TITLE: NOVEL RECOMBINANT MONOCLONAL ANTIBODIES PROTECT THE VIABILITY OF HUMAN RENAL EPITHELIAL AND ENDOTHELIAL CELLS FROM SHIGA TOXIN TYPE 2 CYTOTOXICITY

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ABSTRACT:
Post diarrhea Hemolytic Uremic Syndrome (HUS) is the most common cause of acute renal failure in children in Argentina. Neither a licensed vaccine nor effective therapy for HUS is available for humans. It is well established that Shiga toxin type 2 (Stx2) causes direct damage to glomerular endothelial cells and tubular epithelial cells. Previously, we have reported that Stx2 decreased cell viability of human glomerular endothelial cells (HGEC) and human renal epithelial cells (cell line HK-2) in culture. Recently, recombinant antibodies against Stx2, produced in bacteria, were developed and characterized. In this work, we studied the ability of anti-Stx2 FabC11 antibody to neutralize the Stx2 activity on HGEC and HK-2 cells.
HGEC and HK-2 were plated in 96-well plates and grown to confluence. Then, cells were treated in growth-arrested conditions for 72 h with different pre-incubations (1 h at 37ºC) o co-incubations of FabC11 with Stx2. Antibodies were used from 10 µg/ml to 0.001 µg/ml and Stx2 at the dilution required to kill 50% of cells (0.1 ng/ml). Finally, the viability was assessed by neutral red uptake. Under both conditions evaluated, FabC11 significantly neutralized, in a dose-dependent manner, the cytotoxic effects caused by 0.1 ng/ml Stx2 in HGEC and HK-2 ($p<0.05$, $n=3$). The highest protection of FabC11 against Stx2 was obtained at a concentration of 10 µg/ml. HGEC viability was protected in about 52% by FabC11 at the co-incubation condition, and about 54% at the pretreatment condition. In addition, HK-2 viability protection was 100% and in both conditions. The results show that FabC11 are able to protect human renal epithelial and endothelial cells from the cytotoxic effects of Stx2 with similar effectiveness. This antibody could be used as a therapeutic strategy to prevent renal damage described in HUS patients.

Keywords: Hemolytic Uremic Syndrome, Stx2, recombinant antibodies, therapy

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