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Role of Endogenous Gamma Interferon in Host Defense against *Chlamydia trachomatis* Infections†

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BALB/c mice (6 to 8 weeks old) infected with *Chlamydia trachomatis* serovar L1 were sacrificed, and the yield of *Chlamydia* inclusion-forming units from the liver and lungs was measured in HeLa 229 cells. The yield of inclusion-forming units reached a peak at 3 days postinfection and then progressively declined. The mice infected with *C. trachomatis* had no detectable levels of gamma interferon (IFN-γ) in their sera. However, stimulation of their spleen cells with either concanavalin A or heat-killed *C. trachomatis* resulted in the release of high levels of IFN-γ (600 to 900 IU/ml) at 5 to 8 days postinfection. The increased release of IFN-γ from the spleen cells paralleled the clearance of chlamydia from the liver and lungs. Sera and spleen cells from animals immunized with live *C. trachomatis* were transferred to recipient mice that were subsequently challenged with *C. trachomatis*. Transfer of spleen cells resulted in a reduction of the infection in the recipient animal as measured by the yield of chlamydia from the spleen, but transfer of the sera did not confer protective immunity. In addition, mice infected with *C. trachomatis* serovar L1 were treated with a hamster neutralizing monoclonal antibody to recombinant murine IFN-γ (MAb-MuIFN-γ). In the animals receiving the MAb-MuIFN-γ, the yield of chlamydia from the lungs, spleen, and liver was significantly higher than from the control groups of mice. Histopathological analysis of tissues from the chlamydia-infected mice showed that the animals treated with the MAb-MuIFN-γ had a significantly more extensive inflammatory reaction in their lungs, liver, and spleen.

Analysis of the host defense against chlamydial infections has been an area of intense research in recent years (3, 17, 24, 25). The data indicate that both antibody and cell-mediated immunity play an important role in limiting or eliminating a chlamydial infection (24, 25). However, as for other intracellular parasites such as *Rickettsia* and *Toxoplasma* species (2, 21), cell-mediated immunity may be the predominant component in controlling a chlamydial infection (3, 24, 25). In particular, several lines of evidence suggest that lymphokines limit chlamydial infections (3, 6–9, 11, 14, 20, 22, 26). For example, *Chlamydia* infection induces interferons (IFNs) in tissue culture cells (13), and both crude and recombinant IFNs can limit in vitro the growth and infectivity of different *Chlamydia* strains (6–9, 11, 14, 20, 22). Furthermore, we have recently provided the first evidence of the in vivo antichlamydial activity of exogenously administered recombinant murine gamma IFN (IFN-γ) (r-MuIFN-γ) (26). Intravenous injection of r-MuIFN-γ decreased the yield of chlamydial inclusion-forming units (IFUs) from the liver, lungs, and spleen of mice infected with *Chlamydia* species, and this was accompanied by a significant decrease in the tissue inflammatory reaction to the microorganism.

In 1985, Buchmeier and Schreiber (4) looked at the requirements for endogenous IFN-γ in the resolution of *Listeria monocytogenes* infection. They found that following listeria infection IFN-γ was not detectable in the sera of the experimental mice but that increased production of IFN-γ occurred following stimulation of the spleen cells with antigens or mitogens. This increased production of IFN-γ correlated with the clearance of listeria from the tissues of the infected animals. Furthermore, treatment of listeria-infected mice with a hamster neutralizing monoclonal antibody to rMuIFN-γ (MAb-MuIFN-γ) inhibited the generation of activated macrophages and limited the recovery of mice from their listeria infection. Similarly, Li et al. (15) have recently shown that when Swiss Webster mice, which are naturally resistant to *Rickettsia conorii*, were treated with MAb-IFN-γ, they became extremely susceptible to *R. conorii* infection.

