TYPICAL ENTEROPATHOGENIC *ESCHERICHIA COLI* EVADE MICROBICIDE MECHANISMS AND SURVIVE INSIDE MACROPHAGES

Melo, K.C.M. [1], Borges, M.M. [1], Vieira, P.C.G [1], Santos, H.A. [1], Ruiz R.C. [1]

Laboratório de Bacteriologia, Instituto Butantan (Avenida Vital Brazil 1500, 05503-900, São Paulo, SP, Brazil).

The success of infection by many pathogens has been attributed to various strategies to evade the host's innate immune response. Macrophages are cells equipped with antimicrobial and degradative proteins. Therefore, these cells constitute one of the main targets for microbial evasion. Typical Enteropathogenic *Escherichia coli* (tEPEC) are known to induce an anti-phagocytic mechanism by injecting different effector proteins through the type III secretion system into macrophages. However, the inhibition of phagocytosis is not absolute and bacteria can still be found inside the macrophages. The objective of this research was to study the *in vitro* interaction of macrophages with tEPEC to investigate the entrance and survival of these bacteria in the intracellular environment. J774.A1 macrophages were infected with tEPEC (E2348/69) or non-pathogenic *E. coli* (HB101) at MOI 10, 50 or 100 for 30min. Bacterial survival assays were performed after 24, 48 and 72h of infection. The interaction of bacteria with macrophages was evaluated by determining the percentage of infected cells and by quantifying the number of bacteria present in infected macrophages. The number of cells infected with tEPEC was significantly lower than the number of cells infected with non-pathogenic *E. coli*. While the number of intracellular tEPEC was maintained after 72h of infection, the number of intracellular non-pathogenic bacteria decreases significantly by the same time of the infection. To evaluate whether the intracellular survival of tEPEC is related to a decreased macrophage microbicide response, we verified the production of nitric oxide (NO) and TNF-α by macrophages infected with tEPEC as well as with the control strain. tEPEC, but not non-pathogenic bacteria, resist high levels of NO and TNF-α, even under optical conditions of NO production which occurs in the presence of IFN-γ. The combination of LPS and IFN-γ constitute the most efficient signal to activate macrophages. The results shown here suggest that, besides the described antiphagocytic mechanism, tEPEC presents an additional mechanism to overcome macrophage microbicide activities. Once inside the macrophage, tEPEC presents an additional escape mechanism which ensures its survival within the defense cells. A more detailed understanding of how these bacteria escape phagocyte defense mechanisms is the subject of further studies in course in our laboratory.

Key words: Enteropathogenic *Escherichia coli,* macrophages, nitric oxide, TNF-α, intracellular survive.

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