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SUMMARY OF THESIS

CHINEN, Ludmilla Thomé Domingos - **Estudo da capacidade de apresentação de antígenos e da interação com *Mycobacterium avium* em modelo *in vitro* de células epitelióides.** São Paulo, 2005. (Tese de Doutoramento - Escola Paulista de Medicina/Universidade Federal de São Paulo).

RECOMBINANT INTERLEUKIN-4 TREATED MACROPHAGES, EPITHELIOID CELLS SURROGATES, HARBOR AND ARREST *Mycobacterium avium* MULTIPLICATION *IN VITRO*

Introduction and Objectives: Granulomas are chronic inflammatory lesions composed mainly by mononuclear phagocytes, multinucleated giant cells and epithelioid cells (ECs). Although hallmarks of granulomatous lesions, the precise functional roles of ECs are not clarifyed so far. Beyond containing pathogens spreading, it has been suggested that ECs could act as antigen presenting cells in the granuloma microenvironment. Also, it has been shown its ability to control microorganisms multiplication, at least in a model where T lymphocytes were not involved in granulomatous lesion formation. Our group has previously described that a 7-day treatment of murine peritoneal macrophages with recombinant interleukin-4 (rIL-4) generates a cell population (ECs surrogates) which presents morphological and functional characteristics already described to ECs found in granulomas (Cipriano et al., 2003, *Inflammation, 2:201-211*). Therefore, in this work ECs surrogates were studied concerning both, the antigen presenting capacity and their interaction with *Mycobacterium avium*.

Methods and Results: By immunocytochemistry and immunofluorescence it was observed an increased expression of CD11b, CD54, CD86 and CD40 molecules on ECs surrogates when compared to controls. Although more pinocytic for dextran particles, ECs surrogates were less phagocytic for latex beads (phagocytic index 96 ± 44.4) than controls (195 ± 66.4; $p = 0.024$). Lymphoproliferation assays using OVA and *M. avium* as antigens showed that both cultured macrophages were equally efficient antigen presenting cells. However, *M. avium* was better presented *in vivo* by ECs surrogates (stimulation index 2.0 ± 0.1) than by control cells (1.3 ± 0.1; $p = 0.01$). Both macrophage cultures were similarly infected by *M. avium*, but while the percentage of infection was maintained in ECs surrogates, untreated macrophages showed a progressive increase in bacilli/cell number along the time ($p < 0.1$). At the 96th h after infection, control cells secreted higher amounts of TNF than ECs surrogates (1227.4 ± 155.5 versus 399 ± 65.5 pg/mL; $p < 0.01$) as quantified by a commercial *kit* (*BD™*). Nitric oxide measured by Griess reagent was also higher in supernatants of control macrophages than in ECs surrogates cultures (26.5 ± 3.8 versus 14.7 ± 0.05 µM; $p < 0.05$). Although not attaining statistical significance ECs surrogates showed higher TGF-β production than controls, quantified by sandwich ELISA. The expression of galectin-3 was also increased in rIL-4 treated macrophages after mycobacteria infection, as compared to control cells. Therefore, ECs are antigen presenting cells that harbor but not destroy *M. avium*.

Conclusion: Taken together, our results enable the suggestion that ECs could be key protagonists for the host-parasite equilibrium state sustaining the immune response while limiting the evolution and spreading of the infection.

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