

## EVALUATION OF PRO-INFLAMMATORY RESPONSE CAUSED BY ENTEROAGGREGATIVE *ESCHERICHIA COLI* IN INTESTINAL CELLS: MODULATION WITH ALANYL-GLUTAMINE

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Diarrheal diseases account for nearly 1.5 million deaths a year among children under-five years of age. As mortality related to acute diarrhea was reduced, persistent diarrhea (PD) has become a major enteric infant disease, collaborating to morbidity of affected populations. Both malnutrition and infection caused by *Escherichia coli* enteroaggregative (EAEC) are strongly associated with PD development. The pro-inflammatory responses have been reported by in vitro EAEC infections studies. Conversely, alanyl-glutamine has been shown as an important nutrient for prevention of malnutrition and regulation of the inflammatory response of intestinal cells. The present study investigated the effect of EAEC infection (strain 239-1) in pro-inflammatory responses when compared to commensal *E. coli* HS. Also we evaluated the effect of alanyl-glutamine on pro-inflammatory response to EAEC infection. Rat intestinal cells (IEC-6) monolayers were infected by EAEC at 10<sup>7</sup> CFU/mL for 3 hours and had the medium with bacteria collected. IEC-6 cells were washed three times and medium containing alanyl-glutamine (10 mM) and gentamicin (200 µg/mL) were added to the IEC-6 cells. The supernatant was collected at 6 and 12h after supplementation. The Bio-Plex Rat Cytokine 9-plex 1X kit (Bio-Rad) allowed simultaneous analysis of the mediators IL-1α, IL-1β, TNF-α, GM-CSF, IFN-γ, IL-5, IL -2, IL-6 and IL-10. Analysis was performed using GraphPad Prism by Student t test and the test was considered statistically significant when P<0.05. All cytokines were evaluated but only IL-6 and TNF-α were detected after EAEC infection. We observed that TNF-α was increased significantly after EAEC infection when compared to commensal *E. coli* HS at 12 h post infection. Supplementation with alanyl-glutamine after EAEC infection showed significant increased and reduced levels of TNF-α, at 6 and 12h, respectively, while IL-6 levels increased at 6 and 12h after supplementation. In conclusion, EAEC increased pro-inflammatory response through the increased levels of TNF-α. The treatment with alanyl-glutamine showed different profiles in immune response. Further studies are underway to investigate the possible mechanisms by which EAEC causes this immune response.

Keywords: Enteroaggregative *Escherichia coli*. Alanyl-Glutamine. Pro-inflammatory response.

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