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Endogenous Gamma Interferon Mediates Resistance to *Brucella abortus* Infection

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Depletion of endogenous gamma interferon (IFN-γ) with anti-IFN-γ monoclonal antibody resulted in increased numbers of *Brucella abortus* in the spleen and liver of infected CBA mice. This increase was accompanied by a decrease in splenomegaly and a lower proportion of macrophages in the spleen. Furthermore, treatment of recipient mice with anti-IFN-γ antibody blocked the adoptive transfer of resistance with immune T cells. Together, the results indicated that endogenous IFN-γ plays an important role in mediating resistance to primary and secondary *Brucella* infection.

Resistance to facultative intracellular bacterial pathogens depends on acquired cell-mediated resistance and activation of macrophages by T lymphocytes. Gamma interferon (IFN-γ) is believed to be an important mediator of acquired cell-mediated resistance. Injection of recombinant murine IFN-γ enhanced resistance of mice to infection with the intracellular bacterial pathogens *Brucella abortus* (14), *Listeria monocytogenes* (9), and *Mycobacterium tuberculosis* (8). In vitro studies have also shown that IFN-γ-treated macrophages were better able to inhibit the growth of *B. abortus* than untreated macrophages (5, 6), indicating that IFN-γ is likely to be an important cytokine in controlling *B. abortus* infection. However, the role of endogenous IFN-γ in host defense against this pathogen is unproven. The availability of neutralizing antibody allows this question to be investigated. Indeed, in vivo administration of anti-IFN-γ antibody provides evidence of a requirement for endogenous IFN-γ for resolution of *L. monocytogenes* infection in mice (2, 11). We report here that neutralization of IFN-γ in vivo with a monoclonal antibody (MAb) reduced the in vivo clearance of brucellae from infected CBA/J mice.

To deplete endogenous IFN-γ in vivo, each mouse was given a single intraperitoneal injection of 2 mg of rat anti-mouse IFN-γ MAb, partially purified by ammonium sulfate precipitation from ascitic fluid of the R4-6A2 rat-mouse hybridoma (4). The neutralizing titer of the anti-IFN-γ MAb was 10^5 IU/mg of protein. Normal rat globulin was made from rat serum in the same way as control protein. Both preparations contained 0.3 to 3 ng of endotoxin/mg of protein. Twenty-four hours after injection of the antibody, mice were intravenously infected with 5×10^5 organisms of *B. abortus* attenuated strain 19 as described previously (12). After 14 days of infection, weighed fragments of spleen and liver were homogenized in 5 ml of normal saline with an Ultra Turrax homogenizer (Janke and Kunkel K.G., Breisgau, Germany). The numbers of *B. abortus* in the organs were established by plating serial 10-fold dilutions of organ homogenates in saline on a horse blood agar plate.

The remaining part of the spleen was used to prepare a spleen cell suspension in phosphate-buffered saline (PBS) with 5% fetal calf serum for determination of cell surface phenotype. Spleen cells from four individual mice were incubated with PBS and the anti-Mac-1 (M1/70) (13), anti-Thy-1 (30-H12) (10), anti-L3T4a (GK1.5) (3), or anti-Lyt2.2 (53-6.7) (10) MAb and further incubated with fluorescein-conjugated anti-mouse immunoglobulin. Stained cells were analyzed by flow cytometry on a FACScan (Becton Dickinson, Sunnyvale, Calif.).

To test the role of IFN-γ in adoptive transfer of T-cell-mediated immunity, nylon wool-filtered T cells (7) were prepared from the spleens of normal CBA/J mice and CBA/J mice which had been infected intravenously with 5×10^5 *B. abortus* strain 19 organisms for 6 to 8 weeks. Naive recipient CBA/J mice, which had been injected intraperitoneally with 2 mg of anti-IFN-γ antibody or control proteins 24 h previously, were challenged with 10^6 *B. abortus* and then injected with 2×10^7 nylon wool-purified T cells from normal mice or infected mice in sterile PBS (pH 7.4). The numbers of bacteria in spleen and liver were assayed 14 days later, the optimal time for assessing adoptive transfer in *Brucella* infection (12). Student's *t* test was used for all results in this study to assess statistically the differences in mean CFU, spleen size, and percentage of macrophages between groups.

