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Short Communication

Potentiation of interferon action by mixtures of recombinant DNA-derived human interferons

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Summary

Natural and essentially pure recombinant DNA-derived HuIFN-α and HuIFN-γ were examined for their relative abilities to potentiate interferon action. Potentiation of human interferon's antiviral and antiproliferative activities were studied. The essentially pure recombinant DNA-derived human interferons were found to be as effective in their potentiating interactions as their natural counterparts. The results demonstrate that it is the human interferons themselves which interact to potentiate human interferon's varied activities.

interferon; antiproliferative effect; potentiation

Combinations of MuIFN-α/β and MuIFN-γ have been shown to interact to give a greater than additive activity. This potentiation has been demonstrated for the antiviral [6,12], direct antiproliferative [4], and in vivo antitumor [2,3,7] activities of the murine interferons. Recently, using MuIFN-γ_R which was derived by recombinant DNA technology, the potentiating activity present in MuIFN-γ preparations has been shown to be MuIFN-γ itself [5].

The potentiation phenomenon has been extended to the human system. Potentiation of interferon's antiviral activity [8], direct antiproliferative effect [8] and natural killer cell activation [11] have been shown for combinations of HuIFN-γ and either HuIFN-α or HuIFN-β. Combinations of HuIFN-α and HuIFN-β give only an additive effect [8,11]. However, these experiments have been performed using relative-

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ly crude preparations of interferon. Of particular concern are HuIFN-γ preparations which contain many lymphokines and other biologically active molecules. In the present study, we extend our work with recombinant interferons to the human system. Essentially pure human interferons derived by recombinant DNA technology (HuIFN-γ_R and HuIFN-α_R) were employed to demonstrate that potentiation was the result of the interaction of the human interferons themselves.

The interferons employed in these studies included HuIFN-α_N (10^7 units/mg of protein; prepared by Dr. Kari Cantell and kindly supplied by Dr. Jordan Gutterman), HuIFN-α_R [essentially pure at 10^8 units/mg of protein; HuIFN-α_2 (study drug SCH 30500) prepared by Schering in bacteria using recombinant DNA technology and kindly supplied by Dr. Richard Pollard], HuIFN-γ_N (10^7 units/mg of protein; prepared by Meloy Laboratories [1], and kindly supplied by Dr. Irwin Braude), and HuIFN-γR (greater than 90% pure at $10^{7.3}$ units/mg of protein; prepared in CHO cells using recombinant DNA technology [10]). Interferon titers were determined in a microtiter plaque reduction assay [4] employing WISH cells and mengovirus. All assay results were expressed as units/ml, corrected by comparison with the WHO HuIFN-α international reference preparation G023-901-527 (NIH).

The abilities of natural and recombinant DNA-derived interferons to potentiate interferon's antiviral activity were examined by evaluating their protective effects in single-cycle virus yield reduction experiments [6]. WISH cells were treated for 12 h with the different interferon preparations separately and in various combinations. The interferon was removed and the cells were challenged with mengovirus at a multiplicity of 10 plaque forming units/cell. After a 45 min absorption period, the monolayers were washed twice, overlaid with growth medium and incubated for 24 h. The supernatant fluids were harvested and assayed for virus yields by a plaque assay [9]. For each interferon sample or mixture tested, the virus yield from a single cycle virus yield experiment was compared to a curve developed concurrently for a laboratory standard preparation. This allowed the antiviral potency to be expressed as units/ml observed and permitted potentiation by interferon mixtures to be calculated as the ratio between observed and expected antiviral potencies.

Table 1 presents the results of a representative experiment. No significant differences in potentiation levels were seen, as all of the interferon combinations gave potentiation levels of between 5- and 6-fold. The essentially pure recombinant DNA-derived interferons (HuIFN-α_R and HuIFN-γ_R) were as effective in their potentiating interactions as their natural counterparts (HuIFN-α_N and HuIFN-γ_N). Thus, the factors responsible for the potentiation of interferon's antiviral activity appeared to be the interferons themselves.

