ECUSTEC
Genetics and Optional Samples

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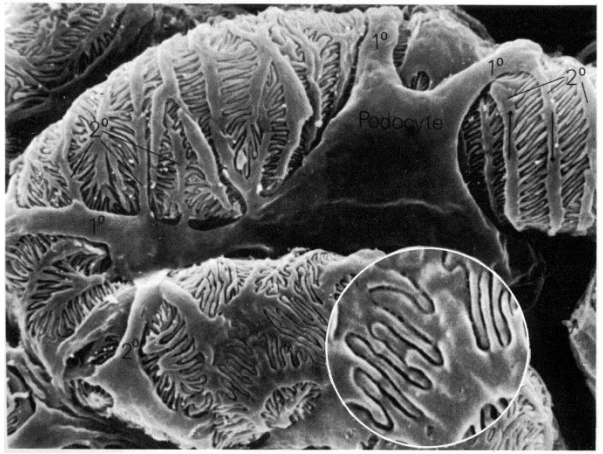
Introduction

- We are interested in two aspects of the pathogenesis of HUS

  - Are there genetic mutations that predispose to a sensitivity to HUS?

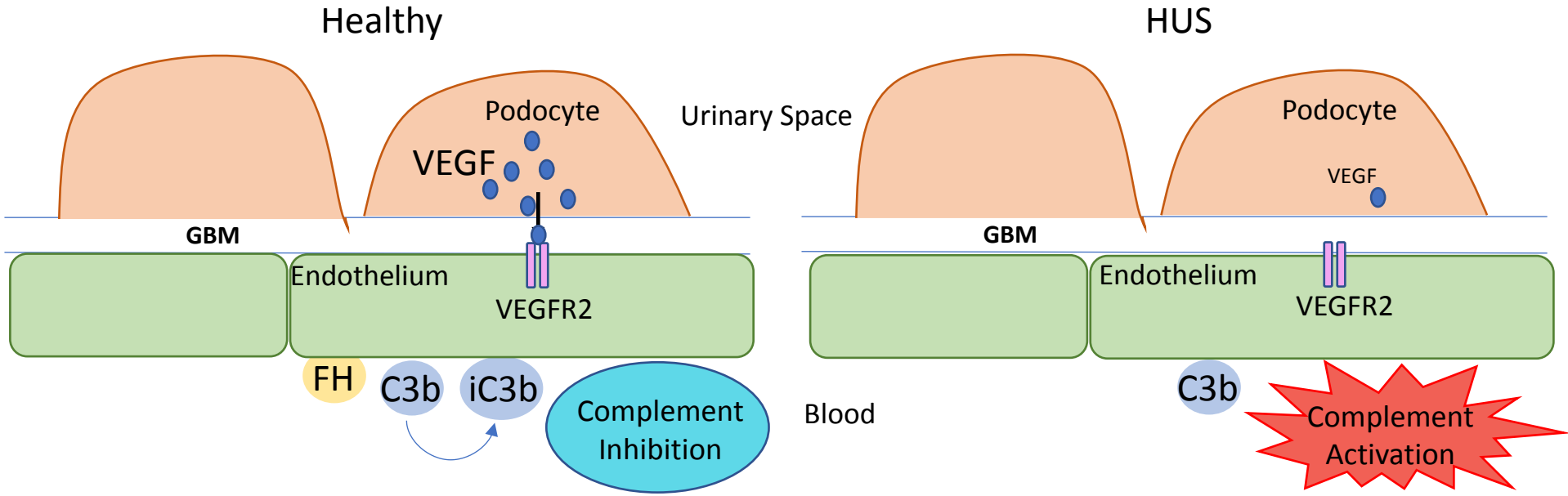
  - What is occurring in the podocyte/endothelial cells in HUS?



Cormack, D.H. Ham's Histology, 9th ed., Lippincott, Philadelphia, 1987, p. 578.

Hypothesis

- The podocyte is a central target of Shiga-toxin damage
- Podocyte prevents complement inhibition via reduction in podocyte VEGF secretion
- Leads to thrombotic microangiopathy



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Genetics blood Sample

- 4ml sample taken between obtaining consent and day 8.
- Investigate genetic susceptibility to STEC-HUS.
- Focus on any polymorphisms associated with altered complement regulation.

