Inhibition of Apoptosis by Gamma Interferon in Cells and Mice Infected with *Chlamydia muridarum* (the Mouse Pneumonitis Strain of *Chlamydia trachomatis*)

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The effect of gamma interferon (IFN-γ) on apoptosis due to infection by *Chlamydia muridarum* (the mouse pneumonitis strain of *Chlamydia trachomatis*) was studied in epithelial cells in culture and in the genital tracts of mice. IFN-γ concentrations that induce the formation of aberrant, persistent chlamydiae inhibit apoptosis due to *C. muridarum* infection. In cells treated with an IFN-γ concentration that leads to the development of a heterogenous population of normal and aberrant *Chlamydia* vacuoles, apoptosis was inhibited preferentially in cells that contained the aberrant vacuoles. The inhibitory effect of IFN-γ appears to be due in part to expression of host cell indoleamine 2,3-dioxygenase activity, since inhibition of apoptosis could be partially reversed through coincubation with exogenous tryptophan. Apoptotic cells were observed in the genital tracts of wild-type mice infected with *C. muridarum*, and a significantly larger number of apoptotic cells was detected in infected IFN-γ-deficient mice. These results suggest that IFN-γ may contribute to pathogenesis of persistent *Chlamydia* infections in vivo by preventing apoptosis of infected cells.

Chlamydia trachomatis strains include a causative agent of ocular infection in humans and the most common cause of sexually transmitted bacterial infections (20, 54). An inflammatory response is required for the resolution of primary *C. trachomatis* infection, but chronic inflammation is also responsible for the scarring process observed in trachoma and chlamydial sexually transmitted disease (2). In addition, the ability of chlamydiae to persist in the host could be an important factor in exacerbating pathogenesis (4, 6). The immune mechanisms responsible for chronic inflammation are not fully understood, but it is believed that repeated exposure to chlamydial antigens contributes to pathogenesis and that bacteria in persistently infected cells may serve as a source of long-lasting pathology (5).

Chlamydia muridarum (also known as the mouse pneumonitis strain of *C. trachomatis*) (14, 48) infects the genital tracts of mice and has been used to characterize the host immune response to chlamydial genital tract infection. In *C. muridarum* infections of mice, a major role for CD4+ T cells secreting gamma interferon (IFN-γ) during clearance of the infection has been described (25, 31, 42, 52). Thus, studies with IFN-γ-deficient mice have shown that IFN-γ is required for prevention of dissemination of genital tract infection (9, 26, 42). Similarly, administration of anti-IFN-γ antibodies or recombinant IFN-γ prolongs or resolves the infection, respectively (45).

Chlamydiae are obligate intracellular bacteria that have a distinct biphasic infection cycle: the small elementary bodies are infectious and metabolically inert, and the larger reticulate bodies (RB) are noninfectious and metabolically active (22, 32, 56). A persistent state of chlamydiae can be induced in vitro by IFN-γ treatment, which leads to altered bacterial forms and an antigen profile that is different from that observed during active infection (4, 6). Significantly, there is down-regulation of the chlamydial major outer membrane protein (MOMP), and expression increases or decreases for several other proteins and genes, whereas expression of the immunostimulatory chlamydial antigen, hsp60, remains at high levels during the persistent state (6, 27, 49). The intracellular chlamydiae undergo striking morphological changes, becoming much larger than conventional RB (5, 49). In IFN-γ-treated infected cells, the chlamydiae are noninfectious, but the bacteria are viable and can revert to the infectious state following removal of IFN-γ (3).

Several *Chlamydia* spp. have joined a growing number of intracellular microorganisms that modulate apoptosis in opposite directions, both promoting and inhibiting apoptosis under different conditions (8, 17, 19, 23, 35, 55). Similar to other pathogens, chlamydiae protect infected cells against apoptosis due to external stimuli during early stages of infection (15) and induce apoptosis of the host cell during later stages (21, 38, 40, 41). Chlamydiae may thus protect infected cells against cytotoxic mechanisms of the immune system, while the apoptosis observed at the end of the infection cycle may contribute to the inflammatory response or facilitate release of bacteria from infected cells.

