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Activation of Mouse Peritoneal Macrophages In Vitro or In Vivo by Recombinant Murine Gamma Interferon Inhibits the Growth of *Chlamydia trachomatis* Serovar L1

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Peritoneal mouse macrophages activated in vitro with recombinant murine gamma interferon (10 ng/ml) or in vivo (10 μg per mouse) showed a significant decrease in the growth and yield of *Chlamydia trachomatis*. The restriction of the growth of *C. trachomatis* paralleled the expression of Ia^d on the macrophages. Mice that received macrophages activated in vitro with recombinant murine gamma interferon showed a significant decrease in the yield of chlamydial infection-forming units from their spleens and peritoneal fluids.

The ability of lymphokines to activate macrophages to inhibit the growth of *Chlamydia psittaci* has been investigated by several authors. Rothermel et al. (16) indicated that the supernatants from concanavalin A-stimulated human peripheral blood mononuclear cells contained a lymphokine activity that blocked the growth of *C. psittaci* in human monocyte-derived macrophages. This activity was suppressed by a monoclonal antibody to human gamma interferon (IFN-γ), thus suggesting that IFN-γ was the main factor in the suppression of *C. psittaci*. The antichlamydial activity of IFN-γ has now been confirmed for *C. psittaci*-infected monocyte-derived macrophages (15). By contrast, Yong et al. (18) have recently shown that recombinant human IFN-γ can only partially restore the microbicidal activity of monocyte-derived macrophages against *Chlamydia trachomatis* serovar L2. These authors also indicated that the differences between their results and those of Rothermel et al. (15, 16) could be explained by the biological differences between *C. trachomatis* and *C. psittaci*. In the present study, we showed that recombinant murine IFN-γ (rMuIFN-γ) administered in vivo or in vitro can activate mouse peritoneal macrophages, as shown by Ia^d expression, and that this activation paralleled the inhibition of the growth and productive replication of *C. trachomatis*. Furthermore, passive transfer of macrophages activated in vitro by rMuIFN-γ resulted in partial protection of the recipient mice against a challenge with *C. trachomatis*.

Seven- to eight-week-old female BALB/C mice (Simonsen Laboratories, Gilroy, Calif.) were used in this study. The *C. trachomatis* lymphogranuloma venereum L1 serovar (strain 440) was propagated in HeLa 229 cells (American Type Culture Collection, Rockville, Md.) as previously described (5, 6, 19). The rMuIFN-γ was a generous gift of C. W. Czarniecki (Genentech, Inc.) (3, 6). The method for obtaining and culturing thioglycolate-elicited peritoneal macrophages was that of Lin and Stewart (10). Briefly, mice were injected intraperitoneally (i.p.) with 3 ml of 3% (wt/vol) sterile thioglycolate medium. Three days later, the peritoneal cells were obtained by injecting i.p. 3 ml of Hanks balanced salt solution containing 10 U of heparin per ml and were incubated for 2 h at 37°C in 5% CO_2. For in vitro activation, adherent macrophage monolayers were treated for 24 h with rMuIFN-γ at concentrations ranging from 0.1 to 10 ng/ml. For in vivo activation, mice were injected i.p. with thioglycolate as described above and 48 h later were injected i.p. with rMuIFN-γ (10^1 to 10^4 ng per mouse), and the peritoneal macrophages were harvested 24 h later. Phosphate-buffered saline (PBS) (0.01 M; pH 7.4) was used as a control. The macrophage monolayers treated in vivo or in vitro with rMuIFN-γ were infected with chlamydiae at a multiplicity of 10 infection-forming units (IFUs) (titers determined in HeLa 229 cells) and incubated for 2 h at 37°C. To determine the number of chlamydia-infected macrophages, the monolayers were fixed 48 h postinfection and stained (5). To assay for the yield of chlamydial IFUs from the macrophage cultures, 1 ml of sucrose phosphate buffer was added to each well at 0, 5, 10, 20, 40, 60, and 80 h following infection, the cells were sonicated for 15 s, and the titers of chlamydiae were determined in HeLa 229 cells (5). The Ia^d antigen was detected by an indirect immunofluorescence assay with a monoclonal antibody against the Ia^d antigen (Becton Dickinson and Co., Mountain View, Calif.), and $F(ab')_2$ fragments of goat anti-mouse immunoglobulin G conjugated with fluorescein isothiocyanate were used as the second antibody (Organon Teknika Corp., Durham, N.C.). For passive transfer of rMuIFN-γ-activated macrophages, mice were injected i.p. with macrophages (2×10^7 cells per mouse) treated in vivo or in vitro with rMuIFN-γ as described above. Two hours following the transfer, the mice were infected i.p. with *C. trachomatis* L1 at a multiplicity of 10^7 IFUs per mouse. Three days postinfection, the peritoneal fluids and the spleens were harvested and the titers of chlamydiae were determined in HeLa 229 cells (19).

