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# Regulation of interferon-β activity by fibroblast cells

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## Summary

Exogenously administered interferons are rapidly cleared from the body. Several pharmacological mechanisms have been implicated in this clearance; however, they do not entirely explain the different clearance rates of the interferons. Cultured cells were studied for their ability to regulate interferon levels in vitro. Preparations of MuIFN-α, MuIFN-β, and MuIFN-γ were exposed to cells in culture and monitored for any loss in titer. MuIFN-β titers were found to be significantly reduced following exposure to mouse L-929 fibroblast cells. The reduction of MuIFN-β activity appeared to be specific for fibroblasts, since the reduction occurred following exposure to L-cells and to mouse embryo fibroblasts, but not to mouse reticuloendothelial cells. Moreover, the ability of the mouse fibroblast cells to reduce MuIFN-β titers was blocked if the cells were pre-treated with actinomycin D, suggesting that de novo RNA synthesis was required. The titers of IFN-α and IFN-γ were not reduced following exposure to either fibroblast or reticuloendothelial cells. Thus, the reduction of interferon titer by fibroblasts was IFN-β specific. Similarly, HuIFN-β titers were reduced following exposure to human fibroblasts. The ability of fibroblast cells to reduce IFN-β titers was also found to be species-specific, since human fibroblast cells reduced the titer of HuIFN-β but not MuIFN-β while murine fibroblasts reduced the titer of MuIFN-β but not HuIFN-β. These results suggest that IFN-β-treated fibroblasts specifically regulate their response to IFN-β by reducing the titer of the IFN-β activity.

fibroblasts; regulation; interferon-β; interferon

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## Introduction

Interferons are natural cell proteins which have been shown to have antiviral, antitumor, and immunoregulatory properties [1]. Three types of interferons have been recognized: IFN-α, IFN-β, and IFN-γ [19]. Improved techniques for the production and purification of these interferons have strengthened the possibility of using interferons as antiviral and anticellular agents.

Pharmacokinetic studies in man have shown that significant levels of HuIFN-α are detectable in the serum for several hours following intramuscular administration [12,20]. The circulating HuIFN-α is then cleared from the body. Bocci et al. [5] have presented evidence that the tubular cells of the kidney play an important role in the catabolism of HuIFN-α by trapping the circulating HuIFN-α and degrading it. Intramuscular administration of HuIFN-β in man results in less efficient dissemination of HuIFN-β as evidenced by its lower relative detectability in the serum [2,3,21].

We have investigated the possibility that this represents a regulatory effect at the local tissue level. Preparations of the three different types of interferons were incubated with fibroblast, or reticuloendothelial cells in culture and were then monitored for loss in activity. The data show that metabolically active fibroblasts reduce the titer of IFN-β but not of IFN-α or IFN-γ and suggest that fibroblasts but not leucocytes play a key role in the clearance of IFN-β but not of IFN-α or IFN-γ.

## Materials and Methods

*Cells*

Mouse cells employed included secondary mouse embryo fibroblast cells; L-929 fibroblast cells; L-1210S and L-1210R leukemic cells which were sensitive and resistant to the antiviral effects of IFN-β, respectively; S-49 lymphoma cells; and J-774 macrophage cells. Human cells employed included secondary lung and foreskin fibroblast cells and WISH cells.

All cells were grown in a growth medium composed of Earle's base Eagle's minimal essential medium (MEM, GIBCO, Grand Island, NY), supplemented with fetal calf serum (10%, Flow Laboratories, McLean, VA), penicillin (100 U/ml, Pharma-Tek, Inc., San Diego, CA), streptomycin (100 μg/ml, Pfizer, New York, NY), and gentamycin (11 μg/ml, Schering, Kenilworth, NJ).