We first tested the effect of the anti-IFN-γ MAb on resistance against primary *Brucella* infection. Mice were injected intraperitoneally with anti-mouse IFN-γ MAb or normal rat globulin (2 mg) 24 h before infection with 5×10^5 *B. abortus* strain 19 organisms, and the numbers of bacterial cells in the spleen and liver of these mice were determined on day 14 of infection (Fig. 1). The bacterial counts increased significantly in the spleen and liver of mice that received an injection of the anti-IFN-γ MAb ($P < 0.01$) compared with controls. The increase in bacterial counts in the liver was greater than in spleen. Multiple injections of anti-IFN-γ MAb after infection did not further exacerbate infection compared with that in mice receiving a single injection of the MAb before infection (data not shown).

The above mice treated with the anti-IFN-γ MAb showed decreased splenomegaly after infection with *B. abortus* strain 19 (spleen weight, 340 ± 54 mg) ($P < 0.01$) compared with untreated mice (440 ± 47 mg) or control globulin-treated mice (439 ± 61 mg). This finding was related to the observation by Stevens et al. (14) that injection of recombinant IFN-γ increased the spleen size of *Brucella*-infected mice. We investigated the change in the cell population in the spleens of the MAb-treated mice. Spleen cells were prepared from normal mice, *Brucella*-infected mice, *Brucella*-infected mice treated with normal globulin, and infected mice treated with the anti-IFN-γ MAb. Subpopulations in

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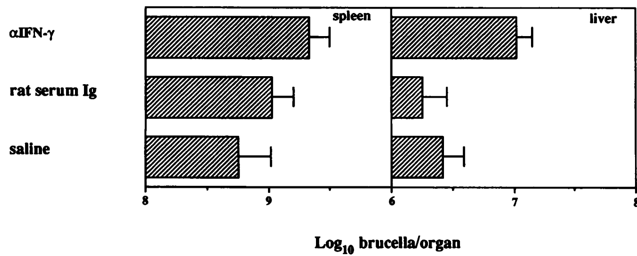

FIG. 1. Effect of the anti-IFN-γ MAb on primary *Brucella* infection. Mice were injected intraperitoneally with 2 mg of anti-IFN-γ MAb or the same amount of normal rat globulin 24 h before infection. Mice were then infected intravenously with 5×10^5 brucellae and killed 14 days after infection. The control group included mice injected with saline before *Brucella* infection. Each point represents the mean and standard deviation for five mice. Results are from one of three repeated experiments.

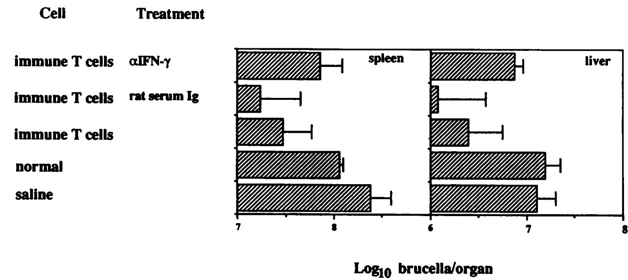

FIG. 3. Effect of anti-IFN-γ MAb on resistance transferred by *Brucella*-immune T cells. Recipient mice were injected intraperitoneally with 2 mg of anti-IFN-γ MAb or normal rat globulin 24 h before transfer and infection. The mice were then given 2×10^7 immune T cells (from 8-week-infected mice) and infected with 10^6 brucellae. Mice given normal T cells or saline were also infected with 10^6 brucellae. Bacterial numbers in spleen and liver were determined 14 days after infection. Data represent the mean and standard deviation for five mice per group. Results shown are representative of two repeated experiments.

