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Effect of Interferon on the Growth of *Chlamydia trachomatis* in Mouse Fibroblasts (L Cells)

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The effect of murine interferon on the growth of the lymphogranuloma venereum biotype of *Chlamydia trachomatis* (strain 440L) in murine fibroblasts (L cells) was examined. Treatment of infected cell cultures with interferon caused a reduction in the number of inclusion-bearing cells as seen by light and electron microscopy and a decrease in yields of chlamydiae as determined by infectivity assays. Interferon also inhibited cycloheximide-resistant (*chlamydia*-specific) protein synthesis in infected cells. The interferon effect was dose dependent, with 80 to 90% inhibition occurring at concentrations of greater than 200 IU/ml. The inhibitory effect was neutralized by anti-murine interferon globulin. Interferon did not inactivate extracellular chlamydiae, and both host cell RNA and protein synthesis were required for the development of the interferon-induced antichlamydial state. Inhibition of chlamydial growth by interferon was demonstrable in cells treated 18 h before infection or up to 4 h after infection. Cells infected after interferon was removed exhibited an antichlamydial activity decline which was complete by 30 h after interferon removal. We show that interferon treatment did not affect either entry of chlamydiae into host cells or chlamydial conversion to reticulate bodies but rather caused a reduction in the rate of reticulate body replication.

The first report of interferon (IFN) acting against an infectious agent more complex than a virus was that of Sueltenfuss and Pollard (23). Using an acridine orange-fluorescence staining technique, they demonstrated the inhibition of *Chlamydia psittaci* maturation in IFN-treated cell cultures. A similar effect has been observed by immunofluorescence staining (15). In other in vitro studies, the IFN effect has been demonstrated by a reduction in the number of chlamydial intracytoplasmic inclusions (8, 9, 12, 13) and by a decrease in infectivity titers in IFN-treated cells (13). IFN has also been shown to inhibit the multiplication of chlamydiae in ovo (18).

In the present study, the inhibitory effect of murine IFN (MuIFN) on chlamydial growth was studied by inclusion counting, electron microscopy, infectivity assays, and measuring the incorporation of [^{3}H]leucine into chlamydial protein. The objectives of these studies were to (i) define host cell metabolic activities which are required for IFN action, (ii) describe the characteristics of the antichlamydial state, and (iii) determine which stage of chlamydial development is inhibited by IFN. The results demonstrate a number of similarities between the antichlamydial and antiviral actions of IFN and suggest that in IFN-treated cells, chlamydial development is restricted at the reticulate body stage.

MATERIALS AND METHODS

Growth of host cells. L929 mouse fibroblasts were grown in plastic flasks containing medium 199, supplemented with 0.1% sodium bicarbonate, 2 mM L-glutamine, and 10% heat-inactivated fetal bovine serum (FBS; Flow Laboratories, Inc.). Medium 199 is hereafter referred to as growth medium. In most experiments, 100 μg of gentamicin sulfate per ml was added. HeLa 229 cells were cultivated in RPMI 1640 medium containing 10% FBS. Cells were counted in a hemacytometer, and viability was determined by trypan blue exclusion (17).

Growth of chlamydiae. The lymphogranuloma venereum biotype of *C. trachomatis* (strain 440L) was provided by James W. Moulder, University of Chicago. The chlamydial inoculum was added to confluent monolayers of HeLa cells (1×10^7 to 2×10^7 cells per 75-cm^2 flask) and allowed to adsorb to the cells for 1 h at 37°C. RPMI 1640 medium containing 10% FBS and 2 μg of cycloheximide per ml was then added to increase yields of chlamydiae (10). The monolayers were reincubated for 48 h, and the medium was then decanted and held at 4°C. The cells were removed from the substrate with 0.2% trypsin (1:250; Difco

Laboratories), and all subsequent operations were carried out at 4°C. The cells and decanted medium were combined and centrifuged at 500 × *g* for 10 min, and the supernatant fluid was decanted and saved. The sedimented cells were suspended in 1 to 2 ml of Dulbecco phosphate-buffered saline (PBS) and disrupted by ultrasonic treatment for 10 min. The lysate was combined with the 500 × *g* supernatant and centrifuged at 10,000 × *g* for 20 min. The sedimented chlamydiae were suspended in sucrose-phosphate buffer (4) containing 2% FBS and centrifuged at 500 × *g* for 5 min to remove cell debris. Samples of the *C. trachomatis*-containing supernatant fluid were then stored at −70°C. Chlamydiae were titrated in L cells, and infectivity was measured as the 50% infective dose (ID_{50}) for 5 × 10^6 L cells, as described by Hatch (10). The inoculum used to prepare chlamydial harvests was diluted in medium so that 1 ml contained 10 ID_{50} of chlamydiae. One ID_{50} per host cell is equal to 0.7 infectious chlamydiae per host cell (14).