It has recently been reported that cells infected with *C. trachomatis* in the presence of IFN-γ resist apoptosis due to external ligands, via inhibition of caspase activation (11). Inhibition of caspase activation has also been shown in the absence of IFN-γ (15), while the apoptosis due to the infection itself is independent of caspases (38, 41). To determine if persistently infected cells are still susceptible to apoptosis at

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the end of the infection cycle, we characterized apoptosis of cells infected with *C. muridarum* in the presence or absence of IFN-γ in vitro. The study was then extended to an in vivo infection by measuring the number of apoptotic cells in the genital tracts of wild-type and IFN-γ-deficient mice infected with the same strain of *C. muridarum*. In both cases, IFN-γ inhibited *Chlamydia*-induced apoptosis, which could contribute to limiting propagation of the infection during the state of persistence.

MATERIALS AND METHODS

Cells and animals. HeLa 229 cells (obtained from the American Type Culture Collection, Manassas, Va.) were cultivated in Dulbecco's modified minimal essential medium with Glutamax-1 (Life Technologies, Inc., Rockville, Md.), supplemented with 10% heat-inactivated fetal calf serum and 25 μg of gentamicin per ml. *C. muridarum*, obtained originally from the American Type Culture Collection, was grown in McCoy cells and purified as previously described (44). Six-week-old female C57BL/6 (C57; *H-2^b*) and IFN-γ-deficient female C57BL/6-Ifg^tm1 (IFN-γ^{-/-}) mice were purchased from The Jackson Laboratory (Bar Harbor, Maine). Recombinant human IFN-γ was obtained from PeproTech (Rocky Hill, N.J.), and L-tryptophan was from Aldrich (Saint Quentin Fallavier, France). All other reagents were previously described (40).

Infections of cells with *C. muridarum* and treatment with IFN-γ. For infections in vitro, HeLa cells were plated at a density of 2×10^4 cells per well in six-well plates (Costar). After 24 h in a humidified incubator at 37°C with 5% CO$_2$, the cell monolayers were washed and infected at a multiplicity of infection (MOI) of 0.5. At 1 h postinfection, the inoculum was removed and replaced with fresh culture medium. Infected monolayers were maintained in the incubator for 2 days or treated 3 h postinfection with various concentrations of IFN-γ. In some experiments, 200 μg of L-tryptophan per ml was added to the cell cultures at the same time as the IFN-γ (3, 28).

Immunofluorescence. Infected or uninfected cells were fixed in 4% paraformaldehyde and revealed with anti-*Chlamydia* fluorescein-conjugated antibodies, consisting of a mixture of two monoclonal antibodies specific for the chlamydial MOMP and chlamydial lipopolysaccharide (1:500 dilution; Argene, Varilhes, France), as previously described (37, 38). Host cell and bacterial DNAs were visualized by staining saponin-permeabilized cells with 5 μg of Hoechst 33258 (Sigma, St. Louis, Mo.) per ml. Samples were examined with an Axiovert 135 TV fluorescence microscope (Zeiss, Oberkochen, Germany) attached to a cooled charge-coupled device camera (Photometrics).

To estimate the number of cells with persistent chlamydiae, infected samples were fixed and incubated with an anti-*Chlamydia* monoclonal antibody, as described above. Using the scale of 1 to 4 for the level of persistent chlamydial development defined previously (3), normal cells correspond to cells with inclusions containing typical chlamydia (level 1), while persistently infected cells included all the cells with inclusions with some evidence of atypical, enlarged chlamydiae (levels 2 and 3) or exclusively atypical chlamydiae (level 4). As the cells were also labeled with Hoechst, apoptotic cells in the same samples were identified by their condensed nuclei.

Analysis of cell death. In vitro, apoptosis was measured by cytofluorimetry using detergent-permeabilized cells stained with propidium iodide (PI), as previously described (13, 29, 33, 36). Since apoptotic cells were present mainly in the supernatant (38), adherent cells were detached with 1 mM EDTA in phosphate-buffered saline at 37°C, and both adherent cells and cells in suspension were collected and centrifuged. After one washing with phosphate-buffered saline, the cell pellet was resuspended directly in PI-detergent buffer (62.5 μg of PI per ml, 0.1% Triton X-100, and 1 mg of sodium citrate per ml in water) and transferred into 12- by 75-mm Falcon 2052 fluorescence-activated cell sorting tubes (Becton Dickinson, San Jose, Calif.). Data from 10,000 HeLa cells were collected on a FACScan flow cytometer (Becton Dickinson) with an argon ion laser tuned to 488 nm. Apoptosis was measured in the FL-3 range, and the marker used to quantify the number of apoptotic cells was set in such a way as to exclude debris and necrotic cells in the low FL-3 values. Each sample was performed in triplicate, and each experiment was repeated at least three times on separate days.

