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Interferon-Induced Inhibition of *Chlamydia trachomatis*: Dissociation from Antiviral and Antiproliferative Effects†

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The yield of infectious *Chlamydia trachomatis* was analyzed in human (HeLa) and mouse (McCoy) cell lines treated with the human interferon (IFN) subtypes IFN-αA and IFN-αD, with their hybrids [IFN-αAD (*Bgl*II), IFN-αAD (*Pvu*II), and IFN-αDA (*Bgl*II)] constructed in vitro from their expression plasmids, or with IFN-β_1 or buffy coat IFN. In HeLa cells, a significant inhibition of *Chlamydia* infectivity was obtained with IFN-αD, IFN-αDA (*Bgl*II), and buffy coat IFN. In McCoy cells, IFN-αAD (*Bgl*II) and IFN-αAD (*Pvu*II) induced a strong degree of inhibition of *Chlamydia* infectivity. In McCoy cells, there was a correlation among the antichlamydial, antiviral, and antiproliferative activities of the different IFNs tested. In HeLa cells, however, the ability of a particular IFN subtype to inhibit *Chlamydia* infectivity did not always correlate with its inhibitory effects on encephalomyocarditis virus replication or with its antiproliferative activity.

Chlamydia spp., the obligate intracellular parasites which replicate by binary fission within intracytoplasmic inclusions, are among the most common pathogens in the animal kindgom (19). This class of organisms is one of the most frequently encountered in sexually transmitted diseases in humans (18). In 1963, Sueltenfuss and Pollard used *Chlamydia psittaci* as an indicator agent for the assay of interferon (IFN) induced by duck hepatitis virus in chicken embryos, providing the first evidence that IFN could inhibit the replication of pathogens more complex than viruses (22). Since then, several reports have shown that crude mouse IFN preparations can decrease the yield of *C. trachomatis* in mouse systems, as measured by infectivity titrations (12–14).

In the present study, we compared the abilities of human IFN subtypes IFN-αA and IFN-αD and their molecular hybrids constructed from their respective expression plasmids (16, 21, 23) to inhibit the infectivity of *C. trachomatis* in homologous (human) and heterologous (mouse) cells. The antichlamydial activities of human IFN-β_1 and buffy coat IFN were also examined in both cell systems. We correlated our findings with the antiviral activities of the different IFN preparations and compared them with previous results on the antiproliferative activities of these IFNs (4).

MATERIALS AND METHODS

Cell lines. HeLa 229 cells were obtained from the American type Culture Collection, Bethesda, Md., and grown in Eagle minimal essential medium supplemented with 5% fetal calf serum. McCoy cells were purchased from Flow Laboratories, Inglewood, Calif., and grown in the same medium at 37°C in a CO_2 incubator.

C. trachomatis. *C. trachomatis* LGV-1(440), obtained from Julius Schachter (Hooper Foundation, San Francisco, Calif.), was used throughout this study. This strain was prepared in HeLa 229 cells grown in Eagle minimal essential medium containing 5% fetal calf serum and gentamicin.

IFNs. The human IFNs (Fig. 1) IFN-αA, IFN-αD, IFN-αAD (*Bgl*II), IFN-αAD (*Pvu*II), IFN-αDA (*Bgl*II), and IFN-β_1 were synthesized in *Escherichia coli* (7–9, 23) and were >95% pure, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Natural buffy coat IFN was obtained from Sendai virus-induced human peripheral blood leukocytes. Titers of IFN-α preparations were determined by measuring the inhibition of the cytopathic effect on bovine kidney (MDBK) cells challenged with vesicular stomatitis virus and are expressed as International Units, based on the human IFN-α research reference standard, G023-9010-527. The specific molecular activities of the bacterium-derived IFN-α preparations were ca. 1×10^8 to 2×10^8 U/mg of protein on bovine cells, whereas that of natural buffy coat IFN was ca. 10^6 U/mg of protein. Human IFN-β_1 was similarly assayed on HeLa cells challenged with vesicular stomatitis virus and standardized against the human IFN-β research reference standard, G023-902-527. The specific activity of IFN-β_1 was ca. 1×10^8 to 2×10^8 U/mg of protein.