IFN. MuIFN was prepared by inducing confluent monolayers of L-cell fibroblasts (Lpa strain) with the synthetic polyribonucleotide polyinosinic-polycytidylic acid, which was purchased from P-L Biochemicals, Inc. Murine Lpa cultures were induced for a 6-h period with polyinosinic-polycytidylic acid (20 μg/ml) complexed with DEAE-dextran (400 μg/ml) in Eagle minimal essential medium (MEM) at 37°C under 5% CO_2. At the end of the incubation period, the medium containing the inducing agent was removed, and the cells were washed three times with Hanks balanced salt solution and then reincubated with MEM containing 1% FBS (vol/vol) at 37°C for an additional 18 h. Titration of IFN antiviral activity was done by the micromethod of Armstrong (2) as modified by Havell and Vilček (11) with L cells (L929B strain) and vesicular stomatitis virus as the indicator virus. The MuIFN titer (antiviral units per milliliter) is expressed as the reciprocal of the highest dilution of the MuIFN preparation which protected 50% of the L929B fibroblasts from the cytopathic effect of vesicular stomatitis virus. The titers of the MuIFN used in these studies have been calibrated against an international MuIFN reference preparation (G-002-904-511) so that the potencies are given in international units per milliliter.

Anti-IFN anti-serum. Rabbit anti-murine L-cell IFN globulin (A-L Inf. no. 12, 6/15/73) was obtained from the Development and Applications Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md. The lyophilized antiserum was reconstituted in medium 199 so that the titer was 6,000 neutralizing units per milliliter. The neutralization titer was defined as the reciprocal of the highest dilution of antiserum which, when mixed with an equal volume of IFN (20 IU/ml), neutralized 50% of the antiviral activity. In the IFN neutralization experiments, a concentration of antiserum capable of neutralizing 2,400 IU of IFN was used.

Measurement of IFN effect: inclusion count method. L-cell monolayers containing 2 × 10^5 cells were grown on cover slips in 35-mm petri dishes. Cultures were incubated at 37°C for 2 to 3 h to allow the cells to attach and spread. MuIFN was diluted in growth medium and incubated with cells at 37°C for 18 h, unless otherwise indicated. Control cells were incubated with fresh growth medium. In general, the chlamydial inoculum was diluted so that the proportion of infected control cells was about 50%. Monolayers were then washed twice with PBS and infected with an appropriate chlamydial inoculum diluted in 0.5 to 1 ml of growth medium. After adsorption at 37°C for 1 h, fresh growth medium was added, and the cells were incubated an additional 22 to 24 h. The cover slips were then stained with Giemsa, and the percentage of inclusion-containing cells per 200 cells was determined. In some experiments, the IFN effect was expressed as percent inhibition: {[(percent infected, control) − (percent infected, IFN treated)]/(percent infected, control)} × 100.

Measurement of IFN effect on cycloheximide-resistant protein synthesis in infected L cells. The method used to measure *Chlamydia*-specific protein synthesis was based on the work of Alexander (1), in which cycloheximide was shown to inhibit host cell protein synthesis without interfering with parasite development. In the present study, confluent L-cell monolayers in 75-cm^2 flasks were incubated with either IFN or growth medium alone for 18 h at 37°C. The cells were then washed twice with PBS, treated with 0.2% trypsin, and resuspended in growth medium to a density of 10^6 cells per ml. Suspensions were incubated for 30 min at 37°C in a water bath-shaker, infected with chlamydiae, and incubated for an additional 90 min. Samples (1 ml) of the infected cell suspensions were transferred to sterile glass scintillation vials and incubated overnight at 37°C. The cells adhered to the bottom of the vials and formed monolayers. To facilitate incorporation of [^{3}H]leucine into chlamydial protein, the cells were exposed to 5 μg of cycloheximide per ml to inhibit host protein synthesis (1). After incubation at 37°C for 30 min, the medium was replaced with leucine-deficient MEM (GIBCO) containing 5% FBS, 1 μCi of [^{3}H]leucine per ml, and cycloheximide (5 μg/ml). The cells were reincubated for 3 h, washed three times with serum-free MEM, and fixed in absolute methanol. Samples were treated with 5 ml of 10% trichloroacetic acid (TCA) for 5 min, washed in two changes of 10% TCA, and dried overnight at room temperature. Toluene-based scintillant (5 ml) was added to each vial, and acid-insoluble radioactivity was measured in a liquid scintillation counter. Results were expressed as counts per minute per culture. The inclusion count method was performed in parallel to confirm IFN activity.

Measurement of IFN effect on chlamydial replication. The IFN effect on chlamydial infectivity was measured by methods similar to those described by Kazar et al. (13). L-cell monolayers in 25-cm^2 flasks (10^6 cells per flask) were exposed to IFN or growth medium alone for 18 h at 37°C. Cells were then washed twice with PBS and infected with a chlamydial suspension diluted in 1 ml of growth medium. After adsorption at 37°C for 1 h, additional growth medium was added and incubation was continued. At various intervals after the addition of chlamydiae, the cells were washed twice with PBS, treated with 0.2% trypsin, and resuspended to a density of 10^6 cells per ml. The infected cells were then disrupted by ultrasonic treatment for 5 min, and appropriate dilutions of the lysates were used to infect 2 × 10^5 L cells grown on cover slips. After incubation at 37°C for 24 h, cover slips were stained with Giemsa, and the percentage of infected cells per 200 cells was determined. Results were expressed as inclusion-forming units per milliliter of inoculum.