*Interferons*

Partially purified natural MuIFN-α ($10^{6.00}$ U/mg of protein) and natural MuIFN-β ($10^{7.04}$ U/mg of protein) were obtained from Lee Biomolecular, Inc., San Diego, CA. The MuIFN-α and MuIFN-β are differentiated on the basis of their chromatographic properties, their electrophoretic mobility, and their antigenic properties. They also can be seen to have different relative antiproliferative activities [9]. MuIFN-γ ($10^{2.5}$ U/mg of protein) was prepared from staphylococcal enterotoxin A stimulated mouse spleen cells in culture [15]. HuIFN-α ($10^{7}$ U/mg protein) prepared by Dr. Kari Cantell, was a kind gift of Dr. Jordan Gutterman. HuIFN-β ($10^{7}$ U/mg of protein) was purchased from HEM Research, Rockville, MD. HuIFN-γ ($10^{2.8}$ U/mg of protein) was prepared by stimulating peripheral blood leukocytes with staphylococcal enterotoxin A.

Titrations were performed in microtiter plates (Falcon Plastics, Oxnard, CA) using vesicular stomatitis virus and mengo virus plaque reduction assay systems for mouse and human interferons, respectively [7]. Interferon titers were expressed as the reciprocal of the interferon dilution which reduced the plaque counts to 50% of the control level. Murine interferon titers were corrected to NIH IFN-α/β reference standard #G002-904-511.

*IFN-cell interaction studies*

The interferons were diluted in growth medium to contain 200-1000 U/ml and incubated in the presence or absence of cells at 37°C for 48 h. Cells were plated on 35 mm diameter plastic Petri dishes (Miles Scientific, Naperville, IL) at $10^{6.08}$ cells/dish. Control interferon preparations were maintained at -20°C. After incubation, the interferon preparations were collected and stored at -20°C until assay.

## Results

*Differential effect of L-cell exposure on MuIFN-α and MuIFN-β*

Preparations of MuIFN-α and MuIFN-β were diluted in growth medium and incubated with confluent monolayers of L-cells at 37°C for 48 h. The supernatant fluids were collected and assayed for residual antiviral activity. Control interferon preparations were incubated at -20°C and 37°C in the absence of cells. Table 1 presents the results of one of four experiments which gave qualitatively identical results.

Incubation of MuIFN-α at 37°C in the absence of cells caused a slight thermal decay of the interferon. The level of the recoverable interferon was reproducibly about 50% of the initial interferon activity. Incubation of MuIFN-α in the presence of L-cells appeared to protect the interferon from the thermal decay. The loss of MuIFN-α activity was observed in the presence of protease inhibitors (leupeptin, phenylmethylsulfonyl fluoride), suggesting that it was not mediated by serum proteases (data not shown). Also, the loss of MuIFN-α activity was unaffected by the use of different types of containers (i.e., borosilicate or polycarbonate containers, with different surface to volume ratios), suggesting that there was no nonspecific adsorption of MuIFN-α to the containers (data not shown). Thus, it appeared that MuIFN-α activity was partially

TABLE 1

Residual antiviral activity of interferons after incubation with L-cells

| Sample tested | Residual titer (U/ml ± S.D.; $n = 6$) | |
|---|---|---|
| | IFN-α | IFN-β |
| Frozen control | 402 ± 26 | 494 ± 34 |
| Incubated at 37°C | | |
| without cells | 113 ± 18 | 386 ± 28 |
| with L-cells | 377 ± 16 | 56 ± 12 |

thermally inactivated by incubation at 37°C for 48 h and that L-cells protected the MuIFN-α from thermal inactivation.

Markedly different results were observed for MuIFN-β. Little or no thermal decay was observed, as 80% of the initial interferon was recoverable following incubation in the absence of cells. However, incubation in the presence of L-cells resulted in the disappearance of 87% of the initial interferon.

*Loss of MuIFN-β activity requires metabolically active cells*

L-cells were pretreated with actinomycin D (5 μg/ml for 1 h) to block their transcriptional activity, washed three times to remove excess actinomycin D, and exposed to MuIFN-β at 37°C for 48 h. This treatment regimen has previously been shown [17] to reduce transcription to 5% of the control level. Alternatively, IFN-β was similarly incubated over a monolayer of untreated L-cells at 4°C. As shown in Table 2, pretreatment of the L-cells with actinomycin D blocked their ability to cause a loss of MuIFN-β activity. Further, MuIFN-β exposed to L-cells at 37°C lost 85% of its activity, while there was no significant (5%) loss in activity at 4°C. These results suggest that (i) transcriptional activity, and (ii) physiological temperature are required for L-cells to mediate the loss of MuIFN-β activity.

