Role In Vivo for Gamma Interferon in Control of Pneumonia Caused by Chlamydia trachomatis in Mice

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In a model of pneumonia caused by murine Chlamydia trachomatis, depletion experiments with monoclonal antibody to gamma interferon (IFN-γ) made mice more susceptible. Repletion experiments giving exogenous recombinant murine IFN-γ were not consistently protective. IFN-γ may be necessary but not sufficient in host defense against the organism.

The relevance of the in vitro activity of gamma interferon (IFN-γ) in inhibiting Chlamydia replication (3, 6, 9, 10) to its function in vivo was examined in our model of pneumonia caused by the mouse pneumonitis agent (MoPn; murine Chlamydia trachomatis) using super-clean MoPn-susceptible nude (nu/nu) mice and resistant heterozygous mice (nu/+)(5,12–15). The mice were on a BALB/c background and maintained as described previously (5, 14).

Initial studies (Fig. 1) using procedures similar to those we have used previously (4) showed that MoPn replication in vitro in L cells was significantly inhibited by prior incubation (24 h) of the L cells with exogenous recombinant murine IFN-γ (MuIFN-γ; a gift of Genentech, Inc., South San Francisco, Calif.).

Since MoPn was clearly susceptible to MuIFN-γ and MuIFN-γ is produced in response to MoPn by infected nu/+ mice (2), we performed depletion experiments giving nu/+ mice antibody to MuIFN-γ (rat hybridoma-produced monoclonal antibody R46A2 [11]). This method was previously shown by Li et al. (8) to inhibit generation of MuIFN-γ in vivo and was shown to be nontoxic. Endotoxin levels in the antibody as measured by a Limulus amoebocyte assay were <10 ng per dose. Control mice were given an inactive monoclonal antibody raised initially against Histoplasma capsulatum antigens. Figure 2 shows the results of four separate experiments each with 8 to 12 mice per group (Fig. 2B is the combination of two identical experiments with a total of 20 to 22 mice per group). In each experiment, treatment with antibody to MuIFN-γ significantly increased mortality caused by MoPn (P < 0.05, Wilcoxon two tail). At a higher dose of MoPn (2 × 10³) at which 60% of control mice were dead by day 6, no significant difference was seen (P > 0.5, Wilcoxon two tail; data not shown).

We next quantitated MoPn in these mice by lung culture in McCoy cell monolayers as in our previous studies (13, 14). The combined totals of IFU per lung on day 8 postinfection in two separate experiments with mice given 10⁴ IFU of MoPn on day 0 were as follows. In eight control mice the IFU per lung were 800, 400, 300, 200, 100, <100, <100; in nine treated mice the IFU per lung were 15,500, 5,200, 3,100, 1,500, 1,300, 1,100, 1,000, 800, and <100. IFU were significantly higher in the anti-MuIFN-γ-treated mice than in controls (P < 0.01 Mann-Whitney).

Several controls were run to rule out possible toxicity of the antibody. Ten uninfected nu/+ mice were given the same five intravenous (i.v.) doses of antibody, and results were compared with those from uninfected controls given the irrelevant monoclonal antibody. No deaths occurred. The antibody-treated mice appeared healthy, did not develop ruffled fur, and gained weight consistently at a rate equal to or greater than the control group.

A second control group was given antibody intraperitoneally in case the i.v. route was toxic. The same antibody dosage given intraperitoneally similarly increased mortality in anti-MuIFN-γ-treated nu/+ mice (P < 0.05, Wilcoxon two tail; data not shown). Finally, mice treated with the control monoclonal antibody were compared with mice given normal saline to assure that death was not being delayed in the antibody-treated control group in a nonspecific manner. There was no difference in mortality in this experiment (P > 0.3, Wilcoxon two tail). Since the control monoclonal antibody was murine, a single experiment was run by using an irrelevant rat hybridoma-produced monoclonal antibody. Anti-MuIFN-γ-treated nu/+ mice were still more susceptible (P < 0.005, Wilcoxon two tail).

Interferon was measured in serum by a bioassay using a cytocidal infection with vesicular stomatitis virus (2). This

FIG. 1. Effect of exogenous MuIFN-γ on C. trachomatis (MoPn) growth in mouse fibroblasts; 1 U of MuIFN-γ is 0.08 mg of protein. Each point represents the mean value of triplicate determinations. Bars indicate standard deviations from the mean.

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The assay has detected production of IFN-γ in response to MoPn antigen in supernatants from immune spleen cells (2), and in our hands it detected MuIFN-γ infused i.v. into nulnu mice but did not detect any IFN-γ in serum in response to infection with MoPn, suggesting only local production of the cytokine. The same monoclonal antibody used in these studies inhibits generation of IFN-γ in serum in response to rickettsial infection, however (8), and, in our hands, it neutralized exogenous MuIFN-γ in serum after it was infused i.v. as described below (data not shown).

We next determined if results with the depletion experiments could be extended to protection experiments in vivo giving exogenous recombinant MuIFN-γ. Since sufficient lymphokine was not available for extensive experiments in both nul+ and nulnu mice, we used the more susceptible nulnu mouse. Available quantities of MuIFN-γ limited maximal doses to 10^3 U each. We performed 12 experiments, giving various doses of MuIFN-γ to nulnu mice by intraperitoneal and i.v. routes (at least 3 separate days) and continuously by osmotic pump subcutaneously. MoPn was always given intranasally. In five experiments, MuIFN-γ was given with the inoculum at the time of infection as well as i.v. on days −1, 3, and 6. With the exception of two experiments with MuIFN-γ given i.v., no significant protection was seen and protective results were not readily reproducible. In those two experiments, mice given MuIFN-γ survived significantly longer (P < 0.05, Wilcoxon two tail). In one experiment, mean day of death in the controls was 8.1 and in the MuIFN-γ-treated mice it was 10.8. In the other experiment, the values were 5.7 and 7.0, respectively. It is of interest that previous studies examining MoPn-caused pneumonia in immunologically intact mice showed that IFN inducers (type of interferon undefined) can delay death but apparently do not significantly influence MoPn titers in the lung in that model (7). The data presented here (which do not confirm the consistently significant protective effect of exogenous MuIFN-γ recently demonstrated by Zhong et al. [16] in an immunologically intact mouse model using C. trachomatis serovar L1 [strain 440]) indicate that the nulnu mouse is a stringent model in which to test this cytokine. In this regard, in a murine model of Mycobacterium bovis infection, exogenous MuIFN-γ protects immunologically intact mice but is ineffective in nulnu mice (1).

Our experiments may indicate that we have not found the optimal method to deliver MuIFN-γ to mice with MoPn-caused pneumonia. Alternately, the fact that our depletion experiments were successful but our repletion experiments in an immunosuppressed mouse were not may suggest that MuIFN-γ is a necessary but not sufficient factor in host defense against MoPn.

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