**The Role of the Podocyte in Shiga Toxin Associated Haemolytic Uraemic Syndrome**

**Importance:** Haemolytic uraemic syndrome (HUS) is a triad of microangiopathic haemolytic anaemia, thrombocytopenia and acute kidney injury; classified histologically as a glomerular thrombotic microangiopathy (TMA). In 90% of cases, HUS follows gastroenteritis secondary to infection with Shiga toxin (Stx) producing enteric pathogens such as *Escherichia coli*. Stx HUS is the leading cause of acute kidney injury in children with an associated mortality of 5%. In adults a more severe clinical course is observed with chronic proteinuria, hypertension, end-stage renal failure and a mortality of 12%. The precise pathophysiological mechanisms following Stx infection leading to TMA are poorly understood. However, in recent years the podocyte has taken centre stage as a possible initiator in the disease process.

**Objective:** To elucidate the glomerular mechanisms underlying the action of Shiga toxin associated HUS and to elucidate the importance of the cellular Gb3 receptor in this process. The ultimate aim of this work was to identify novel pathways activated in the podocyte following Shiga toxin treatment using a non-biased approach.

**Methods:** To determine the mechanism responsible for glomerular TMA in Stx HUS we performed total and phospho-proteomic studies in conditionally immortalised human podocytes following incubation with 0.1ng/ml Shiga toxin at 0.5 hours and 6 hours. Target proteins identified were validated by western blotting and phospho-protein blotting. To control for non-specific, non-Gb3 receptor binding we also studied the proteomic changes in a stable human podocyte Gb3 receptor knockout cell line. This was generated using short hairpin siRNA against Gb3 synthase.

To determine the physiological role of the Gb3 receptor *in vivo* we generated constitutive Gb3 knockout mice through a collaboration with MRC Harwell. Confirmation of Gb3 knockout was achieved using immunofluorescence for Gb3, endpoint PCR for Gb3 synthase mRNA in mouse kidney, liver and heart tissue and tandem mass spectrometry.

**Results:** We confirmed knockdown of the Gb3 receptor in the Gb3 synthase knockdown podocyte cell line using immunofluorescence and RT-PCR. This confirmed a knockdown of 90%. Gb3 synthase knockdown podocytes were resistant to Stx challenge; even at doses 1,000 fold higher (100ng/ml) than concentrations that elicit cell stress and apoptosis in wildtype podocytes. Initial findings have shown that following 0.1ng/ml Stx treatment, wild type human podocytes express markers of ER stress and the unfolded protein response as early as 0.5 hours from exposure. Key pathways identified include the transcription factor CHOP, ATF6 and p38 MAPK pathways. These markers are not elevated in the Gb3 receptor knockout podocyte cell line suggesting they are specific to Stx-Gb3 receptor binding.

We confirmed the critical physiological role of the Gb3 receptor *in vivo* by administering intraperitoneal Stx to constitutive whole body Gb3 knockout mice. These mice were completely protected from developing HUS even when given doses 400 times the LD50 dose (median dose required to kill half the wild-type population).

**Conclusions:** Through the application of an unbiased, rigorous experimental approach using total and phospho-proteomics we have identified several key pathways activated in the human podocyte following Stx exposure. Furthermore, we have demonstrated that Stx exerts its toxic effects via the Gb3 receptor both *in vitro* and *in vivo*. Through further interrogation of our data this work has the potential to lead to novel therapeutic targets to help treat patients with this devastating disease.