Infection of mice and histological procedures. Wild-type or IFN-γ-deficient mice received 2.5 mg of medroxyprogesterone acetate (Depo-Provera; Upjohn, Kalamazoo, Mich.) subcutaneously 7 days before vaginal infection. The mice were infected by placing 30 μl of 250 mM sucrose–10 mM sodium phosphate–5 mM L-glutamic acid (SPG) containing 1.0×10^7 inclusion-forming units of *C. muridarum* per ml into the vagina of each mouse or 30 μl of SPG without

bacteria for negative controls, as described elsewhere (10). Mice were sacrificed 7 days after vaginal infection. Oviducts and uterine horns were removed, fixed in 4% paraformaldehyde, and embedded in 37°C paraffin. Longitudinal 4-μm sections were cut, deparaffinized, and hydrated through xylene and graded alcohol series.

Apoptotic cells were identified by staining with the terminal deoxynucleotidyl-transferase-mediated dUTP-biotin nick end labeling (TUNEL) method using the apoptosis detection kit from Boehringer Mannheim (Meylan, France), following the manufacturer's instructions. Samples were examined with the Zeiss microscope, and images were acquired and analyzed with the IPLab spectrum program (Signal Analytics Corporation, Vienna, Va.). The relative number of apoptotic cells in each microscope field was quantified with the IPLab spectrum program, as previously described (40).

RESULTS

Effect of IFN-γ on survival of *Chlamydia*-infected cells in vitro. Several strains of *C. trachomatis* and *C. muridarum* and the guinea pig inclusion conjunctivitis strain of *Chlamydia psittaci* induce apoptosis of host cells towards the end of the infection cycle (21, 38, 40). IFN-γ has been reported to induce a state of persistence of *C. muridarum*, human strains of *C. trachomatis*, and *Chlamydia pneumoniae* (5, 30, 39), although human strains of *C. trachomatis* are more sensitive than *C. muridarum* (30). When incubated 24 or 48 h before the start of infection, IFN-γ had a partial effect on growth of *C. muridarum* at 0.5 ng/ml and a larger effect at 2.0 ng/ml. The IFN-γ concentrations used in this work had no effect on uninfected cells (data not shown), but IFN-γ concentrations above 5 ng/ml were toxic to the host (HeLa) cells themselves (30). When cells are infected first and then cultured with IFN-γ, growth of the chlamydiae is not inhibited, and instead the chlamydiae assume the biochemical and morphological features of persistence (4). We therefore measured the effect on apoptosis during *C. muridarum* infection in the presence of IFN-γ at 0, 0.2, and 2 ng/ml, which was added 3 h after the start of infection. Apoptosis, defined by DNA content as measured by cytofluorimetry after 48 h of infection, was not affected by IFN-γ at 0.2 ng/ml but was inhibited by approximately half by IFN-γ at 2 ng/ml (Fig. 1).

Correlation between chlamydial persistence and host cell nuclear condensation. Cells persistently infected with some human *C. trachomatis* strains in vitro contain abnormally large noninfectious forms of chlamydiae (5, 6). As for *C. trachomatis* serovar A (3, 49), the number of *C. muridarum*-infected cells containing persistent chlamydiae was heterogenous for cells treated at the low IFN-γ concentration. At 0.2 ng of IFN-γ per ml, cells with both normal and aberrant inclusions were observed (data not shown), while at 2 ng/ml, most of the inclusions had enlarged chlamydiae, as reflected by the appearance of patches in the inclusions, observed by immunofluorescence (Fig. 2). Thus, unlike *C. trachomatis* L2, whose form does not change significantly in response to IFN-γ (49), the morphological development of *C. muridarum* in IFN-γ-treated cells is reminiscent of that of *C. trachomatis* serovar A.

