

TITLE: THE SERINOPROTEASE PIC IS ESSENTIAL TO *Escherichia coli* SURVIVAL ON BLOODSTREAM.

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ABSTRACT:

Some *Escherichia coli* strains are important pathogens responsible for a variety of diseases, and albeit using distinct mechanisms of pathogenesis, have in common the production of Pic, or protein involved in colonization. We have recently shown that Pic mediates immune evasion by the direct cleavage of complement molecules, significantly reducing complement activation by all three pathways. The aim of this study was to investigate the action of Pic in a murine model of sepsis. Six to eight-week-old female Swiss mice were intraperitoneally inoculated with Pic-producing *E. coli* (F5), F5 Δ pic mutant, HB101 (non-pathogenic *E. coli*) or PBS. Animal survival was monitored for 5 days (n= 6 animals/group) and a subset of mice (n= 6 animals/group) was euthanized after 12 h for cytokines and nitric oxide analysis, and differential cell counts. In addition, organs were removed for weighing, determination of colony forming units (CFU's), total cell count and histological analysis. Intraperitoneal inoculation of Pic-producing bacteria induced 100% death within 24 h, different from the other groups where no death was observed. The UFC count of bacteria in the organs was significantly higher in F5 than other groups. Besides, only F5 was viable on bloodstream. NO and cytokines (IFN- γ , TNF- α , IL-6, IL-12, IL-10 and MCP-1) were detected in serum, as well as high levels of IL-6 and MCP-1 on peritoneal lavage of F5 group, significantly higher than other groups. Moreover, histological evaluation showed a significant alteration on spleen of the F5 group. Therefore, these results evidenced that Pic represents an important virulence factor, allowing the survival of the bacterium on bloodstream and several organs, as well as inducing a high production of pro-inflammatory mediators by the host, leading to a sepsis and death.

Keywords: Pic, *Escherichia coli*, sepsis.

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