TABLE 1. Effect of IFN on cycloheximide-resistant protein synthesis in L cells infected with *C. trachomatis*[a]

Treatment of L cells[b]	cpm ($\times 10^3$)[c]
Untreated, uninfected	10.2 ± 1.6
IFN treated, uninfected	9.0 ± 1.3
Untreated, infected	21.4 ± 0.7
IFN treated, infected	8.3 ± 0.9

[a] L cells were infected with approximately 1 ID_{50} of *C. trachomatis*, as described in the text. The cells were exposed to cycloheximide (5 μg/ml) for 30 min and then treated with leucine-deficient MEM containing 5% FBS, [^{3}H]leucine (2 μCi/ml), and cycloheximide (5 μg/ml). After incubation for 3 h, triplicate samples were analyzed for [^{3}H]leucine incorporation into protein.

[b] Cells were incubated with growth medium alone or IFN (1,000 IU/ml) for 18 h before infection.

[c] Mean ± standard deviation.

Electron microscopy. L-cell monolayers (10^6 cells per 35-mm petri dish) were treated with growth medium or IFN (1,000 IU/ml) for 24 h at 37°C. The cells were then washed twice with PBS and infected with *C. trachomatis*. Samples to be examined at 3 h postinfection received 10 ID_{50} of chlamydiae per cell. The 18-h- and 42-h-postinfection samples received 3 ID_{50} of chlamydiae per cell. At the times specified, cells were washed twice with PBS, treated with 0.2% trypsin, and suspended in 2 to 3 ml of growth medium. Suspensions were centrifuged at 1,000 × *g* for 3 min, and the pellets were fixed for 60 min in 3% glutaraldehyde–0.1 M cacodylate buffer. Samples were then prepared for transmission electron microscopy by methods similar to those described by Friis (6). Microscopy was performed with a Philips 201 electron microscope, generously made available by the New York Blood Center. The inclusion count method was carried out in parallel to confirm IFN activity.

Isotopes. [2-^{14}C]uridine (60 mCi/mol), L-[1-^{14}C]leucine (54 mCi/mol), and L-[4,5-^{3}H;^{15}N]leucine (52 mCi/mol) were purchased from New England Nuclear Corp.

RESULTS

Effect of IFN on growth of *C. trachomatis* in L cells. The inclusion count method was used to determine the effect of IFN (1,000 or 300 IU/ml) on the growth of the lymphogranuloma venereum biotype of *C. trachomatis*. Preliminary experiments, in which host cells were treated with either dose of IFN for 18 h before infection, showed an 80 to 90% reduction in numbers of infected cells. This level of inhibition was consistently observed when multiplicities as low as 0.1 ID_{50} of chlamydiae per host cell or as high as 3 ID_{50} of chlamydiae per host cell were used. These results were corroborated by measuring the effect of IFN on cycloheximide-resistant protein synthesis in infected L cells. Untreated and IFN-treated L cells were infected with chlamydiae and incubated overnight in glass scintillation vials. Cycloheximide-resistant counts were measured 3 h after the addition of [^{3}H]leucine. Preliminary experiments revealed that 5 μg of cycloheximide per ml reduced incorporation of labeled leucine by uninfected L cells 90 to 94%. In the absence of cycloheximide, 1,000 IU of MuIFN per ml suppressed protein synthesis in uninfected L cells about 20%. The increased incorporation seen in infected control cells represented *Chlamydia*-specific (cycloheximide-resistant) protein synthesis (Table 1). Pretreatment of cells with 1,000 IU of IFN per ml reduced parasite protein synthesis to background levels. Inclusion counts performed in parallel showed a 92% reduction in numbers of infected IFN-treated cells.

Dose-response curve for IFN. To test the effect of various concentrations of IFN on the growth of chlamydiae, we incubated L cells with medium alone or IFN-containing medium for 18 h before infection. Treatment with 10,000, 1,000, or 100 IU of IFN per ml reduced the number of inclusion-containing cells 95, 92, and 77%, respectively (Fig. 1). A 50% inhibition corresponded to about 25 IU of IFN per ml. At 10 IU/ml, the IFN effect was negligible.

To determine whether IFN inactivated chlamydiae directly, chlamydial harvests were incubated in IFN-containing medium (300 IU/ml), collected by centrifugation, and added to L-cell suspensions, which were subsequently plated on cover slips. No loss of infectivity was observed when preparations incubated with IFN were compared with preparations incubated in growth medium alone (data not shown).

