative MFI was calculated by normalizing post-operative measurements to pre-operative baseline measurements. Experiments done in triplicate. Error bars: ±SEM.

Results

- A transient increase in graft-specific antibodies was detected 7 and 14 days post-operatively in pigs that did not receive immunosuppression. No increase was observed in the tacrolimus group (**A**).
- Different patterns of antibody response were observed in pigs in the no immunosuppression cohort, with both "responders" and "non-responders" (**C**).
- A trend showing a slight decrease in graft-specific antibodies was observed in the clinical trial patients (**B**)

Conclusions

- This method can be used to detect antibody-mediated graft rejection in vivo
- This study provides evidence for a decreased immune response to transplanted intraspinal stem cell grafts with immunosuppression
- Future studies will correlate these peripheral blood findings to immunohistological analysis of transplanted grafts

References

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