Thioglycolate-elicited macrophages treated with PBS can support *C. trachomatis* productive replication (Fig. 1). On the other hand, macrophages treated in vivo (10 μg per mouse) or in vitro (10 ng/ml) with rMuIFN-γ significantly inhibited the growth of chlamydiae relative to that on the control macrophages. For the first 5 h following infection, the yields of chlamydial IFUs from the PBS- and the rMuIFN-γ-treated macrophages were the same. This suggests that rMuIFN-γ did not affect the early stages of infection. As expected, during the transformation from elementary to reticulate body at 10 to 20 h postinfection, the yield of chlamydial IFUs from the monolayers was very low. However, by 40 h postinfection, the yield of chlamydial IFUs from the macrophages treated in vivo or in vitro with rMuIFN-γ was <1 IFU/ml, whereas the control PBS-treated

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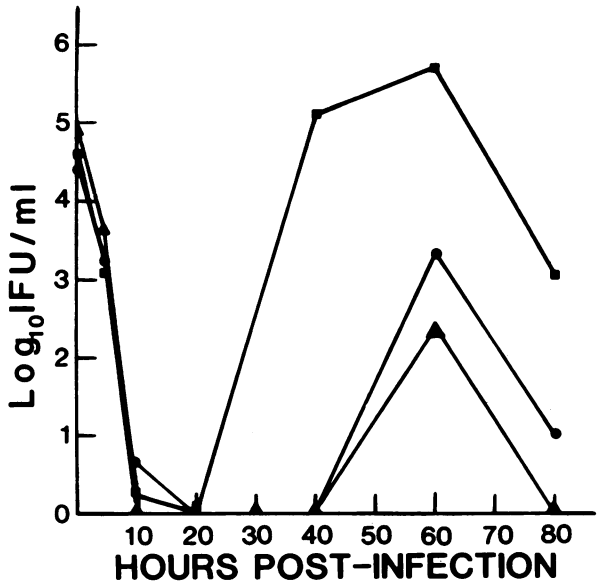

FIG. 1. Yields of chlamydial IFUs from control (■) and rMuIFN-γ-treated macrophages in vivo (●) (10 μg per mouse, i.p.) or in vitro (▲) (10 ng/ml). Macrophages were infected with *C. trachomatis* L1, and the titers of chlamydial IFUs from the macrophages were determined in HeLa 229 cells at 0, 5, 10, 20, 40, 60, and 80 h after infection. Each point represents the average of three to seven experiments.