The present study examined the role played by endogenous IFN-γ in host defense against chlamydial infection. We report here that production by spleen cells of IFN-γ during a chlamydial infection correlated with the clearance of the microorganism from the lungs and liver. Furthermore, inoculation of mice with a hamster neutralizing MAb-MuIFN-γ resulted in a more severe chlamydial infection in the animal, as assessed by the yield of chlamydial IFUs from the spleen, lungs, and liver and by the inflammatory reaction in tissues.

MATERIALS AND METHODS

***Chlamydia* stocks.** The *Chlamydia trachomatis* lymphogranuloma venereum L1 serovar (strain 440) was propagated in HeLa 229 cells (American Type Culture Collection, Rockville, Md.) grown in Eagle minimal essential medium (Irvine Scientific, Irvine, Calif.) supplemented with 5% fetal calf serum (Irvine Scientific), 50 μg of gentamicin per ml, and 1 μg of cycloheximide per ml (7). The HeLa 229 cells were infected with *C. trachomatis* by absorbing the inoculum for 1 h at 37°C. Medium was then added, and the monolayers were harvested 48 h postinfection. The infected monolayers

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were detached with glass beads and disrupted by ultrasound (Braun-Sonic 2000; B. Braun Instruments, Burlingame, Calif.) for 15 s, cellular debris was pelleted at low-speed centrifugation (1,000 × *g* for 5 min), and the *Chlamydia* cells were collected by centrifuging the supernatant at 10,000 × *g* for 30 min at 4°C (26). The *Chlamydia* stocks were stored in sucrose-phosphate buffer at −70°C (26). The stocks contained 1.5×10^9 inclusion forming units (IFUs) of *C. trachomatis* L1 per ml as determined by titration in HeLa 229 cells.

Infection of mice with *C. trachomatis* L1. BALB/c female mice (6 to 7 weeks old) were obtained from Simmonsen Laboratories (Gilroy, Calif.). A nonlethal murine model of systemic infection with *C. trachomatis* (3) was used throughout this study. Each mouse was infected with 10^6 IFUs of *C. trachomatis* L1 in 0.5 ml of phosphate-buffered saline (PBS) by tail vein injection, and the control group was injected with 0.5 ml of PBS. The animals were sacrificed at 1 h postinfection and on different days thereafter by cervical dislocation. Serum samples were collected by retro-orbital bleeding under ether anesthesia at different times following infection and assayed for chlamydial antibodies by immunofluorescence (19). The liver and lungs were harvested to quantitate the yield of infectious chlamydia in HeLa 229 cells as previously described (26). The spleens were removed for IFN-γ induction studies with concanavalin A and heat-killed chlamydia as inducers, and subsequently, the supernatants were used to assay the level of IFN and antichlamydial activity. The experiments were performed three times with two animals in each group.

In vitro production of IFN-γ by spleen cells. Single-cell suspensions of spleen cells were made in RPMI 1640 medium (Irvine Scientific) with 5% fetal calf serum and 50 μg of gentamicin per ml. The cells were washed and adjusted to a cell density of 10^7 cells per ml. The cultures were stimulated with either heat-killed *C. trachomatis* L1 (7×10^7 IFU/ml) or concanavalin A (5 μg/ml; Sigma Chemical Co., St. Louis, Mo.) for 24 h at 37°C. Supernatants from the cultured mouse spleen cells were collected by centrifuging the cells at 1,000 × *g* for 10 min at 4°C, and the supernatants were stored at −20°C until the IFN assays were performed.

Assay and characterization of IFN. Antiviral activity of the sera or spleen cell supernatants was determined by microdilution assay with L cells (L929 strain; American Type Culture Collection) challenged with encephalomyocarditis virus (5, 10). In each assay, IFN titers were corrected against a r-MuIFN-γ standard (specific activity, 2.3×10^7 IU/mg of protein; Genentech, Inc., South San Francisco, Calif.).

The IFN produced by spleen cells was characterized as IFN-γ by assay of residual antiviral activity after 2-ml samples of the spleen cell supernatants were either dialyzed at 4°C against 500 ml of 0.15 M KCl-HCl buffer (pH 2) for 5 days and then dialyzed against PBS (pH 7.4) for 1 day (12) or MAb-MuIFN-γ (4) was added up to a concentration of 5 μg/ml and incubated for 1 h at 37°C.

