BDIAP Student Elective Award Report

Role of HLA Matching for Tolerance Induction in Miniature Swine Models

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Introduction

Intestinal transplants are associated with the worst outcomes of all solid organ transplants and require high levels of maintenance immunosuppression. This carries many risks, including increasing the risk of infection, graft-versus-host disease and malignancies. Inducing tolerance would significantly reduce the level of immunosuppression required but is challenging given frequent human leukocyte antigen (HLA) mismatches between donor and recipient. The relative importance of class I and II major histocompatibility complex (MHC) mismatching in tolerance induction is not fully understood in transplant recipients.

Aims and objectives

Our hypothesis is that MHCII matching improves graft tolerance. Previous intestinal transplants (ITx) in fully mismatched and haplotype matched swine models have been carried out, but only 2 partial mismatches. The aim of the current ITx is to test the reproducibility of our hypothesis.

My personal objectives were to build my clinical knowledge, especially in interpreting lab results and creating management plans in response to clinical observations and daily labs. I was also eager to build upon wet lab skills in running assays and in analysing data from flow cytometry and histology.

Methods

Prior to transplant, a central line was placed, and the pig was depleted for CD8 T lymphocytes with anti-CD8 antibodies. An allographic intestine transplant was then performed on a pair of miniature swine models that were partial SLA mismatches (SLA I^{CD}/II^{DD} and SLA I^{AA}/II^{DD}). A stoma was additionally created in surgery for endoscopic access to the graft. Following transplant, the pig was on an immunosuppressive regimen with the main maintenance immunosuppressant being Tacrolimus; doses were adjusted in response to clinical evaluation and blood levels.

Blood was drawn from a central line to determine Tacrolimus levels and perform flow cytometry. Mixed lymphocyte assays were additionally carried out to determine recipient responsiveness to donor lymphocytes. Samples of intestinal mucosa were collected during endoscopies, stored in formalin and fixed in paraffin prior to Haematoxylin & Eosin (H&E) staining.

Results and role of histopathology

The ITx surgery was successfully performed, and the pig is currently on post-operative day (POD) 50. Chimerism reached a peak of 3.27% on POD5 and has dropped since to 0.42% at POD50. This trend of an early peak in chimerism followed by a gradual decrease to a low but non-negligible level has been observed in prior partial mismatches but is consistently absent in full mismatches. In addition, we observed donor hypo-responsiveness, and this is associated to a steady appearance of CD25+ T regulatory cells in peripheral circulation. The pig has currently developed an infection shown by elevated white blood cell counts and is being treated with antibiotics and antifungals. The pig does not currently demonstrate signs of rejection.

Histopathology played a vital role in this project in determining the post-operative status of the intestinal graft. Whilst endoscopies revealed general status macroscopic level, staining of tissue samples was required to validate observations at microscopic level. This involved biopsy collection through endoscopic collection, followed by H&E staining and assessment with the histology team.

Being involved in many aspects of this process was invaluable in learning about the importance of a multi-disciplinary team and strengthened my skills in analysing histological slides. In particular, I about the main features to look out for when assessing for rejection and comparing these to healthy intestinal mucosa. So far, the tissue samples collected from the pig have appeared healthy and not demonstrated signs of rejection.

Conclusion

I found this project immensely exciting and am so grateful to BDIAP for supporting this project. This is the only centre in the world with the inbred Sachs miniature swine models that enable transplantation experiments that can be controlled for SLA class I and/or mismatching, so it was a huge privilege to be able to travel to New York for this. Weekly updates regarding the status of the pig were presented at meetings with a multi-disciplinary team and being a part of the whole process highlighted to me the importance of histopathology in clinical assessments. This experience has helped me reflect on how my interests and skills might align to pathology as a specialty and I hope to be able to further explore this throughout medical school.