Next, the abilities of natural and recombinant DNA-derived interferons to potentiate interferon's antiproliferative effect were examined. WISH cells were plated and incubated [4] for 6 days in the presence of the different interferon preparations separately and in various combinations. For each interferon sample or mixture tested, the number of colonies was plotted and compared to a curve developed concurrently for a laboratory standard preparation. This allowed the cloning efficiency to be expressed as observed units/ml and permitted the potentiation levels in interferon mixtures to be calculated as the ratio between observed and expected units/ml.

TABLE 1

Potentiation of antiviral activity by mixing preparations of natural or recombinant DNA-derived HuIFN-α and HuIFN-γ

Interferon mixtures applied	Antiviral potency (units/ml)		Potentiation factor[c]
	Observed[a]	Expected[b]	
IFN-α_N	35		
IFN-α_R	21		
IFN-γ_N	25		
IFN-γ_R	35		
IFN-α_N + IFN-γ_N	350	60	5.8
IFN-α_R + IFN-γ_N	278	46	6.0
IFN-α_N + IFN-γ_R	350	70	5.0
IFN-α_R + IFN-γ_R	330	56	5.9

[a] Determined by comparison of virus yield from a single cycle virus yield experiment with a curve determined concurrently for a laboratory standard preparation.
[b] Determined by adding observed potencies of the separate interferons present in the mixtures.
[c] Determined by dividing 'observed' by 'expected' potencies.

Table 2 presents the results of a representative experiment. Again, no significant differences in potentiation levels were seen, as all of the interferon combinations gave potentiation levels of between 9- and 11-fold. The essentially pure recombinant DNA-derived interferons (HuIFN-α_R and HuIFN-γ_R) were as effective in their potentiating interactions as their natural counterparts (HuIFN-α_N and HuIFN-γ_N). Thus, as was seen for the antiviral studies, the factors responsible for the potentiation of interferon's antiproliferative effect appeared to be the interferons themselves.

TABLE 2

Potentiation of antiproliferative activity by mixing preparations of natural or recombinant DNA-derived HuIFN-α and HuIFN-γ

Interferon mixtures applied	Antiproliferative potency (units/ml)		Potentiation factor[c]
	Observed[a]	Expected[b]	
IFN-α_N	0.6		
IFN-α_R	0.8		
IFN-γ_N	0.6		
IFN-γ_R	0.6		
IFN-α_N + IFN-γ_N	13	1.2	11
IFN-α_R + IFN-γ_N	13	1.4	9.3
IFN-α_N + IFN-γ_R	13	1.2	11
IFN-α_R + IFN-γ_R	13	1.4	9.3

[a] Determined by comparison of colony formation efficiency with a curve developed concurrently for an IFN-β laboratory standard [4]. IFN-β was employed since IFN-β gave the sensitivity curve with the steepest slope. The data were expressed as antiproliferative units of interferon where 1 antiproliferative unit was required to reduce the clone number to 50% of the control, untreated cultures.
[b] Determined by adding observed potencies of the separate interferons present in the mixture.
[c] Determined by dividing 'observed' by 'expected' potencies.

It has recently been shown that natural and highly purified, recombinant DNA-derived MuIFN-γ were equally able to interact with natural MuIFN-α/β to potentiate interferon's antiviral and antiproliferative activities [5]. The findings reported here employ essentially pure recombinant DNA-derived HuIFN-γ to extend our observations with recombinant DNA-derived IFN-γ from the mouse system to the human system. They further indicate that natural and essentially pure recombinant DNA-derived HuIFN-α are equally able to participate in the potentiation of interferon's antiviral and antiproliferative activities. Taken together with the recent report that recombinant DNA-derived HuIFN-γ can interact with HuIFN-α and HuIFN-β to potentiate lymphocyte-mediated natural killing [11], these results unequivocally demonstrate that it is the interferons themselves which interact to potentiate interferon's varied activities.

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