In vitro determination of antichlamydial activity of spleen cell supernatants. L929 cells were grown in Eagle minimal essential medium supplemented with 5% fetal calf serum in 24-well plates, and supernatant fluids from stimulated spleen cells as described above were added at 24 h before infection. The cells were then washed with Eagle minimal essential medium–5% fetal calf serum, infected as previously described by centrifugation with *C. trachomatis* serovar L1, and incubated at 37°C for an additional 48 h (26). The infected monolayers were then fixed and stained by an indirect method with a MAb to *C. trachomatis* produced in our laboratory and a horseradish peroxidase-conjugated rabbit anti-mouse antibody (Organon Teknika, Malvern, Pa.) (26). The results were expressed as the $\log_{10}$ reduction in the number of chlamydial IFUs in the L929 cells treated with the spleen cell supernatants from *C. trachomatis*-infected mice versus the L929 cells incubated in spleen cell supernatants from normal control mice. The IFN in all samples was characterized as described above.

Passive transfer of antichlamydial immunity with sera or spleen cells. BALB/c female mice (6 to 7 weeks old) were immunized via the tail vein with live *C. trachomatis* L1 (10^6 IFU) on three occasions at weekly intervals (3). One week following the last injection, the spleens from the six donor mice were aseptically removed and single-cell suspensions were prepared. Blood was collected from the same animals, and the sera were assayed for chlamydial antibody by an immunofluorescence assay (19). Spleen cells and sera from uninfected mice were used as controls. The recipient animals received 0.5 ml of normal or immune pooled sera intravenously (i.v.) or 1.5×10^8 normal or immune spleen cells in 0.5 ml of RPMI 1640 medium 2 h before i.v. challenge with *C. trachomatis* L1 (1×10^6 IFU per mouse). The spleens were harvested 72 h following infection, and the titer of *Chlamydia* IFUs was determined with HeLa 229 cells as previously described (26). The experiments were repeated three times with a total of five recipient mice per group. The results were expressed as the $\log_{10}$ reduction of *Chlamydia* IFUs per spleen.

Treatment of mice with MAb-MuIFN-γ. The hamster neutralizing MAb-MuIFN-γ (H1.5) (2.5 mg of protein per ml; endotoxin, <2 ng/ml) used in all the experiments has been previously described (4). Female BALB/c mice (6 to 7 weeks old) were injected via the tail vein with 10^6 IFUs of *C. trachomatis* L1 per mouse, and 2 h later, the mice were divided into three groups and treated i.v. as follows. Group 1 received 125 μg of MAb-MuIFN-γ per mouse, group 2 received 125 μg of normal hamster immunoglobulin G (NHaIgG; Organon Teknika), and group 3 received 0.5 ml of PBS (pH 7.2). At 3 and 5 days after infection, the animals were sacrificed by cervical dislocation, the liver, spleen, and lungs were harvested, and the yield of chlamydial IFUs was assayed with HeLa 229 cells as previously described (26). The experiments were performed three times, and each group had two to three animals in each experiment. The results were expressed as the $\log_{10}$ *Chlamydia* IFUs per gram of tissue.

Histopathological analysis. Segments of the liver, spleen, and lungs were fixed in 10% Formalin, processed, and stained with hematoxylin-eosin (26). Histological preparations were assessed as blinded specimens by two pathologists.

Statistical analysis. The Student two-tailed *t* test was used for comparative analyses. *Chlamydia* IFUs were transformed to $\log_{10}$ for evaluation.