Approximately 1/10 of the infected cells had persistent chlamydiae at 0.2 ng of IFN-γ per ml, compared to over 3/4 of the cells that had been treated with 2 ng of IFN-γ per ml (Fig. 3A). At the MOI of 0.5 used here and in the absence of IFN-γ, half of the infected cells had apoptotic nuclei after 48 h of infection and the other half still had normal nuclei (data not shown). Interestingly, at the lower IFN-γ concentration, inhibition of
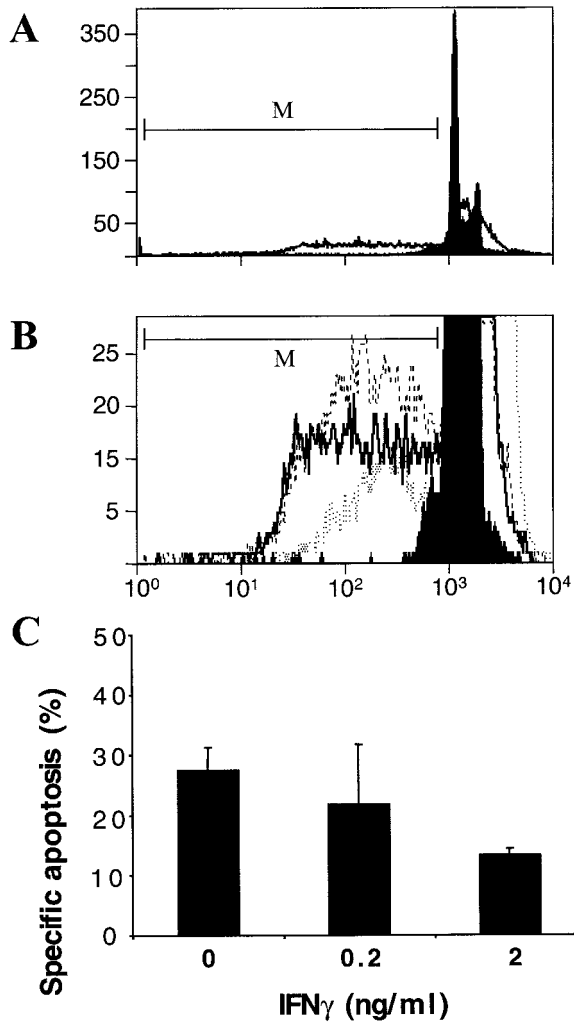
FIG. 1. Effect of IFN-γ on apoptosis and survival of host cells infected with *Chlamydia*. HeLa cells were infected with *C. muridarum* at an MOI of 0.5, and the indicated concentration of IFN-γ was added 3 h after the beginning of infection. After 48 h of infection, specific apoptosis of adherent cells and cells in suspension was measured by cytofluorimetry of PI-treated, detergent-permeabilized cells, as described in Materials and Methods. The marker (M) was used to define the population of apoptotic cells. (A) Cytofluorimetry profile of infected and uninfected cells treated with PI. In the absence of infection or IFN-γ treatment, a spontaneous level of apoptosis of 3 to 5% was always observed after 48 h (filled profile). Over 25% of the cells became apoptotic after a 48 h infection (open profile). (B) Cytofluorimetry profile with expanded *y* axis to show apoptotic cells in a population of infected cells (dashed line) and infected cells incubated with IFN-γ at 0.2 ng/ml (solid line) or 2 ng/ml (dotted line). The fluorescence intensity (FL-3) is given on the *x* axis, and the number of cells is on the *y* axis (A and B). (C) Calculated values of specific apoptosis for infected cells treated with 0, 0.2 or 2 ng of IFN-γ per ml. The values are means and standard deviations for three *Chlamydia*-infected samples treated with IFN-γ on separate days. *P* is <0.01 for 0 versus 2 ng of IFN-γ per ml.

nuclear condensation, as assayed by immunofluorescence of Hoechst-stained nuclei, was limited preferentially to cells containing the aberrant chlamydial inclusions (Fig. 3B). Thus, IFN-γ inhibits apoptosis mainly of persistently infected cells.

Effect of tryptophan on apoptosis of IFN-γ-treated cells.