Optional Samples

- Lithium heparin blood (volume dependent on body weight) at day 1, 2, 4, 6, 8 and 30.
- Urine day 1, 4, 8 and 30 (where possible)
- Patients >15 kg based in Bristol will be asked for an additional 10ml – time critical.

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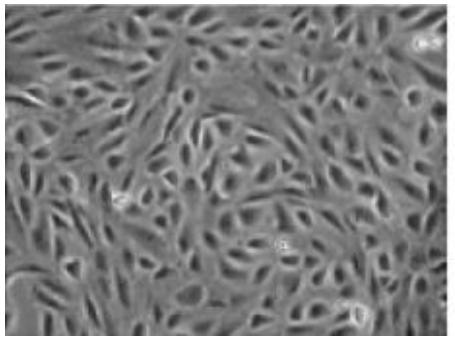
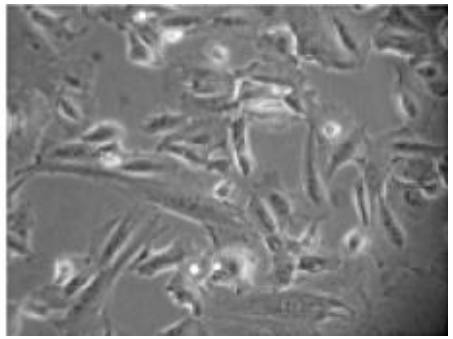
Cell Culture at Bristol Renal

- Isolated and conditionally immortalised cell lines from healthy kidneys
  - replicate at 33°C
  - differentiate at 37°C
- Can co-culture podocytes and endothelial cells together to give a more accurate representation of *in vivo* conditions
- Serum from patients and healthy controls can be incubated with these cells
  - Lyse and blot for C3, VEGF, Factor H etc to see if patient serum causes up/down regulation
  - Run ELISAs on cell culture supernatant to assess complement/VEGF synthesis
- Do these effects decrease with the use of eculizumab?
- Placebo vs treatment