EMCV assays. McCoy or HeLa cell cultures were treated with IFN for 24 h and then infected with encephalomycarditis virus (EMCV) (multiplicity of infection, 10). After virus adsorption, cultures were washed with phosphate-buffered saline to remove nonadsorbed inoculum, and fresh medium was added. Viruses were harvested 24 h after infection, and titers from duplicate samples were determined by a plaque assay on L-929 cells. The results are presented as the $\log_{10}$ percentage of virus controls (virus from cells receiving no IFN treatment).

Assays of antiproliferative activity. HeLa 229 and McCoy cells were seeded into 24-well plates (Becton Dickinson Labware, Oxnard, Calif.) at a density of 4×10^3 to 6×10^3 cells per well. Dilutions of the IFNs were added 24 h after the cells were seeded. After six cycles of replication of the control cells, the monolayer was washed twice with phosphate-buffered saline, trypsinized, suspended in Isotonic II (Coulter Electronics, Inc., Hialeah, Fla.), and counted with an automated cell counter (model D2N; Coulter) (4).

RESULTS

Inhibition of *C. trachomatis* infectivity. Both HeLa and McCoy cells are commonly used to isolate *C. trachomatis*

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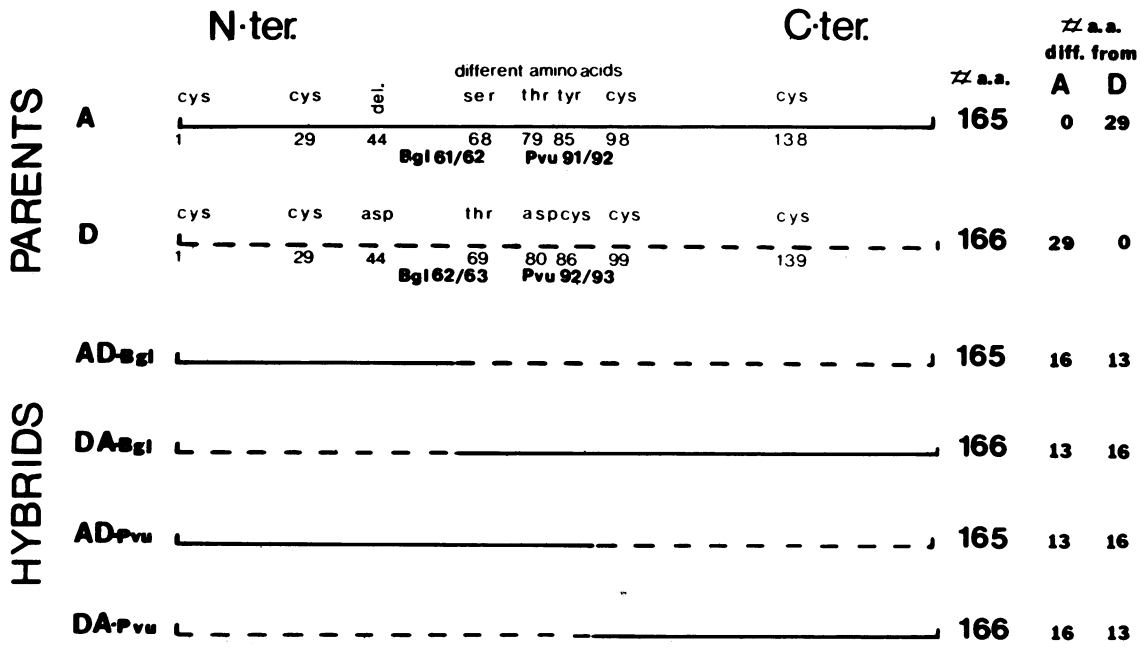

FIG. 1. Schematic representation of IFN-αA and IFN-αD as well as the hybrid IFNs: IFN-αAD (*Pvu*II and *Bgl*II) and IFN-αDA (*Pvu*II and *Bgl*II) constructed from them. Between the *Bgl* and *Pvu* cleavage sites, the products of the parent genes of IFN-αA and IFN-αD differ only at positions 68, 79, and 85 for IFN-αA and 69, 80, and 86 for IFN-αD. N · ter, N terminus; C · ter, C terminus; del., deletion of one amino acid residue at position 44 in IFN-αA; #a.a., number of amino acids; diff., different. This figure is reprinted, with permission, from reference 4.