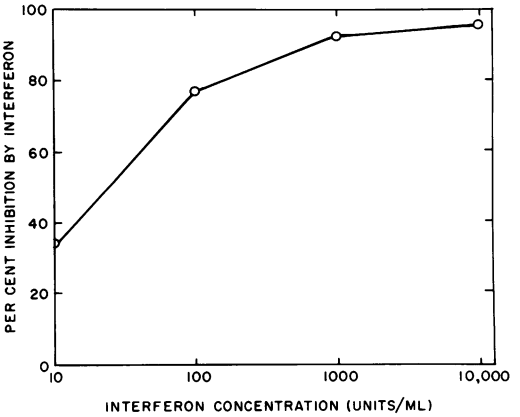

FIG. 1. Dose-response curve for IFN. L-cell monolayers (2 × 10^5 cells per cover slip) were incubated for 18 h with growth medium alone or IFN at the concentrations indicated. The cells were then washed and infected with *C. trachomatis*, and triplicate Giemsa-stained preparations were examined for chlamydial inclusions as described in the text.

TABLE 2. Neutralization of the inhibitory effect of IFN against *C. trachomatis* by anti-IFN antibody (Ab)

Treatment[a]	% of cells with inclusions	
	Expt 1[b]	Expt 2[c]
Growth medium	52	7 ± 1
IFN	6	1 ± 1
Ab	55	9 ± 1
IFN + Ab	60	7 ± 1

[a] Four preparations were assayed simultaneously for antichlamydial activity: 5 ml of growth medium, 5 ml of IFN (1,000 IU/ml), 0.2 ml of Ab + 5 ml of growth medium, and 0.2 ml of Ab + 5 ml of IFN (1,000 IU/ml). The mixtures were held at 37°C for 1 h with occasional shaking. Then 1 ml of the appropriate mixture was added to L-cell monolayers (2×10^5 cells per cover slip). After incubation for 18 h, cells were washed twice with PBS, infected, and incubated for an additional 24 h. Cover slips were stained and examined as described in the text.

[b] Mean counts of at least two samples. Chlamydial inoculum, 1 ID_{50}.

[c] Mean counts of at least three samples ± standard deviations. Chlamydial inoculum, 0.1 ID_{50}.

Neutralization of IFN activity by specific antiserum. To determine whether the antichlamydial effect of IFN could be neutralized by anti-IFN antibody, we treated cover slip cultures of L cells for 18 h with IFN (1,000 IU/ml) or a mixture of IFN and antibody at 37°C. A final concentration of antibody capable of neutralizing 2,400 IU of IFN per ml was used. The cells were then infected with either 1 ID_{50} (experiment 1) or 0.1 ID_{50} (experiment 2) of chlamydiae. The inhibitory effect of IFN against *C. trachomatis* was completely neutralized by anti-MuIFN immunoglobulin (Table 2).

Host cell requirements for IFN action. (i) Inhibition of IFN action by actinomycin D. Actinomycin D (2 μg/ml) was added to uninfected L-cell monolayers for 30 min at 37°C to inhibit RNA synthesis (24). The cells were then treated with 0.25 μCi of [^{14}C]uridine per ml for 45 min. Incorporation of [^{14}C]uridine into RNA was determined after precipitation with cold 10% TCA. Acid-insoluble counts were collected on membrane filters as described by Alexander (1), and radioactivity was measured in a liquid scintillation spectrophotometer. Actinomycin D inhibited L-cell RNA synthesis by 72%, compared with synthesis in untreated control cells. To determine whether host cell RNA synthesis is required for the inhibitory effect of IFN against *C. trachomatis*, chlamydial inclusions were counted in L cells which had been treated with actinomycin D before incubation with IFN. Inhibition of chlamydiae by IFN was partially reversed in cells which had been exposed to the drug (Table 3). This suggested that host cell RNA synthesis was necessary for IFN action. The incomplete reversal was probably due to incomplete shutoff (72%) of host RNA synthesis by the drug. Actinomycin D is not selective in its inhibitory action and, despite careful washing of cells before infection, some inhibition of chlamydiae was apparent in cells treated with the drug alone.

TABLE 3. Effect of actinomycin D on IFN-induced inhibition of *C. trachomatis*

Pretreatment of L cells		% of cells with inclusions[c]
IFN[a]	Actinomycin D[b]	
−	−	79
−	+	56
+	−	10
+	+	34

[a] +, Cells were incubated with IFN (1,000 IU/ml) for 5 h before infection. −, No IFN added.

[b] +, Cells were incubated for 30 min with actinomycin D (2 μg/ml), then washed and incubated with growth medium or IFN for 5 h before infection. −, No actinomycin D added.

[c] Average of two samples.

