TITLE: COMPARISON OF THE CAPACITY OF ATYPICAL ENTEROPATHOGENIC *ESCHERICHIA COLI* TO ADHERE AND INDUCE INCREASE IN THE PRODUCTION OF INFLAMMATORY CYTOKINES IN ENTEROCYTES AND GOBLET CELLS CULTIVATED *IN VITRO*

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ABSTRACT:
Atypical enteropathogenic *Escherichia coli* (aEPEC) are emerging pathogens, whose virulence mechanisms have been intensively investigated. After infecting the host, aEPEC adheres to the intestinal mucosa, while the host immune system is activated. There are few studies on the ability of aEPEC to induce an inflammatory response. This knowledge is important for the management of the disease. In this study, we evaluated the ability of seven aEPEC strains, isolated from feces of diarrheic children, to adhere and induce the production of inflammatory cytokines in enterocytes (differentiated Caco-2 cells) and goblet cells (LS174T) after 3h and 6h of interaction. Six strains adhered in both lineages forming loose clusters, while one adhered randomly. The production of inflammatory cytokines (IL-12p70, TNF, IL-10, IL-6, IL-1β and IL-8) was investigated in bacterial culture supernatant by the Cytometric Bead Array (CBA) kit. Data were evaluated by analysis of variance (ANOVA), assuming as a significant difference $p \leq 0.05$. IL-8 was the cytokine detected at higher levels, followed by TNF and IL-10, which were detected at very low levels. Production of the other cytokines was not observed. Basal levels of IL-8 in uninfected Caco-2 cells were not observed, whereas in LS174T, they were 121.8 pg/ml and 230.0 pg/ml, in 3h and 6h, respectively. In Caco-2 cells, significantly increased IL-8 production was detected in three (42.8%) strains, only after 6h of interaction; in two strains (28.5%), there was an increase up to 6h, and in one strain (14.2%), there was an increase up to 3h, with a subsequent reduction in 6h. In LS174T cells, three strains (42.8%) induced increased IL-8 production up to 6h; one strain (14.2%) induced increase only in 6h; and three strains (42.8%) did not modify IL-8 levels. The maximum IL-8 production in infected Caco-2 cells was 27.3 pg/ml (3h) and 127.6 pg/ml (6h), whereas in infected LS174T it was 691.3 pg/ml (3h) and 2,267.7 pg/ml (6h). Therefore, a greater number of aEPEC strains altered the production of IL-8 in Caco-2 cells than in LS174T, but the concentrations of this cytokine were higher in the latter lineage. These data demonstrate that aEPEC adhere to enterocytes and, for the first time, goblet cells in culture, inducing IL-8 production. The level of induction varies among strains, as a function of the interaction time and cell type analyzed. The molecular basis of differences in cellular interaction and response is under investigation.

KEY WORDS: atypical EPEC, cytokine, enterocytes, goblet cells, bacterial adherence

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