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Short communication

Characterization of Th1 activation by *Bartonella henselae* stimulation in BALB/c mice: Inhibitory activities of interleukin-10 for the production of interferon-γ in spleen cells

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Abstract

This study was conducted to analyze cytokine production mechanisms in mice after *Bartonella henselae* stimulation. BALB/c mice were inoculated intraperitoneally with 3×10^6 colony forming units of *B. henselae* (Houston-1 strain) twice at 10-day interval. Spleen cells were harvested from the mice and stimulated with the organisms. Following the stimulation, interferon-gamma (IFN-γ) and interleukin-4 (IL-4), IL-10, IL-12 and tumor necrosis factor-alpha (TNF-α) were measured in the culture supernatants of the spleen cells by ELISA. The spleen cells specifically secreted IFN-γ, but not IL-4, indicating that T helper 1 (Th1) cells were activated following *B. henselae* stimulation. In addition, IL-10 and TNF-α productions were also detected in the culture supernatants of spleen cells. Neutralization of IL-10 in the culture supernatants significantly enhanced the production of IFN-γ from the spleen cells stimulated with *B. henselae*. These results indicate that *B. henselae* predominantly stimulated Th1 cells and resulted in secreting IFN-γ, however the production was partially inhibited by IL-10, which was produced simultaneously.

Keywords: *Bartonella henselae*; IL-10; IFN-γ; Mice; TNF-α

1. Introduction

Although high levels of bacteremia with *Bartonella henselae* were found in cats for several months to a few years, most infected cats remain asymptomatic (Kordick et al., 1999). Immune responses against *B. henselae* infection in cats have mainly been studied from the standpoints of humoral immunity (Freeland et al., 1999). Analysis of the immune responses, especially cell-mediated immunity (CMI) is indispensable to understand the mechanisms by which the organisms are eliminated from infected animals, since

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B. henselae is an intracellular pathogen (Kordick and Breitschwerdt, 1995). However, little is known about cytokine responses against *B. henselae* in cats as well as in humans (Kabeya et al., 2006; Papadopoulos et al., 2001). The cat naturally *B. henselae*-infected were shown to promote IL4, but not IFN-γ mRNA expression when the emergence of relapsing bacteremia, suggesting that the selective induction of Th2 immune responses may contribute to establishing the persistent infection of *B. henselae* in naturally infected cats (Kabeya et al., 2006).

It has been reported that despite the fact that mice are not the natural reservoir of *B. henselae*, we used a mouse model to analyze the cellular immune response against *B. henselae*, because it has been well known the immune system of such laboratory animals (Arvand et al., 2001; Kabeya et al., 2003; Karem et al., 1999). Previous investigations have been performed to examine the immune responses against *B. henselae* stimulation in BALB/c or C57BL/6 mice (Arvand et al., 2001; Karem et al., 1999). *B. henselae* elicited cell-mediated immune responses mainly $CD4^+$ helper T cells 1 (Th1) in immunocompetent mice. Karem et al. (1999) also reported that *B. henselae*-primed BALB/c mice induced delayed-type hypersensitivity and the secretion of interferon-gamma (IFN-γ) mediated by $CD4^+$ Th1. However, the mechanism by which $CD4^+$ Th1 response in *B. henselae*-primed mice is induced remains unclear. The aim of this study was to better understand how a Th1 response is specifically induced in experimentally *B. henselae*-inoculated mice through analysis of cytokine expression profiles.

2. Materials and methods

2.1. Bacterial strains

B. henselae strain Houston-1 (ATCC49882) was used in this study. The strain was cultured on heart infusion agar (HIA) plates (DIFCO, USA) containing 5% defibrinated rabbit blood at 35 °C in an atmosphere of 5% CO_2 for 1 week. The bacterial cells were re-suspended in PBS and the concentration was adjusted to OD of 1.0 at 600 nm. Ten-fold serial dilutions were made with PBS and 100 μl of each diluted suspension were plated on two HIA plates containing 5% defibrinated rabbit blood to determine the colony-forming unit (CFU) of the inoculums.

2.2. Experimental animals

Female BALB/c mice were purchased from Nihon CLEA Corp. and used for the experiment at the age of 5 weeks. Three animals were caged together and kept under specific-pathogen-free conditions throughout the study. Animal care was carried out in accordance with the guidelines for the care and use of laboratory animals by College of Bioresource Sciences, Nihon University.

2.3. Inoculation of mice with B. henselae

Three of BALB/c mice were used for each experimental group. The mice were inoculated intraperitoneally twice at 10-day interval with 1 ml of the bacterial suspension containing 3.0×10^6 CFU of *B. henselae* Houston-1 suspended in PBS.

2.4. Induction of cytokine production from mice spleen cells

Spleen was harvested from three mice per group and suspended in complete RPMI medium consist of RPMI1640 (Invitrogen, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum, 200 U/ml penicillin, and 200 μg/ml streptomycin at a concentration of 2×10^6 cells/well of 24-well microplate. The cells were stimulated with *B. henselae* Houston-1 (2×10^6 to 2×10^8 CFU/well) with or without anti-mouse interleukin-10 (IL-10) rat monoclonal antibody (ENDOGEN, MA, USA) or IgG_1 rat isotype control (R and D systems Inc., MN, USA) at a concentration of 40 μg/ml for 24–120 h at 37 °C. Following cultivation, the concentrations of secreted cytokines (IFN-γ, IL-4, IL-10, IL-12 and tumor necrosis factor α; TNF-α) in culture-supernatants were measured by using commercial ELISA kits (Quantikine, R and D systems Inc.).

2.5. Statistical analysis

Differences between the amounts of cytokine in culture conditions (stimulation with versus without stimulant, or stimulation with live versus inactivated organisms) were determined by Student's *t*-test. *P*-values of <0.05 were regarded as significant.