(ii) Inhibition of IFN action by cycloheximide. Uninfected L-cell monolayers were exposed to cycloheximide (5 μg/ml) for 18 h at 37°C to inhibit protein synthesis. The cells were then incubated with [^{14}C]leucine (0.25 μCi/ml) for 45 min at 37°C. Incorporation of [^{14}C]leucine into protein was determined after precipitation with cold 10% TCA. Results demonstrated that cycloheximide reduced L-cell protein synthesis 84%, compared with synthesis in untreated control cells. The effect of the drug on the antichlamydial action of IFN is shown in Table 4. L cells that were treated with both IFN and cycloheximide before infection completely lost IFN-mediated antichlamydial activity.

Characteristics of the antichlamydial state. (i) Kinetics of development of the IFN effect. L-cell monolayers were incubated with 1,000 IU of

TABLE 4. Inhibition of IFN-induced antichlamydial effect by cycloheximide

Pretreatment of L cells		% of cells with inclusions[c]
IFN[a]	Cycloheximide[b]	
−	−	22
−	+	20
+	−	7
+	+	23

[a] +, Cells were incubated with IFN (300 IU/ml) for 18 h before infection. −, No IFN added.

[b] +, Cells were incubated with cycloheximide (5 μg/ml) for 18 h before infection. −, No cycloheximide added.

[c] Average of two samples.

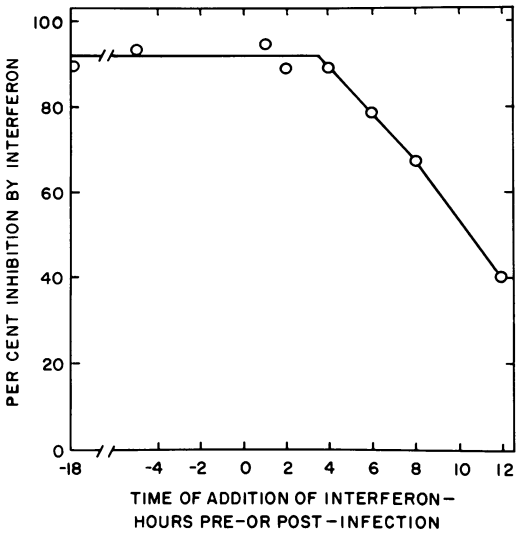

FIG. 2. Kinetics of induction of the IFN effect against *C. trachomatis* in L cells. L-cell monolayers (2×10^5 cells per cover slip) were infected with *C. trachomatis* at time zero. IFN (1,000 IU/ml) was added at the times indicated, and triplicate Giemsa-stained preparations were examined for chlamydial inclusions as described in the text.

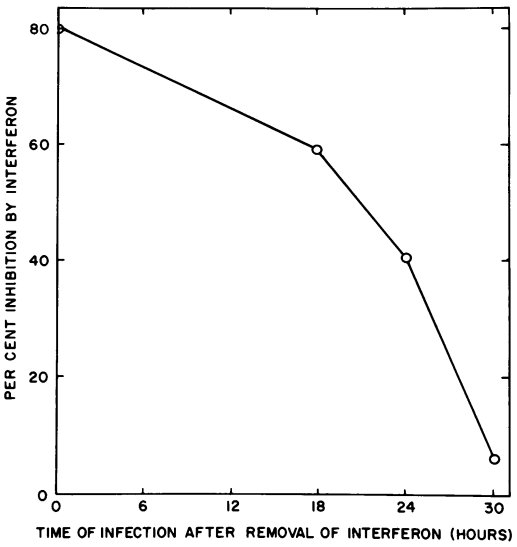

FIG. 3. Decay of the IFN-induced antichlamydial state in L cells. L-cell monolayers (2×10^5 cells per cover slip) were incubated with growth medium alone or IFN (1,000 IU/ml) for 18 h. IFN was removed at time zero, and *C. trachomatis* was added at the times indicated. Triplicate Giemsa-stained preparations were then examined for chlamydial inclusions as described in the text.

IFN per ml for various times before and after infection with chlamydiae (Fig. 2). When IFN was added 18 or 5 h before infection or 4 h after infection, chlamydial growth was inhibited at least 89%. There was a marked decrease in inhibitory effect when IFN was added later than 4 h after infection.

(ii) Duration of the antichlamydial state. IFN-induced antiviral activity is known to decline after IFN is removed from cells (21). We determined that a similar decrease in antichlamydial activity occurred. L cells were infected with chlamydiae at various intervals after IFN was removed. A decrease in protection was observed after 18 h of incubation in IFN-free medium, and by 30 h the cells were completely susceptible to chlamydial infection (Fig. 3).