The persistent phenotype of *Chlamydia*-infected cells can be reversed through incubation of cell cultures with an excess of extracellular tryptophan (3, 28, 53). Similarly, coincubation of *Chlamydia*-infected cells with IFN-γ and tryptophan partially
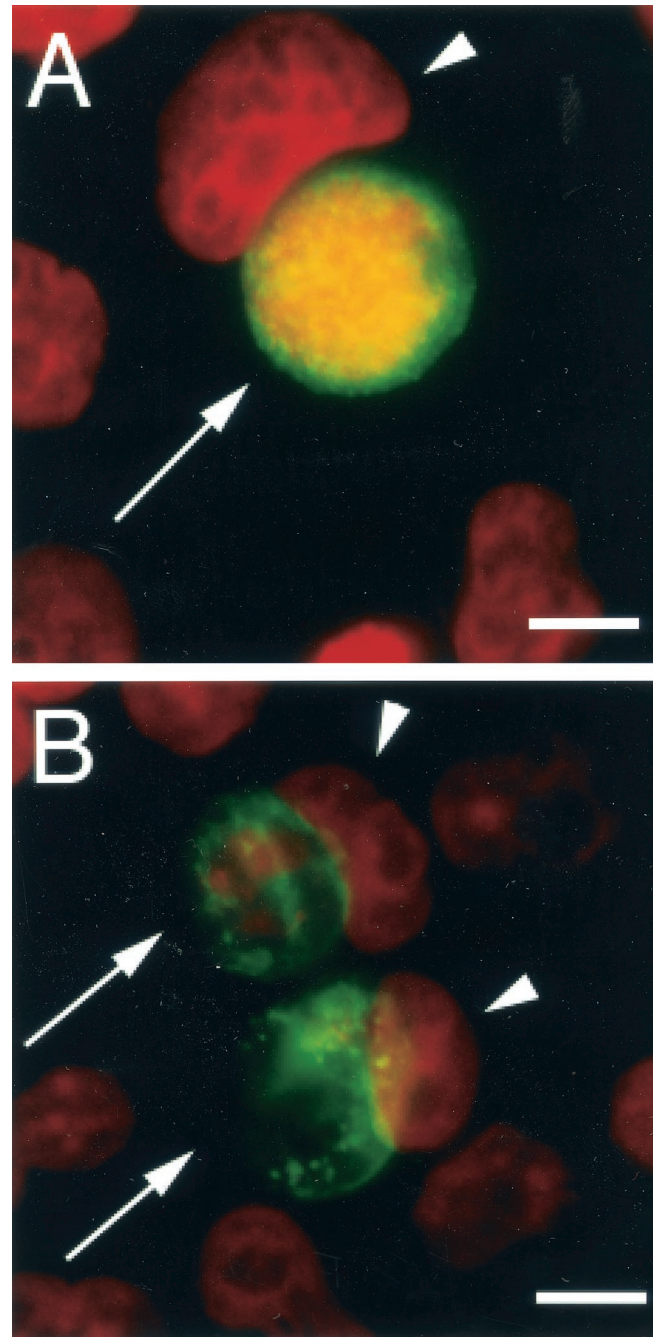

FIG. 2. Appearance of *Chlamydia* inclusions in cells treated with IFN-γ. HeLa cells infected with *C. muridarum* and treated with IFN-γ at 0 (A) or 2 (B) ng/ml were fixed with paraformaldehyde and prepared for immunofluorescence as described in Materials and Methods, using fluorescein-conjugated anti-*Chlamydia* antibodies (green) and Hoechst for DNA labeling (red). *Chlamydia* inclusions were normal in the absence of IFN-γ but were aberrant when infected cells were treated with 2 ng of IFN-γ per ml. Arrows indicate *Chlamydia* inclusions, and arrowheads show host cell nuclei. Bar, 10 μm.
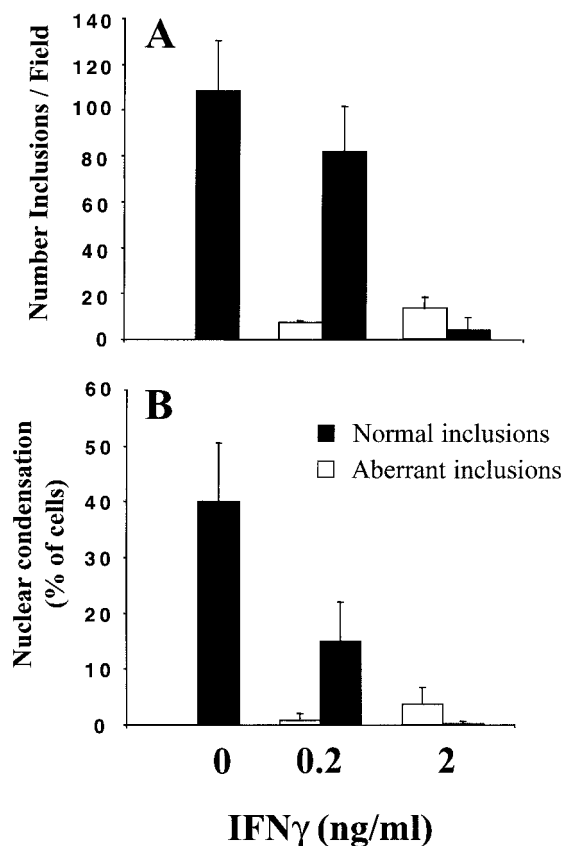
FIG. 3. Sensitivity to the effects of IFN-γ and correlation between the developmental state of *Chlamydia* vacuoles and sensitivity to apoptosis. HeLa cells were infected with *C. muridarum* at an MOI of 0.5 for 48 h in the presence of the indicated concentrations of IFN-γ. (A) The number of cells containing normal inclusions (black bars) or inclusions with persistent chlamydiae (white bars) was determined and defined as described in Materials and Methods. For cells containing normal inclusions, *P* is <0.01 for 0 versus 0.2 and 0 versus 2 ng of IFN-γ per ml. (B) Host cells were identified as containing normal or persistent chlamydiae, and apoptotic cells were counted based on the condensation of Hoechst-labeled nuclei, as described in Materials and Methods. For cells containing normal inclusions and condensed nuclei, *P* is <0.001 for 0 versus 2 ng of IFN-γ per ml and <0.01 for 0 versus 0.2 ng of IFN-γ per ml. Each series of experiments was performed at least three times.