Podocyte 33



Podocyte 37
Differentiated

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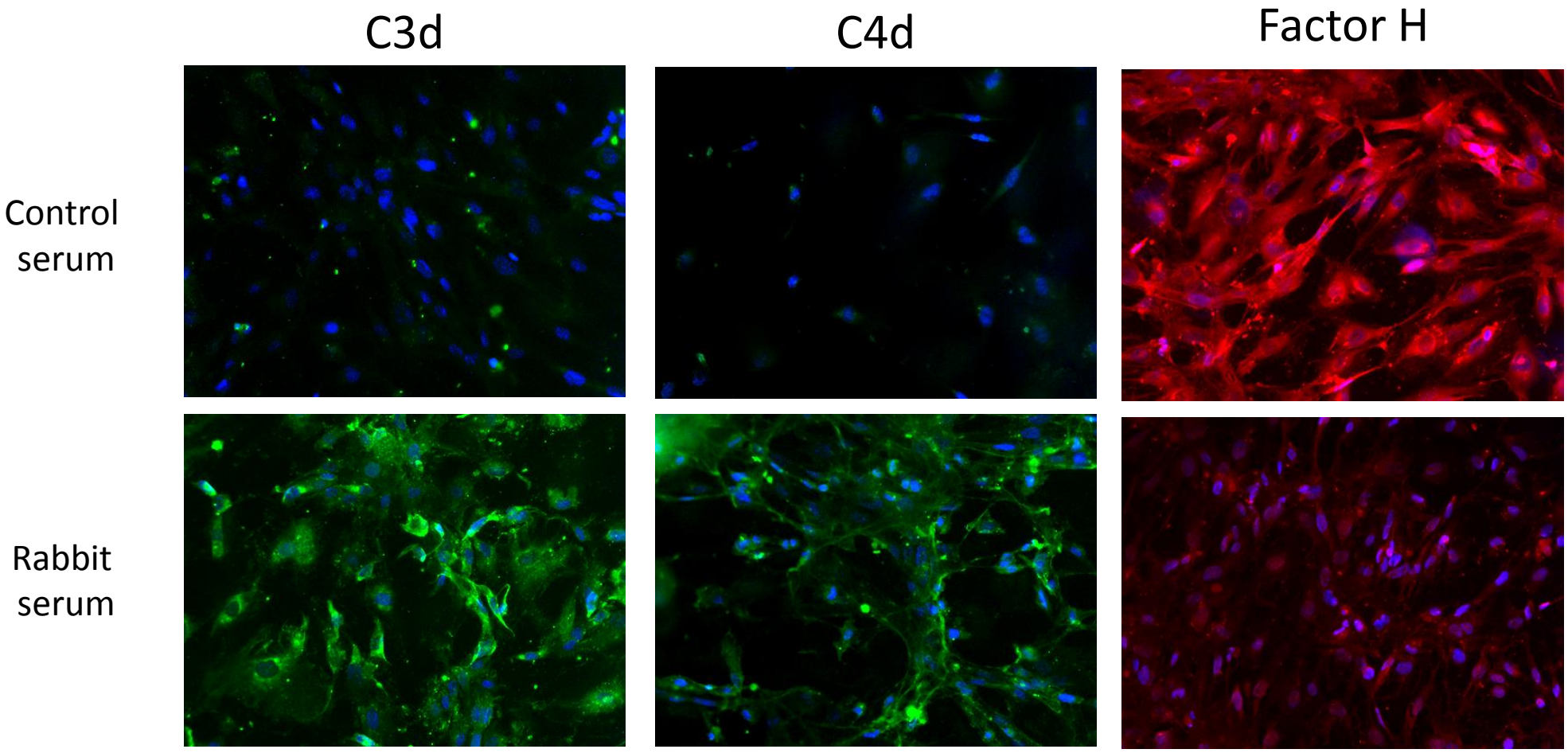
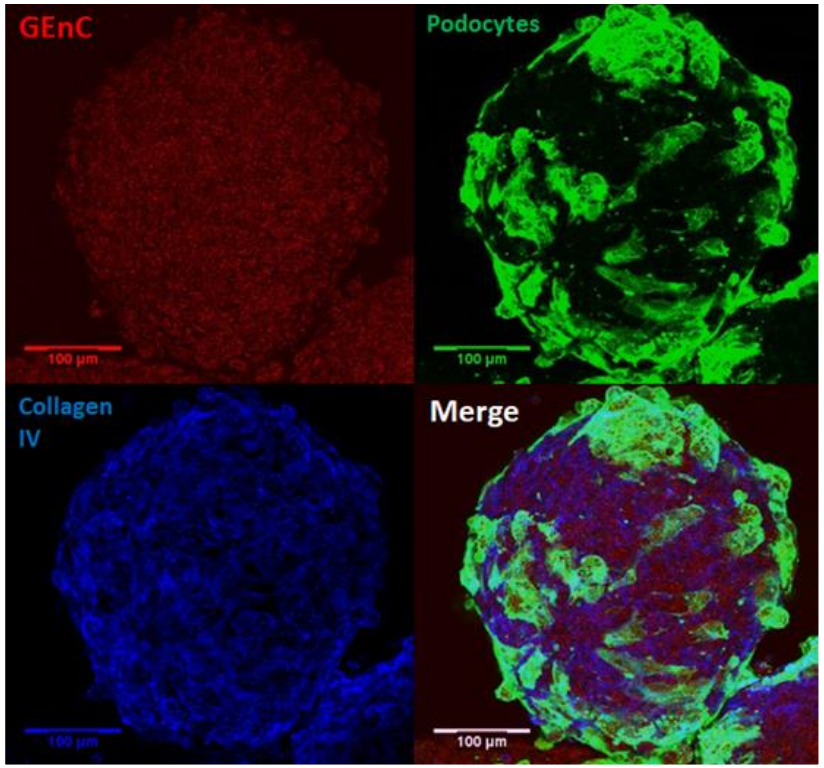
Complement activation