Effect of IFN on the chlamydial developmental cycle. Kazar et al. (13) studied chlamydial development in IFN-treated L cells by measuring infectivity at various times during the growth cycle. Their results suggested that IFN exerts its effect at an early stage in chlamydial development. To determine whether transformation of elementary bodies to reticulate bodies proceeded normally in IFN-treated cells, we assayed for the presence of infectious forms at various times after infection. L-cell monolayers (10^6 cells per flask) were incubated for 18 h with growth medium alone or IFN (1,000 IU/ml). The cells were then infected with 10 ID_{50} of *C. trachomatis*, and yields of infectious elementary bodies from cells harvested at 2, 12, and 24 h postinfection were determined. Inclusion counts performed in parallel showed that IFN treatment reduced the proportion of infected cells 89%. Similar infectivity titers were found in control and IFN-treated cells 2 h postinfection (Table 5). In IFN-treated cells at 12 h postinfection, we found a chlamydial infectivity decrease comparable to that observed in control cells. By 24 h postinfection, a rise in infectivity was observed in control cultures. Infectivity also increased in IFN-treated cells at this time; however, yields were greatly diminished.

IFN treatment of L cells did not prevent the immediate toxicity of high multiplicities of chlamydiae (data not shown). The phenomenon of immediate toxicity (19) requires parasite uptake; thus, our observation provides additional evidence that IFN did not inhibit ingestion of chlamydiae.

Electron microscopic observation of chlamydial development in control and IFN-treated L cells. To determine at which stage of the developmental cycle IFN exerted its antichlamydial effect, we treated L cells for 24 h with growth medium alone or medium with IFN (1,000 IU/ml). The cells were then infected and examined by electron microscopy at 3, 18, and 42 h after infection.

TABLE 5. Effect of IFN on production of infectious *C. trachomatis* in L cells[a]

Time after addition of chlamydiae (h)	Yield of chlamydiae (inclusion-forming units/ml of inoculum)[b]	
	Control	IFN-treated
2	$3.7 \times 10^4 \pm 0.3 \times 10^4$	$4.0 \times 10^4 \pm 1.1 \times 10^4$
12	$4.0 \times 10^3 \pm <0.1 \times 10^3$	$1.3 \times 10^3 \pm 2.3 \times 10^3$
24	$2.0 \times 10^5 \pm 0.3 \times 10^5$	$5.5 \times 10^4 \pm 2.3 \times 10^4$

[a] L-cell monolayers (10^6 cells per flask) were incubated with growth medium alone (control) or IFN (1,000 IU/ml) for 18 h. The cells were then infected with 10 ID_{50} (7×10^6 inclusion-forming units) of *C. trachomatis*, and at the times indicated, yields of chlamydiae were estimated as described in the text.

[b] Mean of three samples ± standard deviation.

The characteristic morphology of chlamydial developmental forms is illustrated in Fig. 4. At 3 h postinfection, elementary bodies were observed at about the same frequency in both control and IFN-treated cells (Fig. 4A and B). At 18 h postinfection, the proportion of inclusion-containing IFN-treated cells was markedly smaller than that of control cells, although the chlamydiae within the IFN-treated cells were normal in appearance (Fig. 4C and D). This was also the case at 42 h postinfection (Fig. 4E and F). Differences in numbers of individual chlamydiae per vacuole in control and IFN-treated samples were quantified by direct counting (Table 6). No elementary bodies or transitional forms were seen in 18-h IFN-treated cells, and the average number of chlamydiae per vacuole was less than half that in the 18-h control. In IFN-treated cells, 66% of the vacuoles contained less than 10 chlamydiae (Fig. 5). In contrast, 78% of the control cell vacuoles contained 10 or more chlamydiae. The presence of reticulate bodies and the reduced number of chlamydiae per vacuole observed in IFN-treated cells provided substantial evidence that replication and not differentiation is affected by IFN-mediated host cell activation.

DISCUSSION

IFN-treated cells develop a state of resistance to the replication of virus and a wide variety of nonviral intracellular parasites (22). In this study, we examined the action of IFN on the multiplication of the lymphogranuloma venereum biotype of *C. trachomatis* in murine fibroblasts.

Infectious chlamydial elementary bodies enter host cells by an endocytic process. Their entire intracellular development is restricted within the confines of a membrane-bound cytoplasmic vesicle. Intracellular elementary bodies differentiate, forming reticulate bodies. This process is completed by 6 h after infection. Reticulate bodies, capable of their own macromolecular synthesis, replicate by binary fission. Replication of reticulate bodies continues until about 20 h after infection, when a second round of reorganization occurs and elementary bodies begin to appear. The entire cycle is completed after about 48 h (depending on the particular chlamydial strain and host cell). Host cell lysis then occurs, and infectious chlamydial progeny are released (16). The results of our infectivity assays and electron microscopic studies indicated that IFN did not affect the entry of chlamydiae into host cells. These results are in accord with those reported by Kazar et al. (13). They reported that in Giemsa-stained, IFN-treated cells, chlamydiae persist as elementary bodies, and the normal sequence of development occurs in only 5% of the cells. Kazar et al. suggested that IFN inhibits the conversion of elementary bodies to reticulate bodies. Sueltenfuss and Pollard (23), however, reported that differentiation of elementary bodies does occur in IFN-treated chicken embryo cells, as evidenced by characteristic staining with acridine orange. We observed a decrease in yields of infectious forms from IFN-treated cells at 12 h after infection. Therefore, either differentiation had occurred or the elementary bodies were rendered noninfectious in these cells. When IFN-treated infected cells were examined by electron microscopy, only reticulate bodies were seen 18 h after infection. There were also far fewer inclusion-bearing, IFN-treated cells, and inclusions that were observed contained significantly fewer individual chlamydiae. These data clearly support the view that IFN-induced antichlamydial activity resulted in an overall reduction in the rate of chlamydial replication.

