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Topic Category: 4110-ASIP Regulation of the epithelial barrier and intercellular junctions

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First Author is a: Undergraduate
First Author is a member of: Not a Member of a Host EB Society
First Author Degree: N/A (for High School Students and Undergraduates)

Presentation Preference: Poster

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Sponsor's Society: Pathology - American Society for Investigative Pathology (ASIP) - Host Society
Keywords: 1. Enteric glial cells 2. GDNF 3. intestinal barrier

Intestinal epithelial barrier maturation by enteric glial cells is glial cell line-derived neurotrophic factor (GDNF)-dependent

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The enteric nervous system is critically involved in the maintenance of intestinal epithelial homeostasis. However, it is still unclear how the interaction between enteric glial cells (EGC) and enterocytes is regulated. Among several potential effectors secreted by EGCs it has been shown that the glial cell line-derived neurotrophic factor (GDNF) significantly contributes to intestinal epithelial barrier maturation and epithelial wound healing. In the present study we tested our hypothesis that GDNF is a key player of EGC-mediated effects on intestinal epithelial barrier maturation.

To analyze the specific contribution of GDNF on EGC-mediated epithelial barrier maturation we used a co-culture model of EGCs (CRL2690) and Caco2 cells in a Transwell-based filter system and established a GDNF-deficient EGC ($EGC^{GDNF-/-}$) cell line using the Crispr/Cas9 system. Control experiments were performed in a co-culture system of ASPC-1 cells which do not express GDNF and Caco2 cells.

Application of 100 ng/ml recombinant GDNF on Caco2 monolayers resulted in increased barrier properties compared to untreated Caco2 cells as revealed by measurements of 4 kDa FITC-dextran flux across epithelial monolayers and transepithelial electrical resistance (TER). This was paralleled by increased immunostaining of the desmosomal junction protein desmoglein2 (Dsg2) and of tight junction proteins claudin1 and claudin5 at cell borders. Similar effects on TER and 4 kDa FITC-dextran flux were observed when Caco2 cells were co-cultured with EGCs. In Western blot analyses and ELISA-based measurements it was confirmed that EGCs express GDNF and consecutively secrete GDNF in significant amounts under these experimental conditions. Accordingly, application of EGC supernatants on Caco2 monolayers resulted in augmented staining patterns of junctional proteins at cell borders and significantly increased TER. In contrast, when Caco2 cells were incubated with EGC supernatants in which GDNF had been depleted by using beads coated with GDNF antibodies, no changes of intestinal barrier properties were evident and increased staining of the junctional proteins Dsg2, claudin1 and claudin5 at the cell borders was absent. Similarly, no effects on epithelial barrier properties were detected when Caco2 cells were co-cultured with $EGC^{GDNF-/-}$ or ASPC-1 cells which both do not secrete GDNF.

Taken together, our data provide evidence that IEB maturation by EGCs is GDNF-dependent. This supports our hypothesis that GDNF is a key player in the interaction between the enteric nervous system and the intestinal epithelium.

Support or Funding Information
SPP 1782 DFG SCHL1962/5-1