Effect of Physiological Flow on Mesangial Cells: Implications for IgA Nephropathy

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Abstract

- Immunoglobulin A (IgA) nephropathy can lead to kidney failure; characterized by IgA accumulation
- Mesangial cells : substructure in glomerulus that helps regulate filtration
- Mesangial role in kidney disease is not yet understood
- Transport of fluids and proteins into and within the mesangial are poorly understood
- Many cell types change phenotype when grown in the presence of physiological flow, versus static conditions
- Mesangial cells produce extracellular matrix and in IgA nephropathy they proliferate and deposit extra matrix

We hypothesized that mesangial cells cultured in the presence of IgA and subjected to physiological flow will show alterations in the matrix. For my UROP project, I undertook a first step by making type I collagen gels and testing them for their permeability. I conducted several studies in which the flow of water through these collagen gels was measured using a perfusion chamber. The long-term goal of this study is to determine how matrix deposition by the mesangial cells changes when cultured in flow conditions.

Introduction

Mesangial cells are one of the specialized cells in the glomerulus of the kidney (Figure 1) [1]. They help regulate the filtration process of the kidney and also provide support for the glomerular structure. They are involved in the kidney's response to injury and disease [1]. Although mesangial cells have been studied in the past by other groups to try to determine their role in the development of long-term renal diseases, there is still very little known about how mesangial cells actually work and what role they play in renal diseases. Furthermore, the transport of fluids and proteins into and within the mesangial region of the kidney has not been well studied [1].

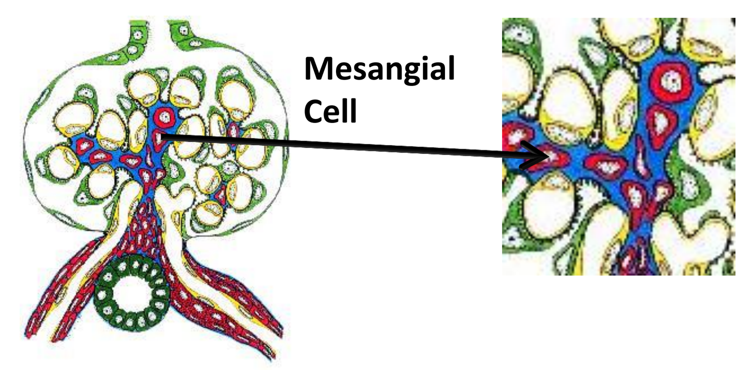

Figure 1: *Glomerular diagram (green = epithelial cells, yellow = endothelial cells, red = mesangial cells). http://www.uncnephropathology.org/jennette/ch1.htm*

IgA nephropathy is a medical disorder in which immunoglobulin A (IgA), a specialized antibody, builds up in a patient's kidneys (Figure 2) [2]. When a patient has IgA nephropathy, their kidneys are no longer able to filter excess water and waste products from their blood, resulting in the presence of protein and blood in their urine. In IgA nephropathy, IgA complexes accumulate within the mesangium. IgA nephropathy is the most prevalent primary chronic glomerular disease of the kidney in the U.S. The exact number of cases of IgA nephropathy is not known, since a definitive diagnosis requires a kidney biopsy. There is no disease-targeted treatment for IgA nephropathy, but physicians can prescribe medications that suppress the patient's levels of angiotensin II and protein-urea, as well as control the patient's blood pressure.

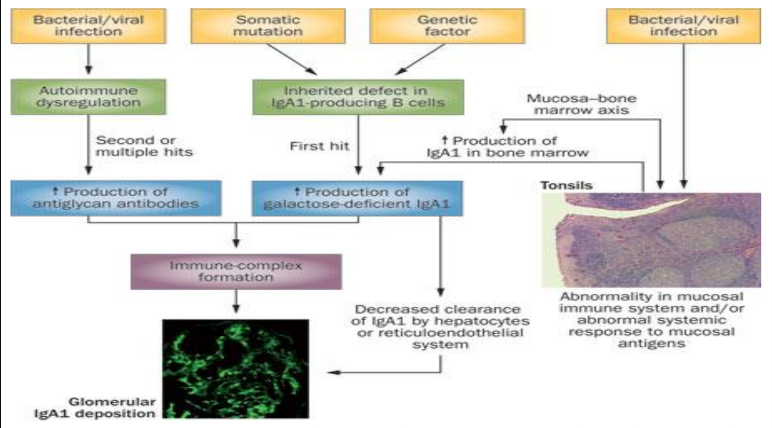

Figure 2: *Proposed pathways involved in the mesangial deposition of IgA1 in IgA nephropathy. http://www.nature.com/nrneph/journal/v8/n5/fig_tab/nrneph.2012.58_F1.html*

