Título: MOLECULAR HOST AUTOPHAGY RESPONSE IN ENTEROINVASIVE *Escherichia coli* INFECTION

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Abstract: Autophagy has been described as an intrinsic host defense system for recognizing and eliminating intracellular-invading bacterial pathogens. Enteroinvasive*Escherichia coli* (EIEC), an important diarrheagenic*E. coli*, are closely related to *Shigella*, showing remarkable phenotypic and genotypic similarities. However, the disease induced by EIEC is less severe. The effector protein IcsB has been shown to play a pivotal role in *Shigella* escape from autophagy. We previously described that EIEC express much less *icsB* than *S. flexneri*, and then, our hypothesis is that EIEC are being efficiently recognized and eliminated by the host cell autophagic process. To extend our investigation, here, we examined the transcriptional profiling of autophagic genes in the intestinal Caco-2 cell line under EIEC or *S. flexneri* infection by microarray analysis. In order to determine gene expression profiles, 44 K DNA microarrrays (Whole Human Genome Microarray Kit, Agilent Technologies, cat no. G2519F, Santa Clara, CA) were used. The procedures for hybridization followed the protocols provided by the manufacturers' instructions (One-Color Microarray-Based Gene Expression Analysis – Quick Amp Labeling). The images were captured by the reader Agilent Bundle according to the parameters recommended for bioarrays and extracted by Agilent Feature Extraction software version 9.5.3. The selected transcripts were used for analysis using the R software version 2.11.1 (R Development Core Team, 2010) and the Lowess test for arrays normalization. We analyzed the following groups: Caco-2 cells not infected (group 1), Caco-2 cells infected with EIEC (group 2) and Caco-2 cells infected with *S. flexneri* (group 3). Protein expression (LC-3B) were analyzed by western blotting. Analyzing the upregulates genes, we observed a quite different genes network to each group at each time point analyzed. These data shows that the host cell response against *S. flexneri* or EIEC is modulated in a completely different way. The genes encoding autophagic proteins were particularly more expressed in group 2. In order to visualize this phenomenon, we evaluated the expression of LC-3B protein, important in the autophagic process and, unlike *S. flexneri*, EIEC induces autophagy in HeLa cells. In conclusion, autophagy appears to be an important mechanism of the host cell to control the infection by EIEC, which could make explain in part the self-limiting disease induced by EIEC.

Keywords: Enteroinvasive*Escherichia coli*, microarray, host-pathogen interactions, Caco-2 cell line

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