The disappearance or loss of MuIFN-β following exposure to L-cells could have been caused by some nonspecific factor or enzyme which was spontaneously secreted by metabolically active L-cells. Such a factor would be expected to be present in L-cell conditioned medium prepared by incubating confluent monolayers of cells at 37°C for 48 h. To test this possibility, MuIFN-β was diluted in L-cell conditioned medium or in fresh growth medium and incubated at 37°C for 48 h. As a positive control, MuIFN-β diluted in fresh medium was exposed to a confluent monolayer of L-cells. As shown in Table 3, incubation of MuIFN-β in the presence of L-cells caused a loss of 86% of the initial acitivity. However, residual activity was unaffected by incubation in L-cell conditioned medium, suggesting that the loss in the MuIFN-β activity was not due to

TABLE 2

Requirement for metabolically active cells to observe reduction of interferon-β titers

| Expt. No. | Sample tested | IFN titer (U/ml ± S.D.; $n = 4$) |
|---|---|---|
| 1. | Frozen control | 224 ± 23 |
| | Incubated at 37°C | |
| | without cells | 157 ± 36 |
| | with L-cells | 38 ± 6 |
| | with actinomycin D treated L-cells | 143 ± 16 |
| 2. | Frozen control | 148 ± 17 |
| | Incubated | |
| | without cells at 37°C | 95 ± 16 |
| | with L-cells at 37°C | 23 ± 1 |
| | with L-cells at 4°C | 140 ± 8 |

TABLE 3

Failure of L-cell conditioned medium to affect interferon-β activity

| Interferon-β sample incubated with | Residual IFN titer (U/ml ± S.D.; $n=4$) after incubation at: | |
|---|---|---|
| | -20°C | 37°C |
| Fresh medium | 338 ± 37 | 282 ± 16 |
| L-cell conditioned medium | 384 ± 12 | 264 ± 51 |
| L-cells | - | 46 ± 1 |

nonspecific degradation mediated by factors or enzymes spontaneously released by L-cells.

*Loss of MuIFN-β activity shows specificity for cell type*

The effect of exposure to several different cell lines was determined for each of the three murine interferons. The cell lines examined included two variant L-929 cell lines which had been carried separately for more than 15 years (L-Pur and L-Gal), a secondary mouse embryo fibroblast cell culture, two variant L-1210 leukemia cell lines (L1210S and L-1210R which were sensitive and resistant, respectively, to MuIFN-α and MuIFN-β), an S-49 lymphoblastoid cell line, and a J-774 myeloblastoid cell line. The results of a representative experiment are presented in Table 4.

Consistent with above observations, MuIFN-α underwent a slight thermal decay (approximately 50%) which was prevented by exposure to either of the two variants of

TABLE 4

Antiviral activity of interferons exposed to different cell types

| Cells to which interferon was exposed | Residual interferon activity (U/ml ± S.D.; $n=4$) | | |
|---|---|---|---|
| | IFN-α | IFN-β | IFN-γ |
| Controls (no cells) | | | |
| -20°C | 1121 ± 210 | 530 ± 23 | 324 ± 26 |
| 37°C | 615 ± 28 | 447 ± 64 | 340 ± 34 |
| L-929 cells | | | |
| L-Pur | 1140 ± 146 | 68 ± 11 | 346 ± 18 |
| L-Gal | 1249 ± 208 | 87 ± 8 | 319 ± 24 |
| Mouse embryo fibroblasts | | | |
| (secondary) | 621 ± 64 | 103 ± 19 | 288 ± 19 |
| Leukemic cells | | | |
| L-1210 S | 487 ± 47 | 476 ± 34 | 324 ± 24 |
| L-1210 R | 546 ± 39 | 526 ± 68 | 298 ± 28 |
| Lymphoblastoid cells | | | |
| S-49 | 475 ± 37 | 368 ± 36 | 336 ± 32 |
| Myeloblastoid cells | | | |
| J-774 | 526 ± 63 | 432 ± 26 | 288 ± 36 |

L-929 cells. None of the other cell lines demonstrated this protective capacity, suggesting that the ability to protect against thermal decay may have been a unique property of L-929 cells.