reverses the inhibitory effect of IFN-γ on *Chlamydia*-induced apoptosis (Fig. 4), suggesting that IFN-γ exerts its effect mainly via host cell indoleamine 2,3-dioxygenase (IDO) activity (5). Thus, IFN-γ inhibits infection-dependent apoptosis by approximately 60% in the absence of tryptophan and by approximately 30% in the presence of tryptophan (Fig. 4). However, tryptophan did not completely reverse the inhibitory effect of IFN-γ, suggesting that this cytokine may have some effects on chlamydiae independent of IDO activity. As controls, addition of tryptophan to the extracellular medium in the absence of IFN-γ did not affect *Chlamydia*-induced apoptosis, and 2 ng of IFN-γ per ml with or without tryptophan had no effect on apoptosis in the absence of infection (data not shown).

Apoptosis in wild-type and IFN-γ-deficient mice. Both *C. muridarum* and human strains of *C. trachomatis* are sensitive to the inhibitory effects of IFN-γ in mouse models, although, as in the case of the chlamydiae in vitro (30), human strains are

more sensitive than *C. muridarum* (43). The effect of IFN-γ on apoptosis during infection with *C. muridarum* in vivo was evaluated by quantifying the apoptotic cells, revealed by the TUNEL technique, in the upper genital tracts of wild-type and IFN-γ-deficient mice infected for 7 days. There was a large increase in the level of apoptosis in infected wild-type mice compared to uninfected controls (Fig. 5), as previously reported (40). The extent of apoptosis increased even further, more than twofold, in the IFN-γ-deficient mice that had been infected (Fig. 5), suggesting that secretion of IFN-γ may protect against *Chlamydia*-induced apoptosis in vivo.

