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Effect of Gamma Interferon on Resolution of Murine Chlamydial Genital Infection

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Mice infected in the genital tract with the *Chlamydia trachomatis* agent of mouse pneumonitis were treated with monoclonal rat anti-gamma interferon (anti-IFN-γ) antibody to determine whether IFN-γ participated in the resolution of the infection. In two experiments, anti-IFN-γ antibody treatment resulted in significantly prolonged infections. In support of these data, passive administration of recombinant IFN-γ to chronically infected *nu/nu* mice was able to bring about resolution of the infection in some animals.

Immune mechanisms operative in protective immunity against human genital infections with *Chlamydia trachomatis* remain largely undefined. Certainly, both humoral and cell-mediated immune responses have been documented, but solid evidence linking one or both limbs of the immune system with a protective response in humans has not been forthcoming. The majority of information on mechanisms of immunity operative in chlamydial genital infections has been derived from guinea pig and murine animal models. In guinea pigs infected genitally with the *Chlamydia psittaci* agent of guinea pig inclusion conjunctivitis, both antibody- and cell-mediated immunity (CMI) have been found to be essential for both resolution of primary genital infections and resistance to reinfection (5, 6, 8, 9). However, in mice infected with the *C. trachomatis* agent of mouse pneumonitis (MoPn), CMI alone has been found to be sufficient to resolve a genital infection and provide immunity to reinfection (4). Thus, the mouse-MoPn model provides an ideal system in which the mechanism of the CMI protective response can be investigated.

We have previously reported that congenitally athymic nude mice were unable to resolve an MoPn genital infection and remained infected virtually indefinitely (7). Furthermore, the adoptive transfer of mixed CD4 and CD8 or solely CD8 MoPn-specific T-cell lines was able to resolve the infection in nude mice (3). Recently, we have demonstrated that a CD4 T_H1 MoPn-specific T-cell clone is also able to resolve the infection in nude mice (2a). One possible mechanism by which both CD4 and CD8 T cells could mediate resolution of chlamydial infection is by the production of gamma interferon (IFN-γ). IFN-γ has been well documented to have antichlamydial activity in vitro (1, 2). In addition, Williams et al. (11) have demonstrated that treatment with anti-IFN-γ antibody prolonged MoPn respiratory infection and that the passive transfer of recombinant IFN-γ was able to provide a degree of protection. Thus, it was the purpose of the current study to determine whether IFN-γ also has a protective role in the resolution of chlamydial genital infection by using the MoPn model.

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Five- to six-week-old female BALB/c and *nu/nu* mice on a BALB/c background were obtained from Harlan Sprague-Dawley, Inc., Indianapolis, Ind. BALB/c mice were maintained under conventional conditions, and *nu/nu* mice were maintained in Plexiglas isolators under pathogen-free conditions. All animals were given food and water ad libitum in an environmentally controlled room with a cycle of 12 h of light and 12 h of darkness.

For infection, mice were inoculated intravaginally on two or three consecutive days with a suspension of HeLa cell- or McCoy cell-grown MoPn containing 10^6 to 10^7 inclusion-forming units (IFU) in 0.03 ml of sucrose phosphate glutamate buffer (pH 7.2) while under anesthesia with sodium pentobarbital (4). The course of the infection was assessed by isolation of MoPn from cervical and vaginal swabs at various times after inoculation (4). Inclusions on coverslips from shell vials were visualized by indirect immunofluorescence, and the numbers of IFU per swab were determined when necessary.

In order to determine the effect of depletion of IFN-γ on the course of MoPn genital infection, mice were treated with rat anti-IFN-γ antibody. Monoclonal rat anti-murine IFN-γ antibody was produced in vitro from the R46A2 hybridoma obtained from the American Type Culture Collection. Supernatant fluid was collected from cultures and enriched for immunoglobulin by fractionation with 50% ammonium sulfate. The immunoglobulin fraction was resuspended in phosphate-buffered saline (PBS) (pH 7.2), dialyzed against PBS, and quantified for the protein content. A similar fraction of normal rat serum was also prepared.

