CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Mar. 2002, p. 491–492 Vol. 9, No. 2
1071-412X/02/$04.00+0 DOI: 10.1128/CDLI.9.2.491–492.2002


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

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Received 13 August 2001/Returned for modification 6 November 2001/Accepted 28 November 2001

**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 $5 \times 10^3$ 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 $5 \times 10^{-5}$ M 2-mercaptoethanol. An aliquot of the resulting cell suspension was plated to determine the number of CFU. To evaluate cytokine production, spleen cells ($2 \times 10^6$) were cultured in 0.5 ml of RPMI 1640 and stimulated with 0.5 ml of heat-inactivated *B. abortus* 2308 at $10^8$ 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 injected 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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TABLE 1. Effect of cytokines on *B. abortus* infection[a]

| Treatment | Spleen wt (mg)[b] | | CFU ($10^3$) in spleen | | Cytokine level (pg/ml)[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | TNF-α | | IFN-γ | | IL-10 | |
| | 1[c] | 5 | 1 | 5 | 1 | 5 | 1 | 5 | 1 | 5 |
| *B. abortus* (untreated) | 89 ± 9 | 144 ± 29 | 31.9 ± 9.9 | 390 ± 336 | 478 ± 49 | 565 ± 263 | 0 | 548 ± 440 | 171 ± 16 | 158 ± 38 |
| *B. abortus* + IL-12 | ND[d] | 133 ± 12 | ND | 379 ± 248 | ND | ND | ND | ND | ND | ND |
| *B. abortus* + IL-18 | ND | 101 ± 9* | ND | 138 ± 42 | ND | ND | ND | ND | ND | ND |
| *B. abortus* + IL-12 + IL-18 | 136 ± 11* | 155 ± 12 | 14.9 ± 12.5 | 21 ± 9* | 719 ± 334 | 286 ± 213 | 114.5 ± 90* | 594 ± 722 | 192 ± 27 | 147 ± 28 |

[a] BALB/c mice were infected with $5 \times 10^3$ CFU of *B. abortus* 2308. Cytokines were administered at 500 ng/mouse 1 day before and on the day of infection. Mice were killed at 1 and 5 days after infection. Data are mean values ± standard deviations of results for four or five animals per group.
[b] An asterisk indicates that results are statistically significant *($P \leq 0.05$)* compared to those for the untreated group.
[c] Day(s) after infection.
[d] ND, not done.

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


1. **Jiang, X., and C. L. Baldwin.** 1993. Effects of cytokines on intracellular growth of *Brucella abortus*. Infect. Immun. **61:**124–134.
2. **Jones, S. M., and A. J. Winter.** 1992. Survival of virulent and attenuated strains of *Brucella abortus* in normal and gamma interferon-activated murine peritoneal macrophages. Infect. Immun. **60:**3011–3014.
3. **Lee, I., S. C. Olsen, M. Kehrli, and C. A. Bolin.** 1999. The adjuvant effect of a single dose of interleukin-12 on murine immune response to live or killed *Brucella abortus* strain RB51. Can. J. Vet. Res. **63:**284–287.
4. **Micallef, M., T. Ohtsuki, K. Kohno, F. Tanabe, S. Ushio, M. Namba, T. Tanimoto, K. Torigoe, K. Fujii, M. Ikeda, S. Fukuda, and M. Kurimoto.** 1996. Interferon-gamma-inducing factor enhances T helper, 1 cytokine production by stimulated human T cells: synergism with interleukin-12 for interferon-gamma production. Eur. J. Immunol. **26:**1647–1651.
5. **Okamura, H., H. Tsutsui, T. Komatsu, M. Yutsudo, A. Hakura, T. Tanimoto, K. Torigoe, T. Okura, Y. Nukada, K. Hattori, K. Akita, M. Namba, F. Tanabe, K. Konishi, S. Fukuda, and M. Kurimoto.** 1995. Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature **378:**88–91.
6. **Okamura, H., S. Kashiwamura, H. Tsutsui, T. Yoshimoto, and K. Nakanishi.** 1998. Regulation of interferon-γ production by IL-12 and IL-18. Curr. Opin. Immunol. **10:**259–264.
7. **Pasquali, P., R. Adone, L. C. Gasbarre, C. Pistoia, and F. Ciuchini.** 2001. Mouse cytokine profiles associated with *Brucella abortus* RB51 vaccination or *B. abortus* 2308 infection. Infect. Immun. **69:**6541–6544.
8. **Romani, L., P. Puccetti, and F. Bistoni.** 1997. Interleukin-12 in infectious diseases. Clin. Microbiol. Rev. **10:**611–636.
9. **Stevens, M. G., G. W Pugh, Jr., and L. B. Tabatabai.** 1992. Effects of gamma interferon and indomethacin in preventing *Brucella abortus* infections in mice. Infect. Immun. **60:**4407–4409.
10. **Trinchieri, G.** 1998. Proinflammatory and immunoregulatory functions of interleukin-12. Int. Rev. Immunol. **16:**365–396.
11. **Ushio, S., M. Namba, T. Okura, K. Hattori, Y. Nukada, K. Akita, F. Tanabe, K. Konishi, M. Micallef, M. Fujii, K. Torigoe, T. Tanimoto, S. Fukuda, M. Ikeda, H. Okamura, and M. Kurimoto.** 1996. Cloning of the cDNA for human IFN-gamma-inducing factor, expression in *Escherichia coli*, and studies on the biologic activities of the protein. J. Immunol. **156:**4274–4279.
12. **Zhan, Y., and C. Cheers.** 1993. Endogenous gamma interferon mediates resistance to *Brucella abortus* infection. Infect. Immun. **61:**4899–4901.
13. **Zhan, Y., and C. Cheers.** 1995. Endogenous interleukin-12 is involved in resistance to *Brucella abortus* infection. Infect. Immun. **63:**1387–1390.
14. **Zhan, Y., Z. Liu, and C. Cheers.** 1996. Tumor necrosis factor alpha and interleukin-12 contribute to resistance to the intracellular bacterium *Brucella abortus* by different mechanisms. Infect. Immun. **64:**2782–2786.