DISCUSSION

We show here that IFN-γ produces a state of persistence of *C. muridarum*, as previously reported for human strains of *C. trachomatis* (5, 6, 39, 49). After treatment with a low concentration of IFN-γ (0.2 ng/ml), we observed a heterogenous population of *Chlamydia* inclusions, some being normal and others being aberrant. A homogeneous population of persistent, aberrant chlamydiae arises after treatment with a higher concentration (2 ng/ml) of IFN-γ, as reported for the human strain *C. trachomatis* serovar A (3). In infected cells in vitro, IFN-γ treatment also results in inhibition of *Chlamydia*-induced apoptosis. As measured by immunofluorescence, there is near-complete inhibition of apoptosis at 2 ng of IFN-γ per ml and partial inhibition at 0.2 ng/ml. However, at the lower concentration, the inhibitory effect is restricted mainly to cells containing inclusions with the persistent phenotype. The inhibitory effect of IFN-γ on apoptosis was smaller when assayed by cytofluorimetry, which allowed us to measure apoptosis of both
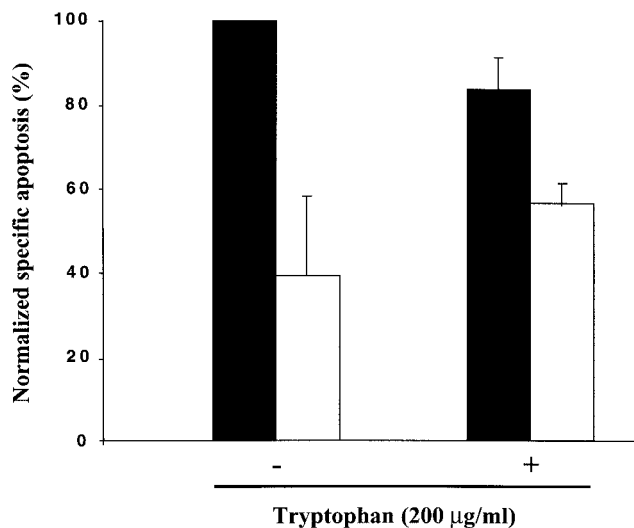

FIG. 4. Reversal by tryptophan of IFN-γ-dependent inhibition of apoptosis. HeLa cells were infected with *C. muridarum* at an MOI of 0.5 for 48 h. Infected cells were treated with (white bars) or without (black bars) IFN-γ (2 ng/ml) and with or without 200 μg of L-tryptophan per ml. Apoptosis was measured by cytofluorimetry using PI-stained, detergent-permeabilized cells, as described in Materials and Methods. The values were normalized to the highest level of apoptosis (infected cells in the absence of IFN-γ or L-tryptophan), and the values are means and standard deviations for three samples infected with *C. muridarum* on separate days.
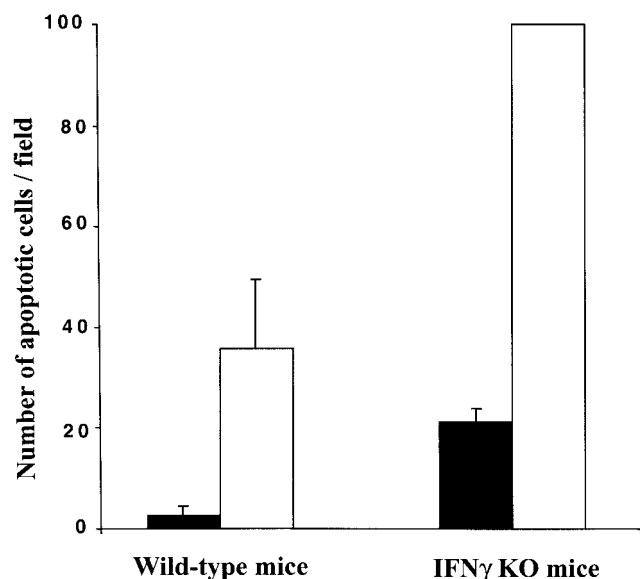
FIG. 5. Level of apoptosis in the genital tracts of wild-type and IFN-γ-deficient mice infected with *Chlamydia*. Mice were infected with *C. muridarum* for 7 days, histological samples were stained by TUNEL, and the number of apoptotic cells in the uterine horns and oviducts was determined as described in Materials and Methods. All apoptosis values were normalized to the value obtained with infected IFN-γ-deficient (IFNγ KO) mice, which was defined as 100. Histological sections were prepared from three uninfected mice and three infected mice from each group.

adherent cells and cells in suspension. Thus, the extent of apoptosis is most likely underestimated when measured by immunofluorescence, which is limited to adherent cells. We also quantified the number of apoptotic cells in the upper genital tract of IFN-γ$^{-/-}$ mice infected with *C. muridarum* and found that, consistent with the in vitro results, there are more apoptotic cells in the IFN-γ$^{-/-}$ mice than in wild-type mice.