RESULTS

Yields of *Chlamydia* IFUs and production of chlamydial antibodies by infected mice. Mice infected with *C. trachomatis* were sacrificed at 1 h or at 1, 3, 5, 8, 11, and 15 days postinfection, and the yield of chlamydial IFUs from the liver and lungs was measured in HeLa 229 cells. The peak yield of IFUs was reached at 3 days postinfection (Table 1). At that point, the liver yielded $\log_{10}$ 6.6 *Chlamydia* IFU per organ and the lung yielded $\log_{10}$ 4.3 *Chlamydia* IFU per

TABLE 1. Yield of chlamydial IFUs from the liver and lungs and levels of chlamydial antibodies in the sera from *C. trachomatis*-infected mice

Time postinfection	Log_{10} of chlamydial IFUs (per organ)[a]		Chlamydial antibody[b]
	Liver	Lungs	
1 h	3.3 ± 0.33	2.5 ± 0.47	0
1 day	5.3 ± 0.9	3.5 ± 0.13	0
3 days	6.6 ± 0.33	4.3 ± 0.35	5
5 days	5.6 ± 0.74	4.1 ± 0.56	218
8 days	1.53 ± 2.1	3.4 ± 1.5	380
11 days	0	0	542
15 days	0	0	1,568

[a] Mean values ± 1 standard deviation from six animals in each group.
[b] Reciprocal of geometric mean titer.

organ. The yield of chlamydial IFUs consequently declined and reached nondetectable levels by 11 days postinfection. In the same animals, the levels of chlamydial antibody in sera progressively increased, and by 15 days the reciprocal of the geometric mean titer was above 1,568 (Table 1).

Production of IFN-γ following chlamydial infection. Sera collected from animals at 1 h or at 1, 3, 5, 8, 11, and 15 days following a chlamydial infection were assayed for IFN. Serum samples from the *C. trachomatis*-infected animals showed no detectable increase in antiviral activity over those from the control noninfected animals. However, when spleen cells from *C. trachomatis*-infected mice were collected and stimulated with heat-killed *C. trachomatis* or concanavalin A, measurable amounts of IFN-γ were induced. The amount of IFN present in those supernatants was assayed in a standard antiviral microdilution assay (Table 2). Spleen cells from noninfected animals produced no IFN when exposed to heat-killed *C. trachomatis* and produced <50 IU of IFN per ml when stimulated with concanavalin A. In contrast, the spleen cells from infected mice responded to both inducers, and the peak of IFN production occurred at 5 to 8 days following infection. Concanavalin A-stimulated cells produced the highest level of IFN (894 IU/ml) at 5 days postinfection, while the peak level of IFN-γ produced by heat-killed *C. trachomatis*-stimulated splenocytes was obtained at 8 days postinfection (613 IU/ml).

TABLE 2. IFN-γ levels and antichlamydial lymphokine activity in the splenocyte supernatants from mice infected with *C. trachomatis*

Days postinfection	Stimulation of spleen cells[a]			
	Concanavalin A		Heat-killed chlamydia	
	Antiviral activity[b]	Anti-chlamydial activity[c]	Antiviral activity[b]	Anti-chlamydial activity[c]
Control (non-infected)	50	0.5	0	0
1	31 ± 24	0.48 ± 0.4	25 ± 23	0.05
3	188 ± 109	2.86 ± 0.28	303 ± 170	2.46 ± 0.69
5	894 ± 652	3.69 ± 1	293 ± 136	1.40 ± 0.29
8	773 ± 410	3.40 ± 2.1	613 ± 572	3.0 ± 0.99
11	420 ± 255	2.17 ± 1.1	30 ± 44	0.50 ± 10
15	216 ± 144	1.12 ± 0.01	50 ± 43	0

[a] Mean values ± 1 standard deviation.
[b] IFN-γ levels (International units per milliliter) in spleen supernatants.
[c] Log_{10} reduction of chlamydial IFUs per milliliter of spleen cell supernatant.