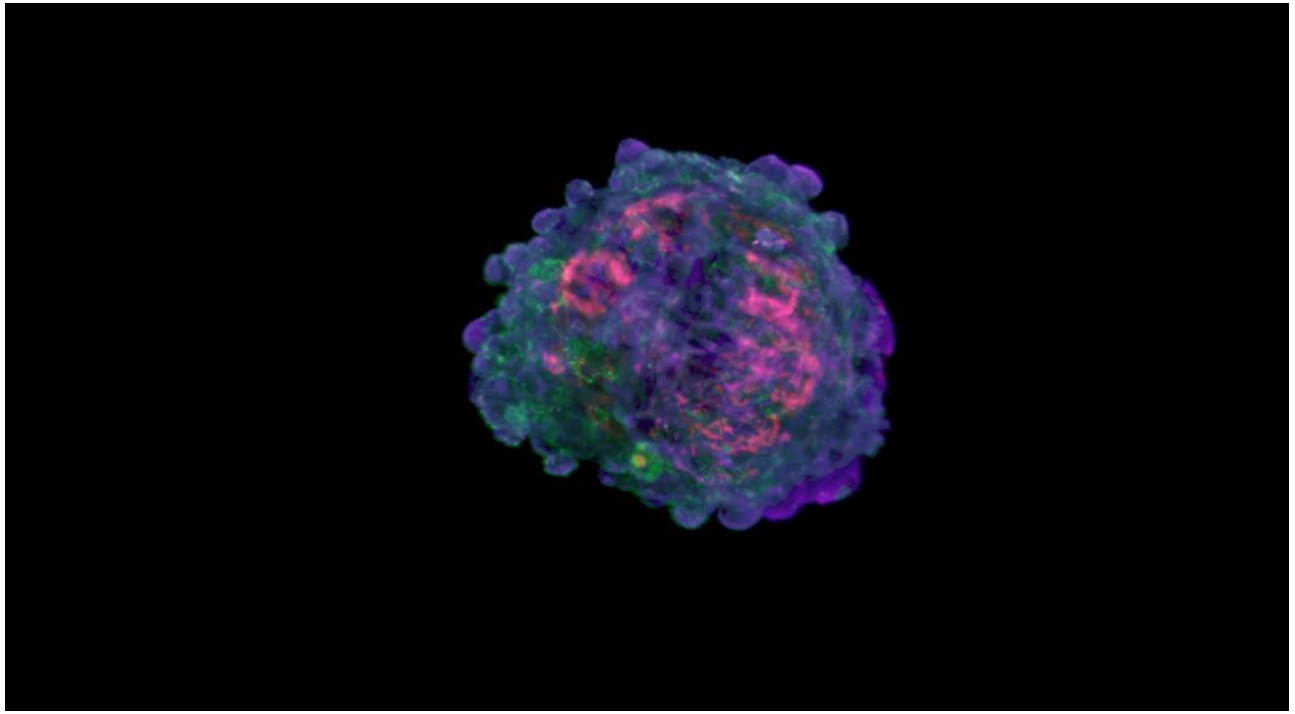
- Proof of principle – complement activation with rabbit serum
- Control is heat inactivated human serum



Spheroids

- Can co-culture podocytes and endothelial cells in the form of spheroids
- Come together to form a GBM





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Courtesy of Jack Tuffin, University of Bristol

Neutrophil Isolation

- The mechanism of delivery of Shiga-toxin (Stx) to the podocyte is currently unknown
- Neutrophils will be isolated from a small number of local patients (local patients only due to time critical nature of isolation)
- These neutrophils can be added to co-cultures and spheroids to see if addition of neutrophils from STEC-HUS patients causes complement activation/VEGF reduction
- Compared to neutrophils from healthy controls and placebo.