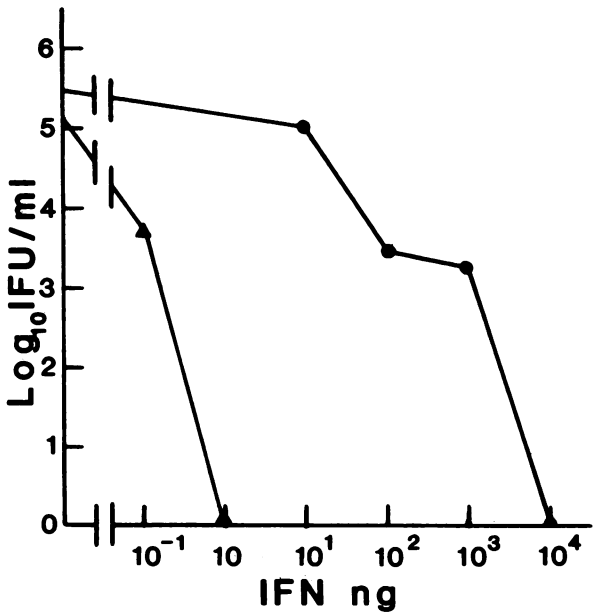

FIG. 2. Effects of the concentration of rMuIFN-γ on the yields of chlamydial IFUs from macrophages. Macrophages activated with different concentrations of rMuIFN-γ in vivo (●) (10^1 to 10^4 ng per mouse) or in vitro (▲) (10^{-1} to 10^1 ng/ml) were infected in vitro with *C. trachomatis* L1, and the titers of chlamydial IFUs were determined in HeLa cells at 40 h postinfection. Each point represents the average of three experiments.

macrophages yielded 1.3×10^5 IFUs/ml ($P < 0.001$). By 60 and 80 h postinfection, the yield of chlamydial IFUs from rMuIFN-γ-treated macrophages was still approximately 100- to 1,000-fold lower than in the control PBS-treated macrophages ($P < 0.001$). Less than 10% of the control thioglycolate-elicited macrophages showed Ia^d positivity (Table 1). On the other hand, macrophages treated in vivo (10 μg per mouse) or in vitro (10 ng/ml) with rMuIFN-γ expressed the Ia^d antigen in more than 30% of the cell population 48 h later ($P < 0.001$). The number of chlamydial inclusions was counted in duplicate monolayers at 48 h postinfection. Of the in vitro control macrophages, 18.6% had chlamydial inclusions, but only 0.16% ($P < 0.001$) of the macrophages treated with rMuIFN-γ had inclusions (Table 1). Macrophages from mice treated in vivo with rMuIFN-γ had few chlamydial inclusions (0.67%), but a high percentage of them (34%) were Ia^d positive. This is in contrast to the macrophages treated in vivo with PBS, of which 21% had chlamydial inclusions ($P < 0.001$) and 10% were Ia^d positive ($P < 0.001$).

TABLE 1. *C. trachomatis* growth and induction of Ia^d expression in peritoneal macrophages treated in vitro or in vivo with rMuIFN-γ

Treatment[a]	% Macrophages[b]	
	Ia^{d+}	Bearing chlamydiae
In vitro		
rMuIFN-γ (10 ng/ml)	32.4 ± 10.5	0.16 ± 0.4
PBS (control)	7.0 ± 3.4	18.6 ± 4.3
In vivo		
rMuIFN-γ (10 μg per mouse)	34.0 ± 6.6	0.67 ± 0.8
PBS (control)	10.0 ± 2.4	21.0 ± 4.3

[a] Five or six mice were tested with each treatment.
[b] Results are the mean ± standard deviation for *C. trachomatis*-infected macrophages or Ia^d-positive macrophages from three separate experiments. By unpaired Student's *t* test, $P < 0.001$ for all values.

From the results described above, we chose 40 h postinfection as the time to study the effect of different concentrations of rMuIFN-γ on the yield of chlamydial IFUs. The macrophages were treated either in vivo by injecting mice i.p. with rMuIFN-γ (10 to 10^4 ng per mouse) or in vitro by adding the rMuIFN-γ (0.1 to 10 ng/ml) to macrophage monolayers. The yield of chlamydial IFUs was assayed in HeLa 229 monolayers 40 h postinfection. In vitro, very low concentrations of rMuIFN-γ (0.1 ng/ml) were needed to decrease the yield of chlamydial IFUs from macrophages by 10- to 100-fold relative to that from the control ($P < 0.001$) (Fig. 2). In contrast, 100 ng of rMuIFN-γ per mouse was required to obtain a similar effect when the mice were injected i.p. with rMuIFN-γ.

Macrophages (2×10^7 per mouse) treated in vivo or in vitro with different concentrations of rMuIFN-γ were transferred to recipient mice by i.p. inoculation. The mice were infected i.p. 2 h later with *C. trachomatis* serovar L1 (10^7 IFUs per mouse), and 3 days following infection the peritoneal fluids and the spleens were assayed for the yield of chlamydial IFUs. Macrophages that were treated in vitro with 10 ng/ml with rMuIFN-γ effectively decreased the yield of chlamydial IFUs from the peritoneal cavities ($P < 0.025$) and spleens ($P < 0.05$) of the recipient animals (Table 2). Macrophages treated in vivo with rMuIFN-γ (10 to 10^4 ng per mouse) did not significantly decrease the yield of chlamydial IFUs from the recipient mice (data not shown).

