Developing an invitro model for immune mediated podocyte injury in Nephrotic Syndrome

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Minimal Change Disease (MCD) results from damage to podocytes - specialized non-mitotic cells that form an integral part of the basement membrane to help regulate blood filtration in the kidney.[1] Despite poorly understood mechanisms, immunosuppressive medications such as Prednisone (broad immunosuppression), Rituximab (anti-B cell therapy) and other immunosuppressive medications have been used with success in some, but not all, patients with MCD. [2,3] Our aim was to elucidate the immunological pathway that leads to podocyte injury.

Methods: Samples from patients with disease or healthy controls were used to test our hypothesis. Patient plasma or serum (which included immunoglobulin, IgG) was added directly to a conditionally immortalized podocyte cell line. The cells were then stained with fluorescent anti-human IgG and imaged using an immunofluorescent microscope. Thymocytes were incubated with patient plasma for 48 hours to make conditioned media to test on podocytes. Immunohistochemistry using immunofluorescence staining was used on podocytes to evaluate vinculin changes as a marker for podocyte adhesion and functionality in vitro.

Results: There was no difference in healthy vs. patient IgG binding specificity to the podocyte cell line. Additionally, we tested focal adhesion complexes as an indicator of podocyte injury after exposure to thymocyte conditioned media from patients versus conditioned media from healthy controls. In our limited sample size, there was no statistically significant difference in the quantity of focal adhesion complexes per cell area, as measured by vinculin expression.

Conclusion: There was no difference between patients and healthy controls in IgG binding to podocytes. Additionally, we found that the podocytes were not affected through a thymocyte mediated mechanism. This study lays groundwork for future studies that investigate the role of immune mediated injury to podocytes, the cell type often injured in glomerular disease.

References

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