Our results also showed that the antichlamydial effects of IFN were similar in some respects to the antiviral activity induced by IFN. The dose-response relationships, typically sigmoidal with a linear portion over a range corresponding to 25 to 75% inhibition in viral systems, were also observed for *C. trachomatis* inhibition in IFN-treated cells. We found that 80 to 90% inhibition occurred when 200 IU/ml was used. For 50% inhibition of chlamydial growth, 25 IU/ml was required. Since, by definition, 1 IU/ml results in a 50% inhibition of virus replica-

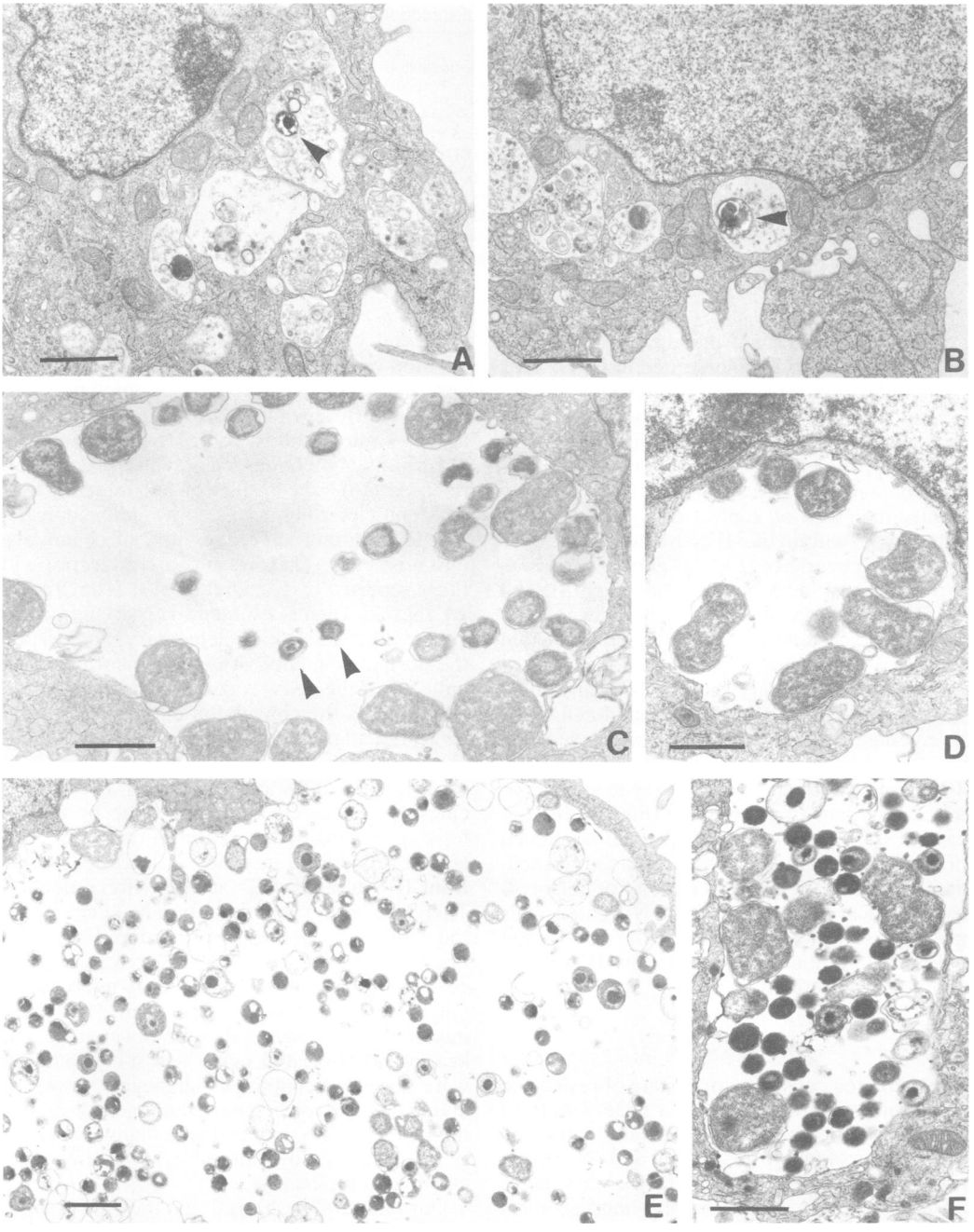

FIG. 4. Electron micrographs of L cells infected with *C. trachomatis*. (A) Untreated control, 3 h after infection; (B) IFN treated, 3 h after infection; (C) untreated control, 18 h after infection; (D) IFN treated, 18 h after infection; (E) untreated control, 42 h after infection; (F) IFN treated, 42 h after infection. The small, dense elementary body is the infectious form, whereas the larger, less rigid reticulate body is the replicative form. Arrows indicate transitional forms. Bars, 1 μm.