because they are highly sensitive to infection with this microorganism. McCoy cells, originally thought to have been derived from a human synovium, now appear to be a subclone of mouse L-cells (2). To determine the effect of IFN on the infectivity of *C. trachomatis*, seeded HeLa or McCoy cells onto cover slips in 1-dram (15 by 45 mm) glass vials, and after 3 h of incubation at 37°C, increasing concentrations of the different IFN preparations were added. After 24 h, the cells were infected with *C. trachomatis* stock by centrifuging the inoculum for 1 h at 1,000 × *g* at 30°C. A dilution of microorganism was used so that ca. 20 to 30% of the HeLa and McCoy control monolayers showed chlamydial inclusions at 72 h on passage 2. The inoculum was adjusted in this manner to avoid possible differences in the ability of the two cell types to support the growth of chlamydiae. Medium containing IFN was added to the cultures immediately after centrifugation, and after 48 h of incubation, the cells were disrupted by being frozen, thawed, and vortexed with sterile glass beads. By adding the IFNs for 24 h before infection and for 48 h after infection, we attempted to eliminate any transient IFN-induced effects. From each test and control vial (receiving no IFN treatment), 0.1 ml was used to inoculate fresh monolayers of HeLa or McCoy cells. The cells were reincubated at 37°C for 72 h; the medium was removed, and the monolayers were stained with iodine. Percentages were calculated by dividing the number of *C. trachomatis* inclusions in IFN-treated cultures by the number of *C. trachomatis* inclusions in cultures receiving no IFN treatment and multiplying by 100. Samples were assayed in duplicate, and each IFN preparation was tested at least three times.

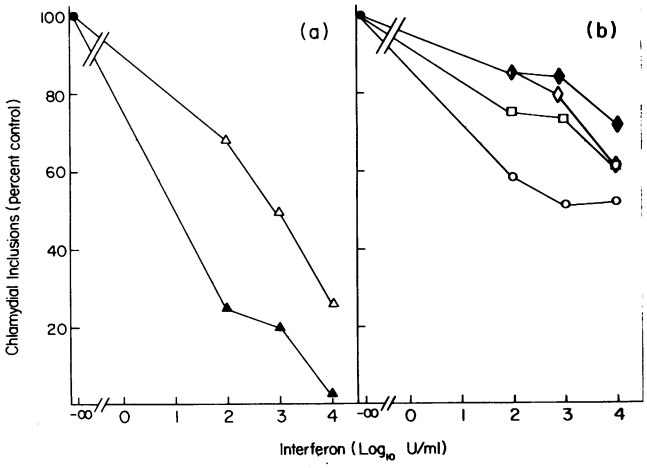

FIG. 2. IFN-induced inhibition of *C. trachomatis* in (a) McCoy cells and (b) HeLa cells. Cell cultures were treated with different IFNs for 24 h and then infected with *C. trachomatis* as described in the text. After 48 h of incubation in the presence of IFN, cells were harvested and disrupted, and infectious yields were determined by infecting new monolayers of McCoy and HeLa cells. The number of inclusions in the IFN-treated monolayers was expressed as a percentage of that in the control cultures. Shown are the yields from cells treated with IFN-αD (○), IFN-αAD (*Bgl*II) (▲), IFN-αAD (*Pvu*II) (△), IFN-αDA (*Bgl*II) (□), IFN-β_1 (◆), or buffy coat IFN (◇). (Only IFNs that inhibited the yields of chlamydiae are shown.)

Of the IFNs examined, only IFN-αAD (*Bgl*II) and IFN-αAD (*Pvu*II) inhibited the yield of infectious *C. trachomatis* in McCoy cells (Fig. 2a and Table 1). The most significant effect was obtained with IFN-αAD (*Bgl*II). A dose of ca. 10 U of IFN-αAD (*Bgl*II) per ml resulted in 50% inhibition, whereas 10^3 U of IFN-αAD (*Pvu*II) per ml was required to obtain a similar inhibition. No inhibition was observed when cells were treated with the other IFN preparations, even at concentrations of up to 10^4 U/ml.

When HeLa cells were treated with either IFN-β_1 or human buffy coat IFN, which is a mixture of IFN-α species,

C. trachomatis infectivity was inhibited (Fig. 2b and Table 2). The two IFN-α subtypes, however, demonstrated differential antichlamydial activity. IFN-αA had no effect even at 10^4 U/ml, whereas IFN-αD was most active, although the effect appeared to plateau at ca. 50% inhibition. Of the hybrid IFN species tested, only IFN-αDA (*Bgl*II) inhibited *C. trachomatis* in this cell system.