The antichlamydial activity of the spleen cell supernatants was determined by assaying the supernatants in L929 cells infected with *C. trachomatis* serovar L1. The reduction of chlamydial inclusions in vitro by the spleen cell supernatants closely paralleled the levels of IFN-induced antiviral activity in those supernatants (Table 2). The peak level of antichlamydial activity with the concanavalin A-stimulated splenocytes was reached at 5 days postinfection (log_{10} 3.69 reduction) and at 8 days with the heat-killed *C. trachomatis*-stimulated splenocytes (log_{10} 3.0 reduction).

To characterize the type of IFN released by the spleen cells, we treated the spleen cell supernatants by acidification or incubated them with a neutralizing MAb-MuIFN-γ. Both treatments resulted in complete loss of antiviral and antichlamydial activity, thus indicating that IFN-γ was the predominant active lymphokine secreted by the spleen cells in response to concanavalin A or heat-killed *C. trachomatis*. The NHaIgG used as a control had no effect on the antiviral and antichlamydial activity of the spleen cell supernatants.

Passive transfer of immunity by sera and spleen cells. To assess the protective role played by antibody-mediated and cell-mediated immunity, we transferred sera and spleen cells from mice immunized three times at weekly intervals with live *C. trachomatis* to recipient mice that were challenged 2 h later with 10^6 IFUs of *C. trachomatis* L1 per mouse. The spleens from the recipient mice were harvested 3 days postinfection, and the yield of *Chlamydia* IFUs was assayed in HeLa 229 cells. The animals receiving sera (titer, 1:640) from immunized mice did not demonstrate a significant decrease in the yield of *Chlamydia* IFUs relative to the control nonimmunized animals (log_{10} 0.21 reduction; $P > 0.05$). On the other hand, mice that received spleen cells from immunized animals showed a greater than 1,000-fold reduction in the yield of *Chlamydia* IFUs per spleen when compared with control mice receiving spleen cells from nonimmunized animals (log_{10} 3.13 reduction; $P < 0.05$).

In vivo administration of a neutralizing MAb-MuIFN-γ. To assess the role played by endogenous IFN-γ in the control and clearance of a chlamydial infection, we treated mice infected with *C. trachomatis* with MAb-MuIFN-γ. Mice were inoculated with 10^6 *C. trachomatis* L1 IFUs by tail vein injection, and 2 h later the experimental group received 125 μg of MAb-MuIFN-γ i.v. Control mice were similarly challenged with *C. trachomatis*, but they were inoculated 2 h postinfection with either 125 μg of NHaIgG or with 0.5 ml of PBS administered i.v. At 3 and 5 days postinfection, the animals were sacrificed, and the yield of chlamydial IFUs from the liver, spleen, and lungs was assayed on HeLa 229 cells. At 3 days postinfection, there was no statistically significant difference between the control and experimental groups in terms of the yields of chlamydial IFUs from the liver and lungs (Table 3). In contrast, the yields of chlamydial IFUs from the spleens of the animals treated with the MAb-MuIFN-γ was significantly higher than the yields found in the two control groups ($P < 0.001$). By day 5, there was a 10- to 100-fold increase in the yield of chlamydial IFUs recovered from the lungs, spleen, and liver of the animals treated with the MAb-MuIFN-γ in comparison with the control groups ($P < 0.002$).

Tissue sections from the organs of the *C. trachomatis*-infected mice showed a marked difference in the inflammatory response between the experimental and the control mice. At 5 days postinfection, the livers from the animals treated with MAb-MuIFN-γ had multiple foci of polymorphonuclear leukocytes and mononuclear cells involving the liver parenchyma (Fig. 1C and D). In contrast, the animals

TABLE 3. Yield of chlamydial IFUs at 3 and 5 days postinfection in mice treated with a neutralizing MAb-MuIFN-γ