In the first experiment, 10 mice each were injected intraperitoneally with 3 mg of anti-IFN-γ antibody or rat immunoglobulin in 0.2 ml of PBS daily beginning 7 days prior to infection. When the infection course was monitored with regard to percentage of animals remaining infected at various times after infection, treatment with anti-IFN-γ antibody significantly prolonged the infection ($P < 0.02$ when compared by a one-tailed Wilcoxon signed-ranks analysis) (Fig. 1A). The experiment was repeated, but the treatment regimen was modified so that each mouse was treated intraperitoneally with 8 mg of supernatant containing anti-IFN-γ antibody in 0.3 ml on days 0, 1, and alternate days thereafter. Beginning on day 9, 6 mg of supernatant was given to each animal on alternate days. In this experiment, PBS was used in the control injections. Once again, treatment with anti-IFN-γ significantly lengthened the time course of the infec-

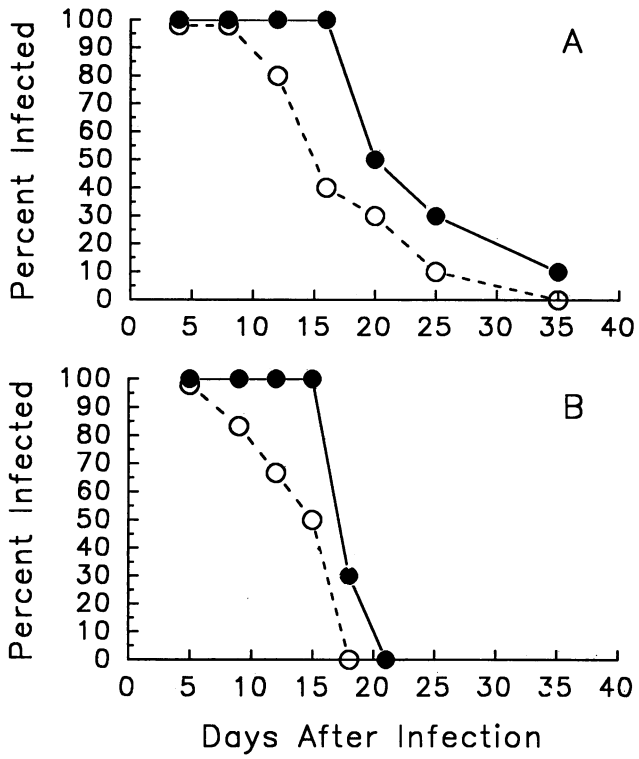

FIG. 1. Effect of treatment with monoclonal rat anti-IFN-γ antibody on the resolution of MoPn genital infection. The data are represented as percentages of animals remaining infected at various times after intravaginal infection with MoPn. (A) Experiment 1. (B) Experiment 2. Closed circles, anti-IFN-γ antibody-treated mice; open circles, control mice.

tion ($P < 0.03$) (Fig. 1B). Therefore, these data suggested that IFN-γ plays a role in the resolution of chlamydial genital infection in the mouse.

To further support such a role for IFN-γ, attempts were made to passively transfer recombinant murine IFN-γ into infected nude mice. Because nude mice are unable to resolve the infection by themselves, any beneficial effect of IFN-γ would be reflected either in a decrease in infection intensity or in the resolution of the infection. Purified recombinant IFN-γ (rIFN-γ) was a kind gift of Genentech Biologics, South San Francisco, Calif. Initially, because MoPn produces a mucosal infection, five infected nude mice were inoculated intravaginally twice per day with 25 μl of rIFN-γ containing 2.6×10^5 U, while control mice were inoculated with PBS. All mice were confirmed to be infected prior to initiation of treatment. The treatment was continued for 15 days, but none of the animals resolved their infections. The same animals, including both experimental and control groups, were then injected intraperitoneally for 10 days with 2.5×10^5 U of rIFN-γ in 0.1 ml of PBS. Two of ten mice resolved the infection before the experiment was halted because of apparent toxicity to the mice, with five of ten mice dying after a rather rapid weight loss.