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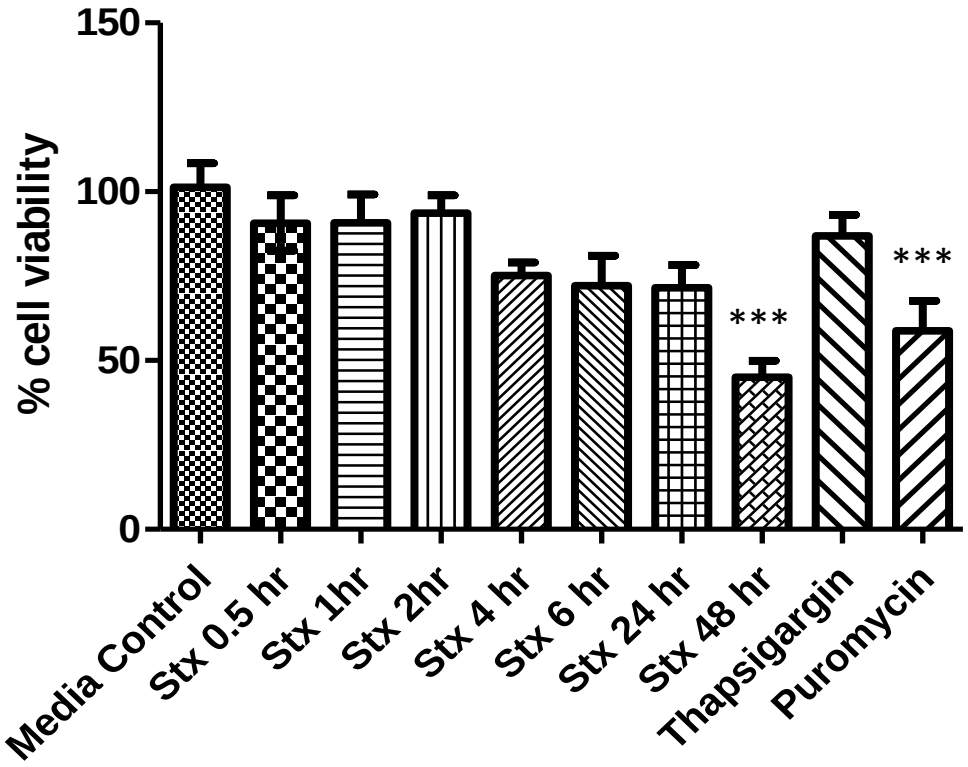
Shiga-Toxin

- We are currently carrying out experiments with Shiga toxin at Bristol Renal
- We can add this to our co-culture experiments to see if addition of shiga toxin directly to cells causes similar effects to addition of plasma/neutrophils from STEC-HUS patients



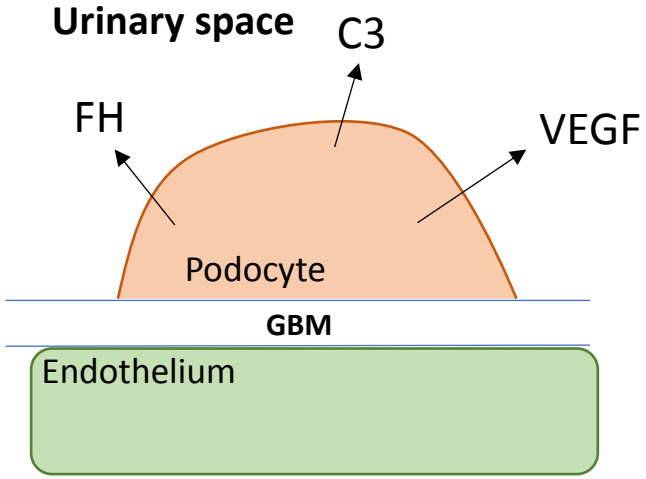
Differentiated human podocytes incubated with 0.1ng/ml of Shiga toxin for 0.5-48 hours as shown.

Viability of podocytes is significantly reduced.

Courtesy of Emily Bowen, University of Bristol

Urine Samples

- Precipitate protein and blot for C3, VEGF and FactorH
- Run ELISA's for C3, VEGF and FactorH against control urines
- As these are across several time points during disease we will look to see if we can identify when podocyte injury occurs
- Could this be a novel biomarker for disease?



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Thank You!

- A huge thank you to the patients and their families for agreeing to participate
- Everyone involved in recruiting patients and taking samples
- Everyone involved in ECUSTEC
- Bristol Renal