Studies have shown that cells grown in the presence of **physiological flow**, versus static conditions, act more like they do within the human body [3]. Mesangial cells produce extracellular matrix molecules, and a key feature of individuals with IgA nephropathy is proliferation and excess matrix production (Figure 3) [1]. Studying the matrix deposition of mesangial cells in conditions of flow may provide us with a better idea of how mesangial cells function.

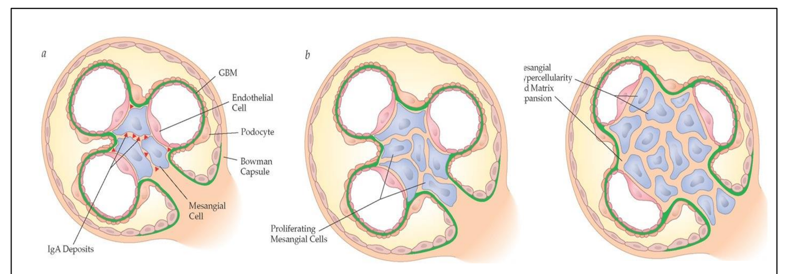

Figure 3: *Events in IgA nephropathy leading to mesangial hypercellularity and matrix expansion. (a) IgA deposition (b) proliferation of mesangial cells (c) mesangial hypercellularity and matrix expansion. http://what-when-how.com/acp-medicine/glomerular-diseases-part-2/*

Hypothesis

The long-term goal of this study is to determine how matrix deposition by the mesangial cells changes in individuals with IgA nephropathy as the flow changes. **My hypothesis** is that mesangial cells that are cultured in the presence of IgA and subjected to physiological flow will show alterations in the matrix deposition due to alterations in flow rate. During my UROP, preliminary experiments were performed to lay the groundwork for testing this hypothesis.

Methods

Collagen gels were made following procedures established by a student who previously worked in the lab. To make 4 mL of a collagen mixture, 2.46 mL of collagen, 568 µL of 1M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 400 µL of 10x Modified Eagle Medium (MEM), 240 µL of Fetal Bovine Serum (FBS), 104 µl of 1M NaOH, 40 µL of L-glutamine, 4 µL of Penicillin-Streptomycin (P/S), and 4 µL of Fungizone were combined, and mixed completely. The collagen mixture was then allocated into smaller tissue culture plates and allowed to incubate at 37° for 24 hours so that they had ample time to solidify.

Perfusion Chambers: In this project, I used a perfusion chamber designed by previous students in the lab (Figure 4)[4]. The gels were tested in three different sizes of perfusion chambers (1 mm, 2.5 mm, and 4.0 mm). The smallest perfusion chamber seemed to contain the gel and hold it in a position which allowed for optimal water flow without destroying the gel or having water flow around it. Therefore the smallest perfusion chamber was used in the preliminary tests.

Figure 4: *Perfusion chamber. Sizes of chambers were 1 mm, 2.5 mm, and 4.0 mm*

Preliminary Data: The gels were placed in the smallest perfusion chamber, 1mm diameter, and the chamber was then placed into a container filled with water and hooked up to a setup of tubing (Figure 5). The tubing was attached to a wooden board and hooked up to the sink allowing water to flow through the tubing. Any bubbles that appeared in the tubing were removed from the tubing to ensure that when measuring the distance the water traveled per second would not be disrupted with an air bubble. Once the tubing was clear of all air bubbles and were lined up perpendicular to the ground, 500 µL of olive oil was added to the top of the tube to ensure that no water evaporated out of the system. A switch was opened which set the water flowing toward the perfusion chamber. Gravity was used to flow water through the chamber to test the permeability of the collagen gels.

Figure 5: *Set up used to test the perfusion chambers*

Transwell Plates: 6 wells Transwell plates were tested for their ability to allow the flow of fluids through the pores. The wells of the plates had filters with 0.4 um diameter pores which served as a model, for the pores observed between the mesangial cells in the kidney. Water flowed through the filters to determine the flow rate of the water through the pores. Collagen gels were then generated in the upper chambers of the Transwell plates.

