MOLECULAR MESSAGES BETWEEN MACROPHAGES AND T LYMPHOCYTES DURING INFECTION WITH *LISTERIA MONOCYTOGENES*

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Abstract: The outcome of listeriosis, caused by the intracellular pathogen *Listeria monocytogenes,* is critically dependent on the initial encounter of the pathogen with the macrophage and then on the specific activation of T lymphocytes. The present study investigated the pattern of cytokine secretion as a hallmark of the macrophage-T cell interaction during the development of an antilisteric response in humans.

INTRODUCTION

For the last two decades, listeriosis, caused by the intracellular pathogen *Listeria monocytogenes*, has become one of the most concerning food-born infections due to the frequent outbreaks recently reported (Aureli et al., 2000, Colodner et al., 2003).

Evidence is accumulating that the outcome of listeriosis is critically dependent on the early events following the initial encounter of the pathogen with the macrophage, Listeria's preferred habitat inside the host. Activation of T lymphocytes under specific listeric stimulation is possible only after antigens are processed and presented by macrophages and is an *in viv*o prerequisite phenomenon to restrict the growth of *L. monocytogenes* (Portnoy et al., 2002). The type of the cytokine microenviroment produced by macrophages and lymphocytes following infection governs whether naive T cells will develop into either a disease-promoting or a protective type of immune response (Marzo et al., 2000).

AIMS OF THE STUDY

The present study aimed at the investigation of macrophage-T cell interactions during the development of an antilisteric response in humans with reactivity for listeric antigens. The functional contact between these two major partners involved in the cellular immunity can be deciphered through the detection and measurement of their cytokine production, as they are molecular mediators of the cellular immunity to infection.

MATERIAL AND METHODS

Bacterial antigens. *L. monocytogenes* EGD was killed thermically at 65°C, 120 min, in a water bath resulting a complex mixture of listeric antigen containing $2x10^9$ colony forming units (CFUs) or 8 mg proteins/ ml. The suspension, named HKL *from heat killed Listeria*, was stored at -70°C as 100µl aliquots until used.

Isolation and culture of peripheral blood mononuclear cells (PBMC). PBMCs were isolated from samples of peripheral blood of one reactive healthy donor by Ficoll Hipaque (Sigma) density gradient centrifugation (300xg, 25 min), then plated on 24-well tissue culture plates (Nunc, Denmark), at a density of $2x10^6$ cells/ml in RPMI 1640 medium (Sigma) supplemented with L-glutamine (2mM), 5% autologous human plasma and antibiotic. Cultures were performed at 37°C, 5% CO_2 with/ without stimulation.

***In vitro* stimulation**. To assess the presence of listeria-specific lymphocytes and the pattern of cytokine production, $2x10^6$ cells were plated with 50µg/ml HKL. In parallel, cells were incubated with no stimulus (the negative control).

Flow cytometry analysis of cellular markers. After seven days of culture (when the peak of proliferation was observed, data not shown), cells were collected, stained for membrane (anti-CD3, for T cells) or intracellular markers (anti-interferon-γ -IFN-γ- and anti-interleukin 4 -IL-4- monoclonal antibodies) and analyzed on a FACSCalibur flow cytometer. For intracellular staining, cultured PBMCs were treated with brefeldin A six hours prior to harvesting, for cytokines' sequestration inside the vacuoles of Golgi apparatus. The cell membrane was then fixed (20 minute in 2% paraformaldehide) and permeabilised (10 minute in 0.2% saponine) than stained for 30 min with (Pharmingen).

Flow cytometry detection of the amount of cytokine in the culture supernatant. To measure the amount of cytokines released by the stimulated cells into the supernatant after 24 and 72 hours of culture, a standardized analysis kit was used (Human Th1/Th2 Cytokine Array, Becton Dickinson). The kit contains capture microbeads coated with monoclonal antibodies against IL-2, IL-4, IL-5, IL-10, TNF-α (tumor necrosis factor -α), IFN-γ. Our results are expressed as average values of four identical experiments carried out in duplicate.

RESULTS AND DISCUSSIONS

In our study, PBMCs of a HKL-reactive subject were cultured in the presence of listeric antigens and intracellularly stained with anti-IFN-γ and anti-IL-4 fluorochrome-conjugated monoclonal antibodies, as described. The flow cytometry analysis was gated on the proliferating cells (HKL-responding cells), and CD3+ cells (T lymphocytes). In parallel, the culture supernatant was collected and analyzed for the concentration of six cytokines (IL-2, IL-4, IL-5, IL-10, TNF-α, IFN-γ) after three days of culture in the presence or absence of listeric antigens.

Detection of Th1 associated cytokines. According to our experiment, in the absence of the antigen there were no significant levels of cytokines in the culture supernatant after either 24 or 72 hours of culture. A clear indication of the macrophages' activation was the high level of TNF-α in the wells with HKL (2800 pg/ml). TNF-α is a proinflammatory cytokine with a protective role through its phagocytic and bactericidal/bacteriostatic functions (Calorini et al., 2002). Secretion of TNF-α was due to the direct effect of the bacterial components present in the assay on macrophages, and to the secretion of IFN-γ, also significantly increased in the positive wells (over 5000 pg/ml). Our results also indicated a proportion of 14% IFN-γ-producing T lymphocytes. The production of IFN-γ (both in the supernatant and intracellularly) and IL-2 (110 pg/ml after 72 hours of culture) is an indication of a Th1 type of immune response in the presence of listeric antigens.

Detection of Th2 associated cytokines. Unexpectedly, markers of a Th2 polarization of the T cell response were also detected in our study, such as the intracellular level of IL-4 in 9 % of HKL-activated T lymphocytes and the secreted level of 42 pg/ml IL-5 in the presence of HKL. On the other hand, the dynamics of IL-10 (also a Th2 promoting marker) production by the activated macrophages in HKL-positive wells (46 pg/ml after 24 hours versus undetectable levels after 72 hours) may be in fact an indication of an early regulatory even. Precedent of similar dynamics during *in vivo* infection in mice exists in literature, attributed to the role to balance the detrimental effect of type 1 cytokine responses, such as tissue damages and increased local reactions (Deckert et al., 2001).

CONCLUSIONS

Our study showed a combined Th1 + Th2 pattern of response, which may reflect the fact that in humans the encounter with *L. monocytogenes* leads to the expansion of a less differentiated subtype of T cells, named Th0, capable of producing both types of cytokines. Our results strongly suggest the fact that in humans the resistance to infection is controlled by this subset of T lymphocytes.

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