Also consistent with above observations, MuIFN-β lost a substantial amount of its activity (approximately 85%) following exposure to either of the two variants of L-929 cells. In addition, exposure to mouse embryo fibroblast cells also resulted in a loss of MuIFN-β activity. None of the lymphoid cells had a significant effect on MuIFN-β activity, suggesting that the ability to cause a loss of MuIFN-β activity was not a general property of metabolically active cells but is rather a characteristic of fibroblast cells.

MuIFN-γ was not susceptible to thermal inactivation by exposure to 37°C for 48 h. Further, none of the cell lines employed in this study, fibroblast or lymphoid in origin, showed any significant effect on MuIFN-γ activity.

*Effect of human cells on human interferons*

To determine whether the loss of IFN-β activity is a unique feature of the murine system or may occur in other species as well, the effect of human fibroblasts on human interferons was studied. HuIFN-α, HuIFN-β and HuIFN-γ were incubated at 37°C for 48 h in the presence or absence of human lung fibroblasts. The supernatant fluids were collected, the interferon activity was estimated, and compared to the activity of an aliquot stored at -20°C. The results of a representative experiment are shown in Table 5. Incubation at 37°C with or without cells did not affect the activity of IFN-α and IFN-γ, since their titers were comparable to the corresponding titers of the frozen samples. IFN-β when incubated at 37°C in the absence of cells retained about 60% of the activity of the frozen aliquot. However, when incubated in the presence of a monolayer of human lung fibroblasts, IFN-β retained only about 12% of its activity when compared with the frozen aliquot. These results suggest that human lung fibroblast cells can reduce the activity of HuIFN-β, but have no significant effect on the activity of HuIFN-α and HuIFN-γ. Similar results were obtained using human foreskin fibroblasts (data not shown). These results suggest that human fibroblasts can reduce the activity of HuIFN-β but not HuIFN-α or HuIFN-γ activity.

TABLE 5

Residual antiviral activity of human interferons exposed to human lung fibroblasts

| Sample tested | Residual antiviral activity (U/ml ± S.D.; $n = 4$) | | |
|---|---|---|---|
| | IFN-α | IFN-β | IFN-γ |
| Frozen control | 659 ± 15 | 233 ± 21 | 88 ± 19 |
| Incubated at 37°C | | | |
| without cells | 770 ± 17 | 137 ± 29 | 74 ± 22 |
| with lung fibroblasts | 725 ± 64 | 27 ± 4 | 66 ± 6 |

TABLE 6

Residual antiviral activity of interferon-β after incubation with homologous and heterologous cells

| Sample tested | Residual antiviral activity (U/ml ± S.D.; $n = 4$) | |
|---|---|---|
| | HuIFN-β | MuIFN-β |
| Frozen control | 251 ± 8 | 371 ± 49 |
| Incubated at 37°C | | |
| without cells | 208 ± 8 | 319 ± 21 |
| with human lung fibroblasts | 49 ± 7 | 311 ± 6 |
| with mouse L-cells | 191 ± 10 | 46 ± 6 |

*Loss of IFN-β activity shows species specificity*

Interferons show high species specificity in exerting their activities. To test whether the loss in IFN-β activity was also restricted by the species compatibility barrier, MuIFN-β and HuIFN-β preparations were exposed for 48 h at 37°C to fibroblastic cells from each species and then assayed for residual activity. As can be seen in Table 6, the activity of MuIFN-β was reduced when the interferon was exposed to murine cells but not to human cells. Similarly, the activity of HuIFN-β was reduced when the interferon was exposed to human cells but not to murine cells, suggesting that fibroblast cells reduce the activity of homologous but not of heterologous IFN-β.