Computational Modeling: In order to observe the effects that the basement membrane thickness, volume fraction of matrix, and different sizes of IgA had on the pressure, a computational model developed by Sarah Hunt was used. The MatLab code Sarah has written stimulates the flow through the mesangial matrix. Different inputs were used and the outputs of the code were observed using Paraview.

Results

The results of a collagen filled perfusion chamber were analyzed to find the hydraulic resistance. This was done through manipulation of Equation 1 into Equation 2, where Equation 2 was applied to the data through a linear fit where a best fit line of the data was applied (Figure 6).

$$\Delta h = \Delta h_o e^{-\frac{\rho g}{R_{hyd} A_{tube}} t} \quad \text{(Equation 1)}$$

$$Ln\left(\frac{\Delta h}{\Delta h_o}\right) = -\frac{\rho g}{R_{hyd} A_{tube}} t \quad \text{(Equation 2)}$$

The best fit line was y=-0.0005x+0.009 with a R^2 value of 0.9994.

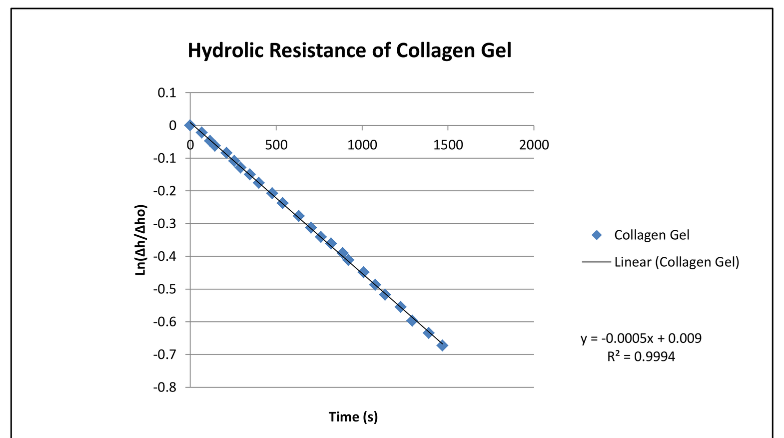

Figure 6: *Water through collagen gel perfusion chamber*

Using the area of the tubing, the density of water , gravity, and the slope of the best fit line, the hydraulic resistance was calcluated to be 2.848[9] $\frac{Pa*s}{m^3}$.

Transwell Plates: The Tranwell plates did not serve to be optimal because the fluid did not flow freely through these chambers. Despite this, collagen gels were generated in the upper chambers of the Transwell plates and were conserved in PBS for future experimentation. This showed that the gels could solidify in the upper chambers of the wells; which can be applied to future studies.

Results

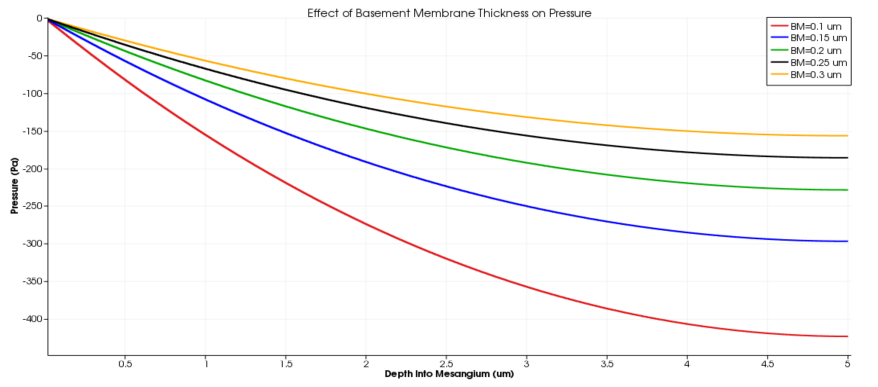

Figure 7: The effects of the basement membrane thickness on the pressure.

As the basement membrane thickness decreases the magnitude of the pressure increases (Figure 7).