Treatment	Log_{10} IFUs/g (±SD)[a] at:					
	3 days			5 days		
	Liver	Spleen	Lungs	Liver	Spleen	Lungs
MAb-MuIFN-γ	7.342 ± 0.592	7.348 ± 0.464	6.760 ± 0.929	7.090 ± 0.595	6.756 ± 0.589	7.290 ± 0.618
NHaIgG	7.111 ± 0.445	6.338 ± 0.504	6.090 ± 0.528	5.213 ± 0.655	5.828 ± 0.472	5.753 ± 0.909
PBS	7.061 ± 0.3625	6.380 ± 0.549	6.243 ± 0.594	5.149 ± 0.115	5.612 ± 0.441	5.706 ± 0.856
MAb-MuIFN-γ- vs. NHaIgG-treated mice	$P > 0.20$	$P < 0.001$	$P > 0.20$	$P < 0.001$	$P < 0.002$	$P < 0.002$

[a] Each value represents the average of six to seven mice. The experiments were done three times with two to three mice in each group.

treated with the NHaIgG showed a less severe inflammatory response in the liver parenchyma (Fig. 1A and B). Similar findings were observed in the lungs (data not shown).

DISCUSSION

We showed here that endogenous IFN-γ plays a significant role in the control of a chlamydial infection. Spleen cells from *C. trachomatis*-infected mice produced significant levels of IFN-γ upon stimulation by concanavalin A or heat-killed *C. trachomatis*. These levels of IFN-γ in the spleen cell supernatants paralleled the clearance of the microorganism from the tissues. In another set of experiments, protection of recipient animals was obtained by transferring spleen cells from *C. trachomatis*-immunized mice, but not by

