Journal of Clinical Neuroscience xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Lab resource

# Peripheral blood detection of systemic graft-specific xeno-antibodies following transplantation of human neural progenitor cells into the porcine spinal cord

Jason J. Lamanna [a,b,*], Juanmarco Gutierrez [a], Jaclyn R. Espinosa [c,d], Jacob Wagner [a], Lindsey N. Urquia [a], Cheryl Moreton [a], C. Victor Hurtig [a], Muhibullah Tora [a,b], Allan D. Kirk [d], Thais Federici [a], Nicholas M. Boulis [a,b]

[a] Department of Neurosurgery, School of Medicine, Emory University, 101 Woodruff Circle, Room 6339, Atlanta, GA 30322, USA
[b] Department of Biomedical Engineering, Georgia Institute of Technology & Emory University, Atlanta, GA 30322, USA
[c] Department of Surgery, School of Medicine, Emory University, Atlanta, GA 30322, USA
[d] Department of Surgery, Duke University, Durham, NC 27710, USA

ARTICLE INFO

*Article history:*
Received 12 July 2017
Accepted 10 October 2017
Available online xxxx

*Keywords:*
Neural stem cell
Pig model
Spinal cord
Immune response

ABSTRACT

Extensive pre-clinical and clinical studies have searched for therapeutic efficacy of cell-based therapeutics in diseases of the Central Nervous System (CNS) with no other viable options. Allogeneic cells represent the primary source of these therapies and immunosuppressive regimens have been empirically employed based on experience with solid organ transplantation, attempting to avoid immune mediated graft rejection. In this study, we aimed to 1) characterize the host immune response to stem cells transplanted into the CNS and 2) develop a non-invasive method for detecting immune response to transplanted cell grafts. Human neural progenitor cells were transplanted into the spinal cord of 10 Göttingen minipigs, of which 5 received no immunosuppression and 5 received Tacrolimus. Peripheral blood samples were collected longitudinally for flow cytometry cross match studies. Necropsy was performed at day 21 and spinal cord tissue analysis. We observed a transient increase in xeno-reactive antibodies was detected on post-operative day 7 and 14 in pigs that did not receive immunosuppression. This response was not detected in pigs that received Tacrolimus immunosuppression. No difference in graft survival was observed between the groups. Infiltration of numerous immune mediators including granulocytes, T lymphocytes, and activated microglia, and complement deposition were detected. In summary, a systemic immunologic response to stem cell grafts was detected for two weeks after transplantation using peripheral blood. This could be used as a non-invasive biomarker by investigators for detection of immunologic rejection. However, the absence of a detectable response in peripheral blood does not rule out a parenchymal immune response.



## 1. Introduction

The use of cell-based therapeutics for treatment of neurodegenerative and traumatic injuries to the Central Nervous System (CNS) presents a promising therapeutic approach [1]. Numerous pre-clinical and clinical studies performed in the brain and spinal cord, conducted by our group and others in large mammals and humans have shown transplantation to the CNS is well-tolerated, safe, and may provide therapeutic benefit [2–10]. For allogeneic sources of clinical cell therapies and pre-clinical xenograft studies, immunosuppressive agents must be used to prolong graft survival [11]. The foundation for these immunosuppression regimens was built from previous experience with solid organ transplantation [12] and limited data from pre-clinical small animal studies in the CNS [13]. However, the interaction between the human immune

*Abbreviations:* APC, allophycocyanin; ANOVA, analysis of variance; BBB, blood brain barrier; CNS, Central Nervous System; CD, cluster of differentiation; FCXM, flow cytometry cross match; FITC, fluorescein; FDA, food and drug administration; GLAST, glutamate aspartate transporter; H&E, hematoxylin and eosin; HLA, human leukocyte antigen; HuNu, human nucleus; hNPC, human neural progenitor cell; Ig, immunoglobulin; IV, intravenous; MRI, magnetic resonance imaging; MHC, major histocompatibility complex; MFI, mean fluorescence intensity; PBS, phosphate buffered saline; PE, phycoerythrin; PSA-NCAM, polysialylated neural cell adhesion molecule; RPMI, roswell park memorial institute; SEM, standard error of measurement.