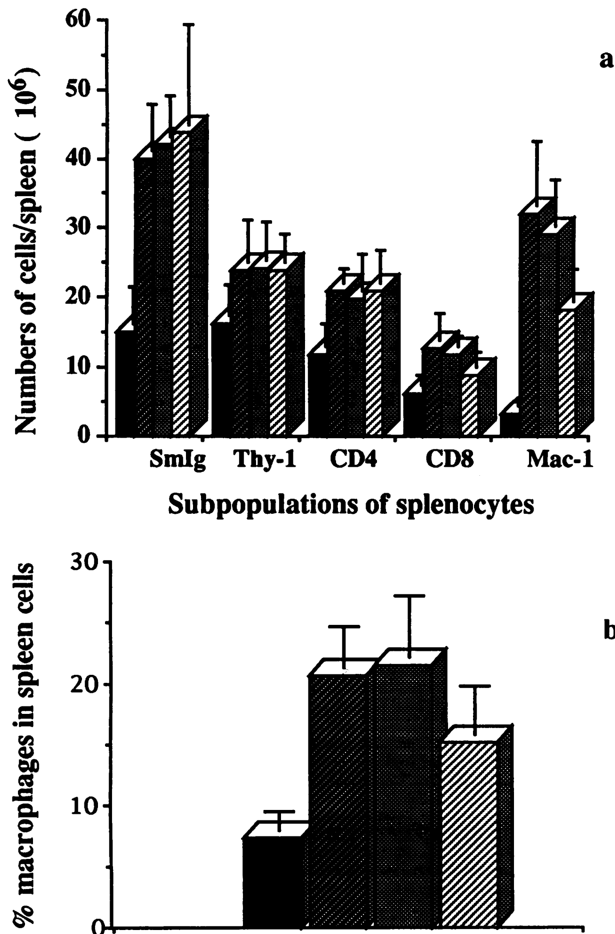

FIG. 2. Effect of anti-IFN-γ MAb on cell composition in spleen during primary *Brucella* infection. Mice were treated and infected as described in the legend to Fig. 1. (a) Cell subpopulations in the spleen. (b) Percentage of macrophages in the spleen. Data represent the mean and standard deviation for five individual mice in a group. Groups included normal mice (solid bars), and infected mice given saline (dark hatched bars), normal rat serum globulin (shaded bars), or anti-IFN-γ MAb (light hatched bars).

the spleen, including B cells (surface membrane immunoglobulin [smIg]), T cells (Thy-1), $CD4^+$ T cells (L3T4a), $CD8^+$ T cells (Lyt-2.2), and macrophages (Mac-1) were analyzed by flow cytometry. Infection led to large increases in the numbers of macrophages and B cells (Fig. 2a). Anti-IFN-γ treatment significantly suppressed this increase in macrophages in infected mice ($P < 0.02$) compared with untreated infected mice or infected mice treated with normal gammaglobulin ($P < 0.03$) (Fig. 2a and b). These results were confirmed in a repeated experiment (data not presented). Evidence from an earlier study (2) showed that depletion of IFN-γ in listerial infection of mice inhibited the activity of macrophages. The present study showed that depletion of endogenous IFN-γ also reduced the numbers of macrophages at the site of infection. For unknown reasons, Thy-1 expression on spleen cells from infected mice was considerably lower than the sum of $CD4^+$ and $CD8^+$ cells, while that of normal spleen cells was equivalent to the sum of $CD4^+$ and $CD8^+$ expression.

In the primary response, IFN-γ may be produced by either natural killer cells or T lymphocytes (1), and the anti-IFN-γ MAb could be acting on either of these. Adoptive transfer of resistance, on the other hand, is mediated by T lymphocytes (12). This system was applied here to study whether protection conferred by immune T cells is mediated by IFN-γ. Mice were injected intraperitoneally with 2 mg of anti-IFN-γ MAb or the same amount of normal rat globulin. Twenty-four hours later, treated mice were given 2×10^7 *Brucella*-immune T cells (from 8-week-infected mice) and infected with 10^6 organisms of *B. abortus* strain 19. Controls included mice receiving immune T cells without anti-IFN-γ MAb, normal T cells, or saline. They were all infected with the same dose of brucellae. Treatment with anti-IFN-γ MAb blocked the protection conferred by *Brucella*-immune T cells (Fig. 3). Bacterial counts in the spleen and liver of anti-IFN-γ MAb-treated mice were significantly higher than in mice receiving normal rat globulin and immune T cells or untreated mice given immune T cells ($P < 0.01$).

Overall, this in vivo study indicated that IFN-γ produced during the natural host response to *B. abortus* infection participates in the clearance of bacteria in vivo. This correlated with the results of in vitro studies showing that IFN-γ has the ability to inhibit *Brucella* replication in macrophages

(5, 6) and in vivo results after injection of recombinant IFN-γ (14). Ironically, IFN-γ also appears to mediate the splenomegaly which is a major pathological effect of brucellosis in humans and mice.

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