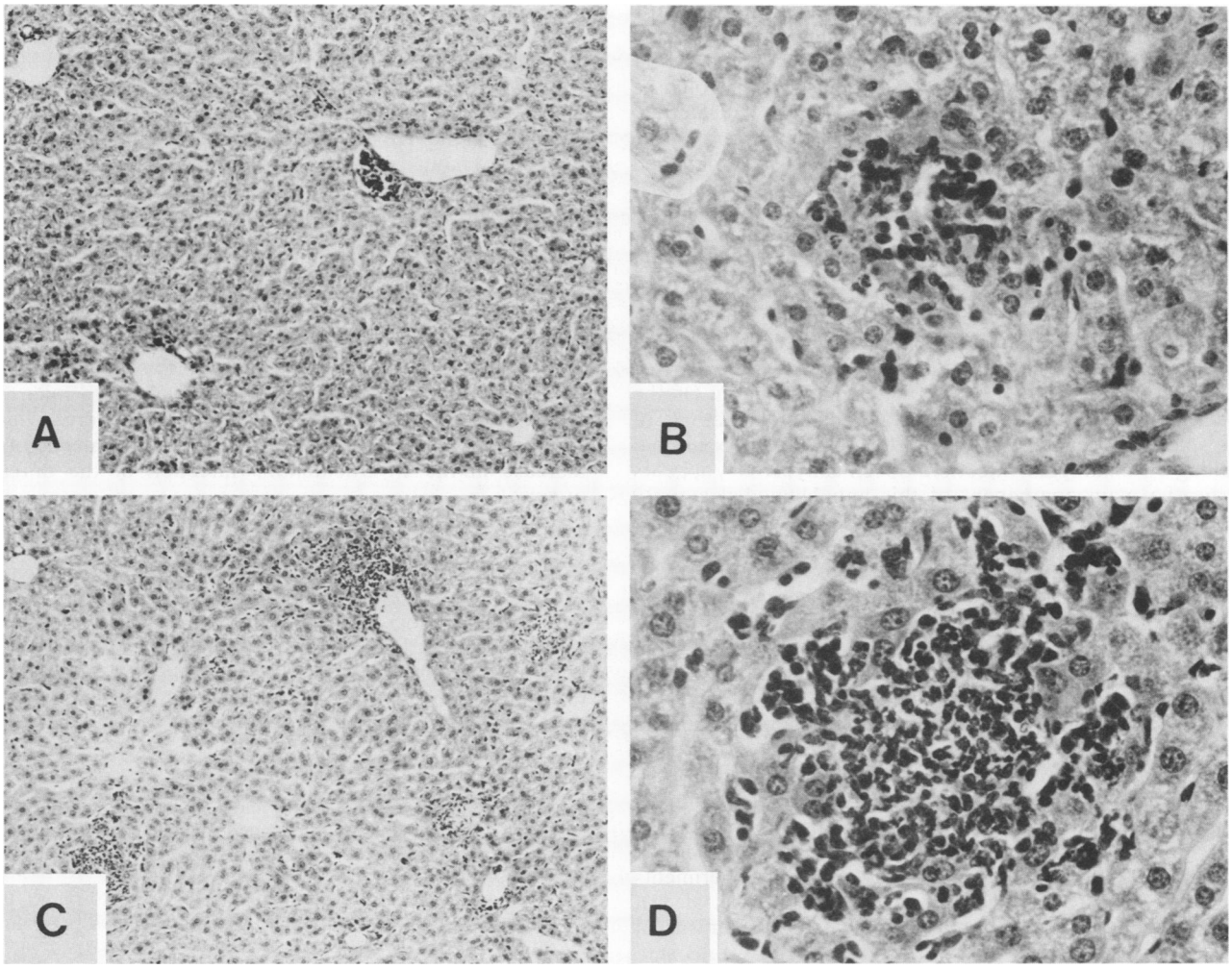

FIG. 1. Histological sections of the liver from NHaIgG-treated mice (A [×600] and B [×1,200]) and mice treated with the MAb-MuIFN-γ (C [×600] and D [×1,200]). Mice were infected with *C. trachomatis* and, 2 h later, treated with NHaIgG or with a neutralizing MAb-MuIFN-γ given via the tail vein. At 5 days postinfection, the animals were sacrificed and histological sections were stained with hematoxylin-eosin. Notice the multiple foci of acute and chronic inflammatory cells in the liver parenchyma of the MAb-MuIFN-γ-treated mice. By contrast, the control animals show moderate foci of inflammatory reaction.

transferring sera. Furthermore, administration of a neutralizing MAb-MuIFN-γ to mice infected with *C. trachomatis* compromised their ability to clear the microorganism from the lungs, liver, and spleen.

In 1963, Sueltenfuss and Pollard (22) first showed that IFN could inhibit the growth of chlamydia in vitro. Since then, data have accumulated indicating that both crude and recombinant IFN preparations can inhibit the growth of *C. trachomatis* and *C. psittaci* in vitro (6–9, 11, 14, 20). Attempts to demonstrate the in vivo antichlamydial activity of IFNs by using IFN inducers such as polyriboinosinic acid · polyribocytidylic acid [poly(I · C)] or Newcastle disease virus failed to show any change in the recovery of infectious *C. trachomatis* from tissues, although there was a marked improvement in the clinical symptomatology (14, 17). We recently showed that i.v. administration of r-MuIFN-γ significantly decreased the yield of *C. trachomatis* L1 IFUs from the liver, spleen, and lungs of infected animals and decreased the tissue inflammatory reaction (26). The apparent discrepancy between our results and those obtained with the IFN inducers may be due to the types or concentrations of IFNs achieved in the tissues or to the different *C. trachomatis* serovars assayed in these systems.

As shown in the present report, the yield of infectious *C. trachomatis* from the liver and lungs of infected mice peaked at 3 days postinfection and then slowly declined up to 11 days, at which point no infectious *Chlamydia* cells could be detected. Assay of the sera from these animals indicated no detectable IFN levels. In contrast, the levels of IFN-γ measured after stimulating the spleen cells from infected animals with either heat-killed *C. trachomatis* or concanavalin A slowly plateaued at 5 to 8 days and then declined for the next 7 days of the observation period. Concurrently, the levels of antibody to *C. trachomatis* in the sera of the infected mice continuously increased over the 15 days of observation. However, at 3 days postinfection, the time at which we observed the peak yield of chlamydial IFUs from both the liver and lungs, the titer of chlamydial antibody was 1:5 and it increased to 1:218 by 5 days postinfection. On the other hand, the significant in vitro antichlamydial activity of the spleen supernatants supports the role of IFN-γ in controlling the chlamydial infection. Specifically, IFN-γ appears to be the main component of this antichlamydial activity since treatment of the supernatants with a neutralizing MAb-MuIFN-γ or pH 2 significantly reduced its antichlamydial activity. We observed a correlation of the IFN-γ levels and the antichlamydial activity in the supernatants from splenic cells, a finding consistent with earlier studies (6, 11, 14, 20, 22) and our own in vitro and in vivo studies that identify IFN-γ as the main lymphokine that activates host cells to express antichlamydial activity (7–9, 26).