* Corresponding author at: 101 Woodruff Circle, Room 6339, Atlanta, GA 30322, USA.

*E-mail addresses:* jlamann@emory.edu (J.J. Lamanna), juanmarco.gutierrez@emory.edu (J. Gutierrez), jaclyn.espinosa@duke.edu (J.R. Espinosa), jacob.l.wagner@emory.edu (J. Wagner), lindsey.n.urquia@emory.edu (L.N. Urquia), clmoret@emory.edu (C. Moreton), cvictorhurtig@gmail.com (C. Victor Hurtig), mtora@emory.edu (M. Tora), allan.kirk@duke.edu (A.D. Kirk), thais.buchman@emory.edu (T. Federici), nboulis@emory.edu (N.M. Boulis).

https://doi.org/10.1016/j.jocn.2017.10.033
0967-5868/

system, the CNS, and transplanted cell-based therapies is poorly understood. Furthermore, due to the nature of CNS relative immune privilege [14], surveillance [15,16], and the blood-brain barrier (BBB) [17], data and experience from solid organ transplantation may not be as applicable as previously thought.

The goal of immunosuppressive regimens in cell therapies is to suppress the host immune response to transplanted grafts. In theory, grafted donor cells should then have improved engraftment, survival, longevity, and functional outcomes (e.g. differentiation, functional connections). The most common immunosuppressive regimen utilizes inhibition of T lymphocyte function. This is based on previous experiences with solid organ transplantation, as T lymphocytes are a primary mediator of acute graft rejection. In preclinical and clinical studies, regimens consisting of multiple agents targeting the activation and function of T lymphocytes are employed. For example, in the Neuralstem Phase I/II clinical trial, allogeneic human fetal-derived neural stem cells were transplanted directly into the spinal cord of 18 patients [2,3]. An immunosuppressive regimen consisting of tacrolimus (calcineurin inhibitor, attenuates T cell receptor signals and prevents T cell activation), basiliximab (monoclonal antibody antagonist specific for high affinity IL-2 receptor), and mycophenolic acid (purine analog, limits lymphocyte cell division) was employed. Moreover, these drugs have significant side effect profiles that require explicit management [2]. Data from pre-clinical studies show that even with immunosuppression, a significant amount of cell grafts do not survive or integrate into the tissue [18,19]. Furthermore, methods for quantifying transplanted cell graft survival in clinical studies are critically limited.

Due to a combination of forces, including the physical process of injection, mechanical forces in the parenchyma [20], transient lack of adequate nutrient support, and interaction with the host innate immune system, a large percentage of the transplanted cell graft dies shortly after injection. After injection into the spinal cord parenchyma, it is likely that the BBB is disrupted for up to 5 days [21], thus exposing the donor stem cells to peripheral blood leukocytes [22]. Furthermore, the intact CNS has the capability to directly present antigen to the peripheral immune system with specific CNS-associated macrophages and dendritic cells [23–25]. Additionally, during transplantation, it is likely that a certain percentage of the donor stem cells reflux out of the parenchyma spill into the cerebrospinal fluid [26], which is known to be capable of initiating an immune response via professional antigen presenting cells [27,28]. Lastly, with the recent description of CNS lymphatics, the BBB is not as absolute as previously thought [29]. These factors provide the peripheral immune system with exposure to transplanted antigens, including donor allogeneic or xenogeneic stem cells, and mount an adaptive response from the periphery to sites within the CNS containing donor tissues [30], likely causing immune-mediated graft rejection. The mechanisms underlying this process are poorly defined, and unlike transplanted organs, there are no markers for non-invasive, *in vivo* detection of immunological rejection of transplanted autologous, allogeneic, or xenogeneic cell grafts transplanted into the CNS.

