**Effect of Exogenous Interleukin-18 (IL-18) and IL-12 in the Course of Brucella abortus 2308 Infection in Mice**

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In this study we demonstrated that combined inoculation of interleukin-12 (IL-12) and IL-18 reduced the number of bacteria in the spleens of mice infected with *Brucella abortus* 2308 and that the effect of the treatment was mediated by an increased capability of spleen cells to produce gamma interferon at the early phase of infection.

Resistance to *Brucella abortus* is largely dependent upon the bactericidal effect of activated macrophages, which is mediated by sensitized T lymphocytes. Several cytokines can orchestrate the immune system and function in key roles to influence the outcome of infections. Of these, gamma interferon (IFN-γ) is a prominent mediator in conferring protection both in vitro (1, 2) and in vivo (7, 9, 12).

Interleukin-12 (IL-12) is pivotal for the development of Th1 responses (10), and it is involved in the outcome of resistance against many infections (8). Mice depleted of IL-12 have been shown to be more susceptible to infection with *B. abortus*, and this susceptibility has been correlated with decreased IFN-γ production (13, 14). This suggests that IL-12 contributes to resistance to *Brucella* infection mainly via an IFN-γ-dependent pathway. However, mice treated with a single administration of recombinant murine IL-12 did not clear more efficiently an infection with the vaccine strain *B. abortus* RB51. In addition, treated mice did not display augmented cellular immune responses to *Brucella* antigen, nor did they respond more efficiently to a challenge infection (3).

IL-18 is a newly cloned cytokine synthesized mainly by activated macrophages. IL-18 is able to stimulate IFN-γ production (5, 11) and can synergistically act with IL-12 on T cells (4, 6). In this study, we explored the effect of exogenous IL-18 given alone or in combination with IL-12 on *B. abortus* 2308 infection in an attempt to better understand the immune mechanisms that control *Brucella* infections.

Female BALB/c mice were purchased from Charles River (Milan, Italy) and used at 10 to 12 weeks of age. Mice were infected intraperitoneally with 5 × 10⁸ CFU of *B. abortus* 2308 in 0.2 ml of phosphate-buffered saline (PBS). Mice were injected intraperitoneally with 500 ng of cytokines (R&D Systems, Minneapolis, Minn.) in 0.2 ml of PBS 1 day before and on the day of the infection. Equal amounts of PBS were injected into control mice. At 1 and 5 days after infection, the mice were sacrificed and their spleens were collected and then dispersed in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) containing 2 mM L-glutamine, 25 mM HEPES, and 5 × 10⁻³ M 2-mercaptoethanol. An aliquot of the resulting cell suspension was plated to determine the number of CFU. To evaluate cytokine production, spleen cells (2 × 10⁹) were cultured in 0.5 ml of RPMI 1640 and stimulated with 0.5 ml of heat-inactivated *B. abortus* 2308 at 10⁶ CFU/ml. Supernatants were collected 72 h after culture for measurement of tumor necrosis factor alpha (TNF-α), IFN-γ, and IL-10 production. Mouse cytokines were detected by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (R&D Systems).

As shown in Table 1, combined inoculation of IL-12 and IL-18 reduced the number of bacterial cells in the spleens of treated mice compared to the number in untreated controls at 1 and 5 days after infection (P = 0.07 and P < 0.05, respectively). IL-18 alone induced a decrease in the bacterial count in the spleens of infected and infected mice compared to that in the spleens of untreated infected mice. However, this reduction was not statistically significant. In contrast, a single inoculation of IL-12 did not exert any effect on the course of the infection. The combined treatment also induced a marked change in spleen weight. Treated animals showed an enlargement of their spleens as early as 1 day after infection. In addition, there was a significant reduction in spleen weight in IL-18-treated infected animals compared to that in untreated infected mice at 5 days after infection. The reason for this difference was not investigated.

When in vitro cytokine production was tested, it was observed that combined treatment with both cytokines induced IFN-γ production as early as 1 day after infection. The levels of IFN-γ were similar in treated and untreated mice 5 days after infection, suggesting that the effects of the cytokines are transient and are more prominent in the early phases of infection. Levels of TNF-α were not statistically different between treated and untreated mice throughout the experiment. However, there was a slight increase of TNF-α 1 day after infection and a slight decrease 5 days after infection in treated mice compared to the levels in untreated mice. Finally, IL-10 levels were not different between treated and untreated mice at either 1 or 5 days after infection.

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Overall, our results demonstrate that the combined administration of IL-12 and IL-18 induces protection against a *B. abortus* 2308 infection in mice, while treatment with either IL-12 or IL-18 alone resulted in little or no effect. It is interesting to note that the treatment induced an increased production of IFN-γ during the early phases of infection. These findings are consistent with recent observations demonstrating that IFN-γ is involved early in *Brucella* infections (7). In addition, these results support the concepts that IL-18 has effects similar to those of IL-12 regarding the induction of IFN-γ production by Th1 and NK cells (5) and that IL-18 may act synergistically with IL-12 in defense mechanisms against infectious agents (6).

These results show that exogenous treatment with IL-12 at the tested dose does not significantly induce resistance to a *B. abortus* 2308 infection, confirming the reports of others (3). The reason why exogenous IL-12 is not able to confer protection against *Brucella* infection has not been ascertained. It is possible that IL-12 alone does not promote increased IFN-γ production at a level that is effective in influencing the outcome of the infection.

**REFERENCES**