Treatment of mice with sera (titer, 1:640) from immunized mice inoculated three times with live *C. trachomatis* did not protect mice from subsequent challenge. In contrast, transferred spleen cells effectively protected recipient animals, thus suggesting that, at least in this experimental model, the protective role conferred by antibodies in a primary infection is minimal. The results of these passive transfer experiments are similar to the findings reported by Brunham et al. (3), who postulated a T-lymphocyte-mediated mechanism of resistance during a primary chlamydial infection, thus supporting the role of IFN-γ in host defense against infection. Similarly, Williams et al. (24) have shown that T-lymphocyte responses are critical for recovery from chlamydial pneumonia induced in athymic mice. However, Williams et al. (25) showed that protection against invasive *C. trachomatis* pneumonitis correlated with high serum levels of antibody to *Chlamydia* species. Furthermore, both sera with high antibody titers and purified immunoglobulins were protective in these studies. Our results suggest that IFN-γ is more important than antibodies in eradicating a primary chlamydial infection. However, *Chlamydia* antibodies may play a significant role in controlling the infection at a later stage of the disease. Also, the different results may reflect the types of animal models and *Chlamydia* serovars used in these experiments. Williams et al. (24, 25) used the mouse pneumonitis serovar of *C. trachomatis*, a serovar known to be biologically significantly different from the L1 serovar that we used in our experiments. It is also possible, however, that the IFN-mediated protection against *Chlamydia* species is enhanced by the presence of specific antibodies, a situation similar to that described for the protozoan *Trypanosoma cruzi* (18).

Treatment of *C. trachomatis*-infected mice with a hamster neutralizing MAb-MuIFN-γ significantly increased the yield of chlamydial IFUs from the lungs, livers, and spleens of the treated animals. These results suggest that endogenous IFN-γ is an important factor in the resolution of a *C. trachomatis* infection and that its activity is exerted at multiple sites. Similar results were obtained by Buchmeier and Schreiber (4) in an *L. monocytogenes* mouse model of infection. This antichlamydial effect of IFN-γ could be exerted by activating macrophages in vivo or by direct effect on the different tissues. In this regard, in vitro experiments have shown that IFNs can exert an antichlamydial activity in human and mouse cell lines of different tissue origin (6–9, 11, 13, 14, 20, 22, 26). Tissue sections of the three organs show marked differences in the inflammatory response between the groups of mice treated with the MAb-MuIFN-γ and the control animals injected with NHaIgG. We have previously shown that animals treated with r-MuIFN-γ have a decrease in the yield of chlamydial IFUs from their liver, lungs, and spleen that is accompanied by a decrease in the inflammatory reaction in those organs (26). It was then not unexpected that treatment of *C. trachomatis*-infected mice with the neutralizing MAb-MuIFN-γ resulted in an increase in the inflammatory response, most likely as a consequence of the increase in the *C. trachomatis* infection of the different organs.

Further studies are necessary to elucidate the mechanism by which IFN-γ exerts an antichlamydial activity in vivo. IFN-γ has been shown to regulate the immune system by exerting multiple effects including enhanced expression of I-A and H-2 antigens on lymphocytes, macrophages, and endothelial cells and increased cytotoxic T-cell and NK-cell killing activity (1, 16, 18, 23). It is possible that many of these functions contribute to the potent antichlamydial activities of IFN-γ observed in vivo.

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