The concentration dependence and the timing of the effects of IFN-γ on *Chlamydia*-induced apoptosis are consistent with previous work on the persistent state of chlamydiae in vitro. It has been reported that multiplication of *C. trachomatis* is inhibited in a dose-dependent manner when IFN-γ treatment begins before infection (50). However, when treatment begins concomitantly with infection or shortly after infection, as in our experiments, a persistent state develops in which the RB are morphologically abnormal and are unable to develop into elementary bodies (4), remaining in an undividing but viable state. In addition, pretreatment of HeLa cells with IFN-γ before infection with the murine species used in this study, *C. muridarum*, had little effect on the production of infectious chlamydiae at IFN-γ concentrations of ≤0.2 ng/ml, but there was more than a threefold reduction in the number of infectious chlamydiae starting at 0.5 ng/ml (30). The concentrations of IFN-γ that cause persistence in vitro are thus comparable to those found in endocervical secretions from women infected with *C. trachomatis* (1, 5).

Chlamydia spp. have been reported to modulate apoptosis in opposite directions (19, 55), both protecting acutely infected cells against apoptosis due to external ligands and inducing apoptosis towards the end of the infection cycle (15, 38, 40, 41). In a recent study, the effect of persistence on the protective

activity of *C. trachomatis* serovar A was evaluated in HeLa cells incubated with 0.2 and 2.0 ng of IFN-γ per ml (11). During acute infection in vitro, infected cells are resistant to apoptosis due to inhibition of cytochrome *c* release from mitochondria and inactivation of host cell caspases (15). Similarly, cytochrome *c* was not translocated from mitochondria to the cytosol in persistently infected cells, suggesting that caspase-9 and consequently caspase-3 were not activated (11). Thus, the mechanism of protection in persistently infected cells appears to be the same as that observed in the absence of IFN-γ (15). The effect of IFN-γ on apoptosis at the end of the infection cycle was not evaluated.

Chlamydia species are endowed with a type III secretion apparatus that could be used by the bacteria to secrete virulence factors into the inclusion or the host cell cytosol (16, 24, 51). Although specific *Chlamydia* factors that could induce or inhibit apoptosis have not been identified yet, it is tempting to speculate that proteins secreted by *Chlamydia* may modulate apoptosis in the infected cell, as reported for other intracellular bacteria (18, 55). It also remains to be seen whether IFN-γ could alter the expression of chlamydial proteins that modify survival pathways in the infected cell. A recent proteomic analysis of the regulation of chlamydial proteins revealed that the expression of chlamydial MOMP and several other proteins decreases markedly early in the developmental cycle of *C. trachomatis* serovar A, while modifications were also found for other proteins (49). However, no significant down-regulation was observed for the expression of MOMP or other proteins in cells infected with *C. trachomatis* serovar L2. If it turns out that chlamydial virulence factors are involved in IFN-γ-dependent inhibition of host cell apoptosis, then one may expect that IFN-γ affects *Chlamydia*-mediated apoptosis differentially, depending on the *Chlamydia* strain.

In human epithelial cells, IFN-γ treatment results in depletion of tryptophan via IFN-γ-induced expression of IDO. IDO activity leads to tryptophan catabolism, thus decreasing the intracellular concentration of tryptophan available for chlamydiae (3). *C. trachomatis* and *C. pneumoniae* can be rescued from IFN-γ-treated or tryptophan-depleted cell cultures through the addition of extracellular tryptophan (3, 28, 53), but IFN-γ may also induce or inhibit apoptosis of eukaryotic cells in the absence of infection (7, 34, 57). In order to distinguish between the effects of IFN-γ on host cell apoptosis and chlamydial development, apoptosis of IFN-γ-treated infected cells was measured in the presence and absence of exogenous tryptophan. IFN-γ by itself (at 2 ng/ml) or with tryptophan in the absence of infection had no effect on survival of the host cells. However, reversal by tryptophan of the effect of IFN-γ in infected cells suggests that IDO activity is responsible for the IFN-γ-dependent inhibition of apoptosis observed during *C. trachomatis* infection of human host cells.

The increase in apoptosis observed in the genital tracts of infected IFN-γ-deficient mice may also have been due to their inability to induce IDO. Unlike the case for infected human host cells (3, 4), the anti-*C. muridarum* activity of IFN-γ in murine host cells was not dependent on induction of IDO and tryptophan catabolism in vitro (12). However, in a murine model of *C. pneumoniae* infection, IFN-γ was necessary for accumulation of IDO transcripts in the lung (46), and in a murine model of malaria, IDO was induced in wild-type mice

but not in IFN-γ-deficient mice (47). It is thus likely that IDO activity may also be responsible for IFN-γ-dependent inhibition of apoptosis in the murine genital tract.

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Editor: S. H. E. Kaufmann