In 1958 Benedict and McFarland (1) showed that the *Chlamydia* meningopneumonitis strain could grow in normal guinea pig monocytes, while it was inhibited in monocytes obtained from guinea pigs that had been infected with *C. psittaci*. In contrast, Kuo (9) could not demonstrate an

TABLE 2. Passive transfer of antichlamydial activity by rMuIFN-γ-activated macrophages in vitro

rMuIFN-γ concn (ng/ml)	Yield of chlamydial IFUs (log_{10} IFU/ml or g of tissue) from[a]:	
	Peritoneal cavity	Spleen
0.1	3.35 ± 0.37	6.77 ± 0.33
1.0	3.05 ± 0.53	6.52 ± 0.28
10	1.99 ± 0.50[b]	5.27 ± 0.90[c]
0 (control)	3.60 ± 0.50	6.70 ± 0.45

[a] The data are a composite of three separate experiments. The results represent the mean yield of chlamydial IFUs from five to six animals ± standard deviation.
[b] $P < 0.025$.
[c] $P < 0.05$.

increased resistance to the intracellular growth of *C. trachomatis* B or L2 on peritoneal macrophages obtained from mice immunized with chlamydiae in comparison with that on macrophages from nonimmunized control mice. More recently, Manor and Sarov (11) showed that infection of human peripheral blood monocytes with *C. trachomatis* L2 resulted in an abortive infection, while it could replicate in human monocyte-derived macrophages. On the other hand, infection of human peripheral blood monocytes with *C. psittaci* resulted in productive infection (11). These data suggest that monocytes and macrophages are under the influence of several factors that result in changes in their antichlamydial activity. In addition, the data indicate that *C. trachomatis* and *C. psittaci* may respond differently to the immune defense mechanisms. Among these immune defense mechanisms, several studies have shown that mitogen-induced lymphokines and IFNs can restrict the intracellular replication of *C. psittaci* and *C. trachomatis* in a variety of host cells, including macrophages (4–6, 15, 16).

Our studies show that mouse peritoneal macrophages activated in vitro or in vivo with rMuIFN-γ can restrict the growth and productive replication of *C. trachomatis* serovar L1. In vivo administration of IFN-γ has been shown to increase antibody synthesis (13), enhance H_2O_2 production by monocytes (12, 14), and activate in macrophages their microbicidal activity against intracellular parasites such as *Toxoplasma gondii* (2). Any or all of these factors could play a role in the antichlamydial activity induced by rMuIFN-γ. We have also shown that passive transfer of macrophages activated in vitro with rMuIFN-γ results in partial protection of recipient mice against a subsequent challenge with *C. trachomatis*, thus lending further support to the concept that IFN-γ-activated macrophages play a significant role in protection against *C. trachomatis* infections. The failure of the in vivo-activated macrophages to passively transfer protection to recipient mice may be the result of a quantitatively inadequate rMuIFN-γ stimulus. Alternatively, substances present in the peritoneal cavity may rapidly neutralize the exogenously administered rMuIFN-γ or deactivate stimulated macrophages.

Steeg et al. (17) showed that IFN-γ is the major lymphokine responsible for the induction of macrophage Ia expression. More recently, it has been found that T-cell-derived IFN-γ dramatically increases macrophage Ia expression in mice infected with *Listeria monocytogenes*, suggesting that an increase in Ia-positive macrophages during infection may increase the immunoregulatory capacity of the macrophages (8). Jerrells (7) found that Ia-bearing-macrophage influx is associated with the genetic resistance of mice to infection with *Rickettsia tsutsugamushi*. In our study we have shown that an increase in Ia-positive macrophages induced by rMuIFN-γ parallels an increase in the antichlamydial activity of the macrophages. The question of whether Ia antigen expression is necessary for macrophages to kill or inhibit chlamydiae should now be investigated by analyzing the antichlamydial activities of the Ia-positive and Ia-negative macrophages.

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