The purpose of this study was to characterize the host immune response to stem cells transplanted into the CNS and develop a non-invasive method for detecting immune-mediated graft rejection. To achieve this, we transplanted human neural progenitor cells into the spinal cord of healthy Göttingen minipigs with and without immunosuppression. In this report, we demonstrated the ability to detect circulating, graft-specific xeno-reactive antibodies in the peripheral blood following transplantation of stem cells into the spinal cord and characterized the local inflammatory response. Furthermore, we showed that these systemic antibodies are transient and the response is attenuated with administration of tacrolimus immunosuppression. However, this attenuation was not directly correlated with improved cell graft survival.

## 2. Materials and methods

### 2.1. Ethics Statement

All procedures were conducted at the Emory University Division of Animal Resources in accordance with protocol #2002806 "Overcoming the Practical Barriers to Spinal Cord Cell Transplantation for ALS" approved by the Emory University Institutional Animal Care and Use Committee.

### 2.2. Cell Culture and Preparation

Stocks of human fetal cortex-derived neural progenitor cells (hNPCs) from passage 24–34 were kindly provided by the Clive Svendsen laboratory at Cedars-Sinai Regenerative Medicine Institute [4,9]. The hNPCs were originally isolated from eight-week-old *post-mortem* fetal cortex of an aborted fetus with Institutional Review Board approval. Briefly, the intact cortical mantel was isolated and dissociated to a single cell suspension. The resulting cell line was expanded to free floating neurospheres of hNPCs [31].

On the morning of transplantation, neurospheres were chemically dissociated with trypsin (TrypLE Express, 12604, ThermoFisher) and DNase (D4527, Sigma-Aldrich) and filtered with a 50 μm separation filter (130-041-407 Miltenyi Biotech) to a single cell suspension in Magnesium and Calcium free hibernation medium (Proprietary, provided by Svendsen lab). The cells were concentrated to $1 \times 10^4$ cells/μL and stored on ice. Cell viability was calculated using a trypan blue exclusion assay. >80% viable cells were required for transplantation.

### 2.3. Flow Cytometry Characterization

hNPCs from passage 24, 26, and 32 were used for characterization studies. Flow cytometry quantification of antigenic surface markers were quantified and compared to control cells. The cells were processed with the following antibodies: anti-Human Leukocyte Antigen (HLA)-DR conjugated allophycocyanin (APC) (BD Biosciences 340549), anti-β2 microglobulin conjugated fluorescein (FITC) (BD Biosciences 551338), anti-CD80 conjugated phycoerythrin (PE) (BD Biosciences 560925), anti CD86 PE (BD Biosciences 560957), anti-glutamate aspartate transporter (GLAST) (Miltenyi Biotech 130–098-804), and anti-polysialylated neural cell adhesion molecule (PSA-NCAM) (130-093-273). The samples were run on the LSRFortessa flow cytometer. Gating and quantification was performed using FlowJo software.

### 2.4. Transplantation

A three-level $T_{14}$ to $L_2$ laminectomy was performed in the thoracolumbar spine of ten healthy female Göttingen minipigs. An incision was made into the dura mater and three spinal cord segments were exposed. A stereotactic injection platform was used to insert a 29-gauge needle into the spinal cord, as previously described [32]. Five 10-μL injections (10,000 cells/μL for $1 \times 10^5$ cells/injection) were unilaterally infused at 5 μL/minute. Inter-graft spacing was 4 mm.

### 2.5. Postoperative management and immunosuppression

Female Minipigs were either immunosuppressed with tacrolimus (Prograf, 0.025 mg/kg, BID, IV) (n = 5) or did not receive immunosuppression (n = 5). Methylprednisolone bolus (Solume-

Please cite this article in press as: Lamanna JJ et al. Peripheral blood detection of systemic graft-specific xeno-antibodies following transplantation of human neural progenitor cells into the porcine spinal cord. J Clin Neurosci (2017), https://doi.org/10.1016/j.jocn.2017.10.033