tion, chlamydiae were 25 times less sensitive than virus to the activity induced by IFN. Other studies (8, 15) also reported that chlamydiae are 1 to 2 logs less sensitive to the action of IFN than is virus. Chlamydiae are significantly more complex than viruses. They are capable of using their own ribosomes for macromolecular synthesis and are bounded by both a membrane and an

TABLE 6. Numbers of chlamydial forms present 18 h after infection in IFN-treated and control L cells

Treatment[a]	No. of chlamydial forms[b]			Avg no. of chlamydiae/ vacuole
	Elementary body	Transitional form	Reticulate body	
Growth medium	5	12	904[c]	18
IFN	0	0	428[c]	9

[a] L-cell monolayers (10^6 cells per dish) were incubated with growth medium or IFN (1,000 IU/ml) for 24 h before infection with *C. trachomatis*.

[b] Number of chlamydiae per 50 cytoplasmic vacuoles as seen by transmission electron microscopy.

[c] These values were significantly different ($P < 0.001$).

outer envelope. Their decreased sensitivity to the action of IFN may therefore be related to their decreased dependency on the host cells (13).

The virus growth suppression mediated by IFN is not due to direct inactivation of the virus (21). Similarly, IFN did not reduce the infectivity of extracellular elementary bodies (8, 18, 23). Thus, just as in viral systems, IFN activated host cells so that they no longer supported significant chlamydial growth. The development of antiviral activity in IFN-treated cells requires the synthesis of protein and RNA, and several de novo proteins are produced that act to inhibit virus replication (7). Our results indicated that a similar series of events may be involved in the development of the antichlamydial state. Actinomycin D and cycloheximide each interfered with IFN-mediated antichlamydial activity. If novel proteins are produced in IFN-treated cells to directly inhibit chlamydiae, then these molecules must penetrate not only the chlamydial wall and membrane but also the cytoplasmic inclusion membrane, a structure that sequesters chlamydiae and prevents them from direct contact with host cell cytoplasm. These formidable barriers are not encountered in virus-infected cells, and this fact alone is sufficient to suggest that IFN mediates other, as yet unknown, antimicrobial activities.

In virus systems, the effect of IFN is generally greater when cells are exposed to IFN for longer periods of time before virus challenge. Some inhibition may occur when IFN is added after the addition of virus, but inhibition is usually reduced, compared with inhibition in pretreated cells (22). We found that when IFN was added to L cells 5 to 18 h before or up to 4 h after infection, significant inhibition of chlamydial growth occurred. The inhibitory effect decreased when IFN was added later than 4 h after infection. The effects of IFN activation were no longer demonstrable in cells infected 30 h after removal of IFN.

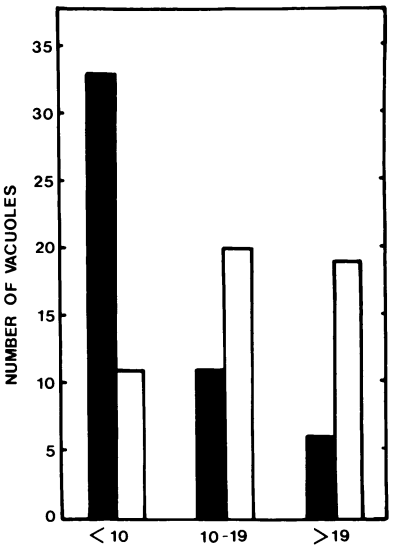

FIG. 5. Direct counting by electron microscopy of chlamydiae within control (open bars) and IFN-treated (closed bars) L cells. L-cell monolayers (10^6 cells) were incubated for 24 h with growth medium or IFN (1,000 IU/ml). The cells were then washed and infected with *C. trachomatis*, and at 18 h postinfection, 50 vacuoles per sample were examined to determine numbers of chlamydiae per vacuole.

The *Chlamydia*–L-cell model is well suited for the study of the biochemical basis of IFN action. A procaryote-eucaryote system is particularly useful because selective inhibition of host or parasite metabolic activities can readily be achieved. This circumvents the limitation encountered in virus systems, in which inhibition of essential host cell functions may result in termination of the infection (5).

It has been established (3) that the IFN system is an important determinant in certain persistent viral infections. There is no information regarding the role of IFN in naturally occurring chlamydial infections. Persistence is a common feature of all chlamydial infections (20), and since chlamydiae both induce (22) and are inhibited by IFN, it is likely that this potent defense mechanism acts to modulate the interaction between chlamydiae and their hosts.

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