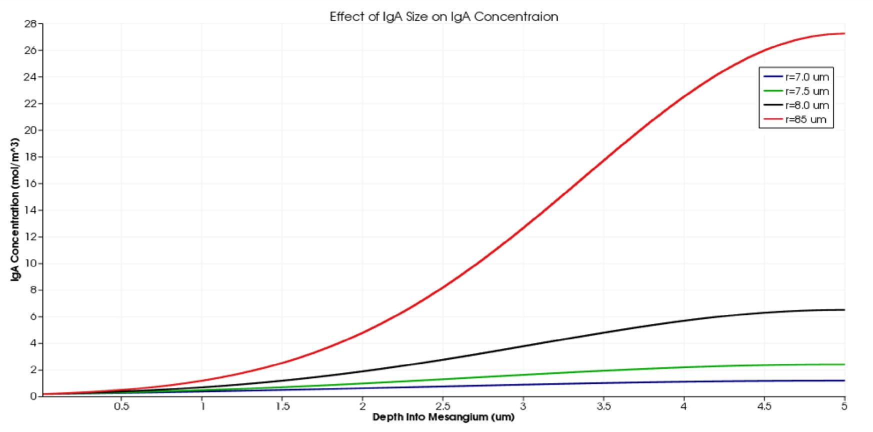

Figure 8: The effects of the IgA radius on the concentration

IgA concentration is found to increase when the size of the IgA is increased (Figure 8). The concentration is modeled to be much greater than is physiologically possible in the body. This is due to the code not including an increase in osmotic pressure from IgA concentration. Increasing IgA concentrations to the level simulated here would lead to large osmotic pressure increases, which would decrease filtration and counteract IgA accumulation.

Conclusions

During the course of these experiments, a better procedure was developed for testing the permeability of collagen gels in the perfusion chambers. The new procedure established that certain precautions must be taken before the gels are to be tested and certain materials must be available before performing the trials. When the commercially purchased mesangial cells are purchased and tested, the procedures developed throughout these trials will most likely be used to ensure that the flow is properly measured through the device and across the gels.

The long-term goal of this study is to determine how matrix deposition by the mesangial cells changes in individuals with IgA nephropathy as the flow changes. It is hypothesized that mesangial cells cultured in the presence of IgA and subjected to physiological flow will show alterations in the matrix deposition due to alterations in flow rate. Plasma derived from the blood of individuals with and without IgA nephropathy will be added to culture the mesangial cells. By using fluorescently tagged IgA, the movement of the IgA complexes will be monitored as they flow through the chip. Changes in IgA transport that occur when the pressure gradients are changed will be monitored. By time-lapse photography, differences in the way that the mesangial cells appear when cultured in the presence of sera from patients with and without IgA nephropathy will be detected. In future studies, the matrix porosity will be monitored; the porosity will be quantified and possibly the different molecules that are secreted by the mesangial cells during the experiments will be identified.

References

[1] Abboud, Hanna E. Mesangial Cell Biology. Experimental Cell Research 318.9: 2012; 979-85. PMID: 22414873

[2] Moura IC, Benhamou M, Launay P, Vrtovsnik F, Blank U, Monteiro RC. The glomerular response to IgA deposition in IgA nephropathy. *Seminars in Nephrology*. 2008;28(1):88–95.

[3] Jang KJ, Mehr AP, Hamilton GA, McPartlin LA, Chung S, et al. Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment. Integr Biol 19: 2013; 1119–29. PMID: 23644926

[4] Lai KN, Leung JC, Chan LY, Saleem MA, Mathieson PW et al. Activation of podocytes by mesangial-derived TNF-alpha: glomerulo-podocytic communication in IgA nephropathy. Am J Physiol Renal Physiol 294: 2008; F945-955. PMID: 18256312

[5] Li CY, Wood DK, Huang JH, Bhatia SN. Flow-based pipeline for systematic modulation and analysis of 3D tumor microenvironments. Lap Chip. 2013; 13(10):1969-78. PMID: 23563587

[6] Mene P, Stoppacciaro A. Isolation and propagation of glomerular mesangial cells. Methods in molecular biology 2009; 466: 3–17. PMID: 19148614

[7] Stockand JD, Sansom SC. Glomerular mesangial cells: electrophysiology and regulation of contraction. Physiol Rev.1998; 78:723–744. PMID: 9674692

[8] Weinberg E, Kaazempur-Mofrad M, Borenstein J. Concept and computational design for a bioartificial nephron-on-a-chip. Int J Artif Organs. 2008; 31:508–514. PMID: 18609503