Antiviral activity. The relative antiviral activities of the IFN preparations were assessed by single-cycle EMCV yield reduction assays. Only IFN-αAD (*Bgl*II) and IFN-αAD (*Pvu*II) effectively inhibited EMCV replication in McCoy cells (Fig. 3a and Table 1). IFN-αD had a slight antiviral activity, because treating cells with 10^4 U/ml resulted in ca. 0.7-log reduction in EMCV yield. At the highest concentration tested (10^4 U/ml), buffy coat IFN, IFN-β_1, IFN-αDA (*Bgl*II), and IFN-αA each inhibited EMCV yield by ca. 0.5 log. These results are consistent with those in previous reports on the antiviral activities of human IFN-α preparations in mouse L-cells (16, 23). All of the IFN preparations that were tested effectively protected HeLa cells against EMCV infection (Fig. 3b and Table 2). The relative potencies, however, were not equivalent.

Antiproliferative activity. The effects of IFN treatment on the growth of McCoy and HeLa cells have been previously described (4). These results are summarized in Tables 1 and 2. At the highest concentration tested (10^4 U/ml), IFN-αAD (*Bgl*II) and IFN-αAD (*Pvu*II) inhibited the growth of McCoy cells by 49 and 40%, respectively, whereas the same concentration of IFN-αD resulted in a 28% inhibition of cell growth. The growth of HeLa cells was inhibited by treatment with each of the IFN preparations; however, the degree of inhibition varied. IFN-β_1 was the most active, resulting in 84% inhibition, whereas the same concentration of IFN-αDA (*Bgl*II) inhibited cell growth by only 17%. Treatment with the other IFNs resulted in intermediate degrees of cell growth inhibition.

DISCUSSION

Comparisons of the relative potencies of various IFN preparations in terms of different biological activities should be done within the same cell system. The data shown in Table 1 suggest that antichlamydial, anti-EMCV, and antiproliferative activities in McCoy cells are correlated with each other. In McCoy cells, IFN-αAD (*Bgl*II) and IFN-αAD (*Pvu*II) inhibited *C. trachomatis* infectivity, and these IFNs also inhibited EMCV replication and cell growth. In contrast, a comparison of the same IFN preparations in terms of the three biological parameters assayed in human cells indicates that antichlamydial activity can be dissociated from both antiviral and anti-cell growth activities induced by IFN (Table 2). IFN-αA and IFN-αD were equally effective in terms of the inhibition of EMCV replication and HeLa cell growth. However, of the two, only IFN-αD demonstrated antichlamydial activity. The results of treating HeLa cells with the hybrid IFN species provide further evidence that these biological activities can be dissociated and suggest that IFN molecules can be modified such that antiviral or antigrowth activity can be altered independently of antichlamydial activity. IFN-αAD (*Bgl*II) and IFN-αAD (*Pvu*II), whose NH_2-terminal portions are derived from IFN-αA, had increased antiviral and antigrowth activities but showed no antichlamydial activity. IFNαDA (*Bgl*II), whose NH_2-terminal portion is derived from IFN-αD, had significantly decreased antiviral activity and slightly decreased antigrowth activity. Its antichlamydial activity, however, was similar to that of IFN-αD but not to that of IFN-αA. Thus, it appears that the NH_2-terminal portion of IFN-αD may confer on the IFN molecule the ability to inhibit *C. trachomatis* within this cell system.

An interesting finding in this study was the observation that IFN-αAD (*Bgl*II) and IFN-αAD (*Pvu*II) can induce antichlamydial activity in heterologous cells while having no effect on the human cell system. The ability of IFNs to be active against phylogenetically nonrelated cells has been previously demonstrated, not only for their viral action, but also for their antiproliferative and immunomodulatory activities (4, 5, 10, 11, 15, 20). Furthermore, as in our report,

TABLE 1. IFN activities in McCoy cells

IFN	EMCV-inhibiting IFN concn (U/ml)[a]	Degree of inhibition[b] of:		
		EMCV (log)	Chlamydiae (%)	Cell growth (%)
αA	>10,000	0.3	0	2
αD	>10,000	0.7	0	28
αAD (*Bgl*II)	125	4.2	99	49
αAD (*Pvu*II)	355	2.0	73	40
αDA (*Bgl*II)	>10,000	0.3	0	2
β_1	>10,000	0.5	0	8
Buffy coat	>10,000	0.4	0	0

[a] IFN concentration resulting in 90% inhibition of EMCV yield.
[b] Degree of inhibition, as compared with controls, achieved with 10^4 U of IFN per ml.