The experiment was repeated with six rIFN-γ-treated nude mice and four control mice. Again, all mice were confirmed to be infected before the experiment was begun. The treated group received 2.6×10^5 U of rIFN-γ intraperitoneally for seven days. Because mice began to show signs of a toxic effect, the dose was reduced on days 7, 9, and 10

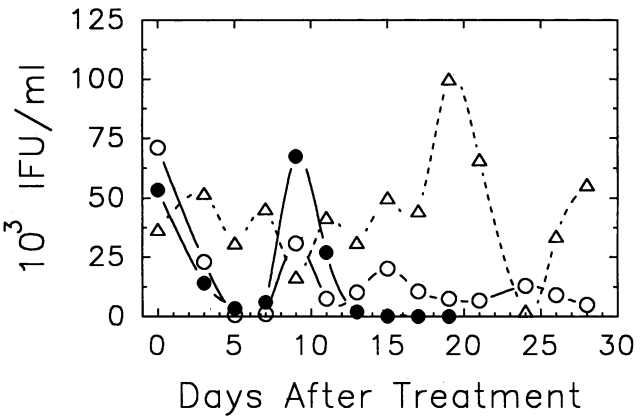

FIG. 2. Effect of passive administration of rIFN-γ on the course of MoPn genital infection in infected *nu/nu* mice. Each point represents the mean number of IFU for the group. Closed circles, rIFN-γ-treated mice which resolved their infections; open circles, rIFN-γ-treated mice which did not resolve their infections; open triangles, PBS-treated mice.

to 10^5 U. No treatment was given on day 8. The original dose of 2×10^5 U was resumed on day 11 and continued to the end of the experiment. Three of the six rIFN-γ-treated mice resolved the infection, while the others remained infected but had lower numbers of IFU on cervical swabs in comparison to the control mice (Fig. 2). None of the control mice resolved the infection. Interestingly, when the rIFN-γ dose was decreased, a marked increase in the IFU was observed in these animals, but IFU decreased again upon resumption of the original dose of rIFN-γ. All three of the animals that had successfully resolved the infection eventually died, possibly as a result of the rIFN-γ treatment. The reason for the apparent toxic effect is not clear. It could be related to the rIFN-γ interacting with or stimulating the production of other cytokines in the animal. The effect does not appear to be related solely to the infection, since one of two uninfected nude mice became sick after 6 days of treatment with the same regimen of IFN-γ. A similar effect was not obvious in the lung model, possibly because fewer treatments were given (11). The endotoxin level of the rIFN-γ was sufficiently low (<0.016 endotoxin units per mg) that it was an unlikely cause of the reaction. Nevertheless, these data suggested that the passive administration of rIFN-γ is capable of altering the course of murine chlamydial genital infection.

The data presented in this study indicate that IFN-γ plays a role in the resolution of chlamydial genital infection in the mouse. The specific deletion of IFN-γ from mice by treatment with anti-IFN-γ antibody was able to prolong the infection, while the passive transfer of rIFN-γ to immunocompromised mice was able to bring about the resolution of the infection in some animals. These data are supportive of those reported by Williams et al. (11) in which they altered the outcome of respiratory infection with MoPn in similar experiments. They also demonstrated that MoPn is indeed sensitive to IFN-γ in vitro. A potential effector function for IFN-γ in a model for *C. trachomatis* intravenous challenge has also been described (13). Moreover, IFN-γ is a major cytokine active in the inhibition of intracellular chlamydial growth in the systemic *C. psittaci* model (2).

While both series of experiments in this study do indeed indicate a protective effect of IFN-γ treatment, the measured effects are not dramatic. This suggests that while IFN-γ is

able to provide some protective function, it is not the only factor involved. Tumor necrosis factor alpha has been found to have antichlamydial activity in vivo (12) and in vitro (10). It is also possible that it is difficult to attain high titers of either anti-IFN-γ antibody or rIFN-γ at the site of infection in the superficial epithelial cells of the genital mucosa. Particularly in the passive transfer of rIFN-γ to nude mice, high levels were required in order to have a positive effect. Previous attempts to measure IFN-γ in the genital tracts of normal infected animals have not met with success (2b), so it was impossible to confirm that the anti-IFN-γ antibody was indeed eliminating the IFN-γ from the system.

The exact mechanism responsible for this effect cannot be determined from the experiments presented here. It is certainly possible that a variety of effects of IFN-γ participate in the resolution of the infection. Since CMI plays a major role in the protective response to chlamydial genital infection in the mouse (4), IFN-γ may exert its effects through macrophage activation or through the release of other cytokines from host cells such as macrophages, or it may be directly bacteriostatic for MoPn, as has been demonstrated in vitro (11). It is interesting that protective T-cell lines (3) and T-cell clones (2a) in this model all elaborate IFN-γ. Thus, these studies provide further information on the mechanism of CMI in a protective role against a localized mucosal infection in the genital tract.

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