## Discussion

Pharmacokinetic studies in man have shown that serum levels following intramuscular administration of antiviral activity of HuIFN-β are low when compared to HuIFN-α [2,3,21]. This may be due to the presence of serum inactivators, such as those described by Cesario et al. [8]. Another alternate possibility is that HuIFN-β might be cleared by fibroblasts present at the site of injection. This possibility was tested by in vitro experiments.

The three different murine interferons were exposed to a variety of fibroblast and reticuloendothelial cells in culture and monitored for loss in activity. The data show that murine fibroblasts have the capacity to reduce MuIFN-β activity, but are not able to reduce MuIFN-α or MuIFN-γ activities. Murine cells of lymphoid origin were found unable to affect the activity of MuIFN-β.

The results further showed that the murine fibroblast-mediated disappearance of MuIFN-β requires cell metabolic activity. Exposure of MuIFN-β to L-cell conditioned medium did not affect interferon activity. Similarly, cells whose transcriptional activity has been blocked by actinomycin D pretreatment were found unable to affect interferon activity. However, exposure to a metabolically active cell by itself was not sufficient to cause the loss of MuIFN-β activity since murine cells of lymphoid origin and fibroblast cells of human origin did not affect MuIFN-β activity.

The high degree of specificity observed with our system (the requirement for

metabolically active murine fibroblasts) suggests that by reducing the titer of the MuIFN-β activity in the surrounding fluid, fibroblasts regulate their responsiveness to the interferon. Further, the observation that HuIFN-β activity is lost following exposure to human fibroblast cells suggests that this may represent a general mechanism of IFN-β regulation by fibroblast cells.

It is of interest to note that pharmacokinetic studies of HuIFN-β have shown divergent results in various animal species [4]. For example, in humans and in rabbits, HuIFN-β is markedly less able than HuIFN-α to become systemically distributed following intramuscular administration. In monkeys, HuIFN-α and HuIFN-β are distributed equally effectively. These results may be reflective of cross-species effects such as those observed in our studies when MuIFN-β was shown to disappear following exposure to mouse, but not to human fibroblast cells.

The specific cellular event which is responsible for this disappearance of IFN-β activity is unknown. It could be related to the phenomenon observed by Hanley et al. [12] who noted the rapid inactivation of HuIFN-β by muscle tissue homogenates. However, this is considered to be unlikely since the IFN-β disappearance observed in our system required the transcriptional activation of an IFN-β-treated fibroblast cell. It could also be related to the phenomenon observed by Robert-Galliot et al. [16] who noted the phosphorylation of HuIFN-γ by a protein kinase released by cells into the culture medium. However, this is considered to be unlikely since IFN-β did not disappear upon exposure to cell culture supernatants and since, unlike in the case for HuIFN-γ, the IFN-β activity was lost as a consequence of fibroblast cell exposure.

Previous reports have indicated that exposure of interferons to cells could result in the disappearance of interferon activity [11,14] implying that large scale "uptake" of interferon was required to mediate an antiviral state. However, Buckler et al. [6] showed that detectable loss of interferon ("uptake") was not necessary for the induction of an antiviral state. The results reported here confirm the original observations of interferon disappearance and provide possible explanations for the failure of other scientists to observe large scale interferon disappearance [6,10].

Perhaps more importantly, these results suggest a possible explanation for the observation that intramuscular administration of HuIFN-β in some animal species does not result in the efficient systemic distribution of the interferon. The data suggest that fibroblasts at the site of interferon administration may cause specific removal of IFN-β activity and be responsible for the low IFN-β titers.

This function of fibroblasts may have a natural role during localized viral infections. Virally infected fibroblasts produce predominantly IFN-β [18]. Local virus infections may thus induce local IFN-β production. For example, in picornavirus epidemic conjunctivitis, the interferon produced in tears has been characterized as IFN-β [13]. In this or similar situations, fibroblasts may function to restrict the spread of IFN-β away from the site of localized viral infection. This restriction of IFN-β spread may serve to limit the potentially harmful systemic effects of INF-β, such as flu-like symptoms and immunosuppression.

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