TABLE 2. IFN activities in HeLa cells

IFN	EMCV-inhibiting IFN concn (U/ml)[a]	Degree of inhibition[b] of:		
		EMCV (log)	Chlamydiae (%)	Cell growth (%)
αA	30	3	0	29
αD	30	3	47	23
αAD (*Bgl*II)	5	3	0	63
αAD (*Pvu*II)	2	3	4	74
αDA (*Bgl*II)	300	1	41	17
β_1	1	3	29	84
Buffy coat	5	3	41	50

[a] See Table 1, footnote *a*.
[b] See Table 1, footnote *b*.

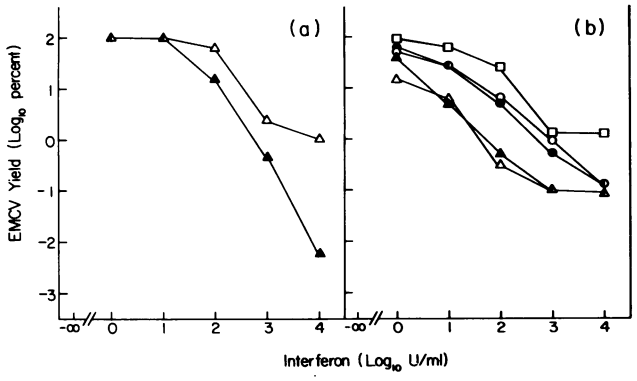

FIG. 3. IFN-induced inhibition of EMCV replication in (a) McCoy cells and (b) HeLa cells. Cell cultures were treated with different IFNs and infected with EMCV as described in the text. Shown are the virus yields from cells treated with IFN-αA (●), IFN-αD (○), IFN-αAD (*Bgl*II) (▲), IFN-αAD (*Pvu*II) (△), or IFN-αDA (*Bgl*II) (□). Titers of virus controls were ca. 10^8 PFU/ml. (Only αIFNs that inhibited the yields of EMCV are shown.)

Desmyter and Stewart (6) showed the modified human IFNs did not have any antiviral activity in homologous cells but were highly active in feline cells. They proposed that the IFN receptors of a particular species may have different affinities for IFNs, independent of their species of origin. Thus, our findings support the claims that the concept of species specificity should be abandoned and that the host range should be defined for each IFN and specific activity under study (1, 20).

It is important to note that in our studies, the IFN-induced antichlamydial effect was never as potent as the antiviral effect. These results are consistent with those in earlier reports, in which crude mouse IFN-α/β was used and in which it was suggested that the decreased sensitivity of *C. trachomatis* might be due to the complexity of the microorganism (12, 17). The difference in sensitivities may be related to the fact that any IFN-induced molecule which might act directly on chlamydial metabolic pathways would have to first penetrate the cytoplasmic inclusion membrane and then the chlamydial cell wall and membrane.

In conclusion, we have demonstrated that the infectivity of *C. trachomatis* is inhibited in cells treated with highly purified preparations of bacterium-derived human IFNs. IFN-αD, IFN-αDA (*Bgl*II), and IFN-β_1 were effective in HeLa cells, and IFN-αAD (*Bgl*II) and IFN-αAD (*Pvu*II) were effective in McCoy cells. The hybrid IFN-αAD should thus be useful in mouse model systems designed to evaluate the in vivo efficacy of IFNs as antichlamydial as well as antiviral and antitumor agents. To gain a better understanding of this model system, the ultrastructural characterization of the effects of human IFN-α preparations in mouse cells has been initiated (3). Furthermore, our results indicate that the antichlamydial effect can be dissociated from the antiviral and antigrowth activities in HeLa cells and suggest that these biological effects are mediated by different mechanisms. Therefore, it may be possible to engineer modified IFN molecules whose activities are targeted towards the treatment of specific human diseases.

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