Independent Regulation of the Antiviral States Induced by MulIFN-α/β and by MulIFN-γ

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SUMMARY

The stability profiles of the antiviral states induced in L-929 cells against mengovirus by murine interferons MulIFN-α/β and by MulIFN-γ were shown to be different. Treatment of cells with combinations of IFN-α/β and IFN-γ have previously been shown to result in a potentiated expression of the antiviral state. Here we report studies of the stabilities of the antiviral states induced by various combinations of IFN-α/β and IFN-γ and that the regulation of the antiviral states induced by IFN-α/β and IFN-γ were independent of each other.

Combinations of interferon-γ (IFN-γ) with IFN-α or IFN-β have been shown to potentiate the biological activities of the interferons including the antiviral (Fleischmann et al., 1979; Samuel & Knutson, 1983), antiproliferative (Fleischmann, 1982; Czarniecki et al., 1984; Oleszak & Stewart, 1985), antineoplastic (Fleischmann et al., 1980; Brysk et al., 1981) effects and natural killer cell activation (Weigent et al., 1983). Combinations of MulIFN-α and IFN-β, on the other hand, result in an additive effect only (Fleischmann et al., 1984). These data suggest that the mechanisms by which interferons induce these varied effects may be different for type I (IFN-α and IFN-β) and type II (IFN-γ).

We have reported earlier that the modes of regulation of the antiviral states against mengovirus in L-929 cells induced by murine interferons MulIFN-α/β and MulIFN-γ are different. The MulIFN-α/β-induced antiviral state was found to be unstable and decayed upon interferon removal, whereas the MulIFN-γ-induced antiviral state showed an enhancement upon interferon removal (Ramamurthy & Fleischmann, 1986). In this report, we have examined the stability of the antiviral state induced by combinations of MulIFN-α/β and MulIFN-γ to gain an insight into the possible mode(s) of regulation of the antiviral states induced by interferon combinations.

Dilutions of MulIFN-α/β and MulIFN-γ were employed separately or in combinations in single-cycle virus yield reduction experiments. Mouse L-929 cells were overlaid for 24 h with different interferon combinations, washed to remove the interferons and were challenged with mengovirus (Franklin isolate) at an m.o.i. of 10 p.f.u./cell at various times after interferon removal. The virus yields were harvested 24 h post-infection and assayed by plaquing on L-cells. The interferons employed in this study were relatively crude. In the previous study with this system (Ramamurthy & Fleischmann, 1986), these interferon preparations showed results identical to those of highly purified interferons. The mixture of interferons used was such that the relative ratios of the interferons either greatly favoured one interferon type, or the other type, or were equivalent. It should be noted that the mixtures of interferons were specifically chosen such that the resulting levels of the antiviral states at the time of interferon removal were approximately the same in each case.

The results of a fully representative experiment are shown in Fig. 1. It can be seen that mixtures of MulIFN-α/β and MulIFN-γ at concentrations of 30 and 3, 3 and 30, or 10 and 10
Fig. 1. Kinetics of the stability of the antiviral state induced by MuIFN-α/β, MuIFN-γ, and their combinations. Mouse L-929 cell monolayers were treated for 24 h with medium only or with medium containing MuIFN-α/β or MuIFN-γ separately or in combination. Following interferon (or medium) removal, the cells were washed two times with fresh nutrient medium and re-incubated. The cell cultures were challenged at the indicated time with mengovirus at a multiplicity of 10 p.f.u./cell. Virus yields were harvested 24 h post-infection and titrated by plaquing on L-cells. The ratio of virus yield in interferon-treated cells to untreated cells was expressed as the log_{10} reduction of virus yield and was plotted as a function of time. (a) 100 units/ml MuIFN-α/β (○) and MuIFN-γ (▲) separately. (b) 30 units/ml MuIFN-α/β (●) and 3 units/ml MuIFN-γ (▲) separately or in combination (▲). (c) 3 units/ml MuIFN-α/β (●) and 30 units/ml MuIFN-γ (▲) separately or in combination (▲). (d) 10 units/ml MuIFN-α/β (●) and MuIFN-γ (▲) separately or in combination (▲). The profile of the stability of the antiviral state induced by 100 units/ml interferon (a) is retraced in panels (b), (c) and (d) for comparison (——).
slightly but consistently lower in cells treated with this combination of interferons when compared to cells treated with 100 units/ml MulFN-α/β. In contrast, the decay rate of the antiviral state in cells treated with 30 units/ml of MulFN-α/β alone was significantly lower than in cells treated with combinations of interferons or with 100 units/ml MulFN-α/β alone. Treatment of cells with 3 units/ml of MulFN-γ alone conferred insignificant protection to L-cells against mengovirus challenge.

Fig. 1(c) shows the stability profile of the antiviral state for combination interferon treatment when the ratio of MulFN-α/β to MulFN-γ was 1:10 (3 units/ml MulFN-α/β and 30 units/ml MulFN-γ). The stability profile of the antiviral state was very similar to the enhancement profile observed in cells treated with 100 units/ml MulFN-γ alone. However, the enhancement of the antiviral state in cells treated with 30 units/ml MulFN-γ alone was very moderate, and 3 units/ml MulFN-α/β did not induce any significant level of antiviral state.

Fig. 1(d) shows the stability profile of the antiviral state for combination interferon treatment when the ratio of MulFN-α/β to MulFN-γ was 1:1 (10 units/ml MulFN-α/β and 10 units/ml MulFN-γ). The stability profile of the antiviral state was a composite curve which reflected some aspects of both the enhancement profile seen for MulFN-γ treatment and the decay profile seen for MulFN-α/β treatment. Initially (up to 8 h) the level of the antiviral state showed an enhancement, and this was followed by a rapid decline. The rate and level of the enhancement observed was lower than that observed in cells treated with 100 units/ml MulFN-γ alone. During the decline phase (after the 8 h point), the rate of decay of the antiviral state was more rapid than in cells treated with 100 units/ml MulFN-γ alone and was reminiscent of the decay observed in cells treated with 100 units/ml MulFN-α/β alone. Treatment of cells with 10 units/ml MulFN-α/β or MulFN-γ alone resulted in the induction of significantly lower levels of the antiviral states, and the decay or the enhancement following interferon removal were moderate to inapparent.

These data indicate that the response following removal of interferon in cells treated with combinations of interferons reflected the response following the removal of the type of interferon dominant in the mixture. Even though the enhancement or the decay of the antiviral state was not apparent when cells were treated with lower concentrations of interferons separately, the presence of small amounts of another type of interferon helped the detection of the change by potentiating the effects. Moreover, the stability profile of the antiviral state induced by combinations of interferons most closely followed the stability profile of the dominant ingredient in the mixture. Thus, the influence of the regulation of the antiviral state induced by one interferon type on the regulation of the mixture of the antiviral state induced by the other interferon type was minimal, suggesting that the regulatory mechanisms are independent.

Use of interferons to combat viral and neoplastic diseases is presently at an experimental stage. Understanding the regulatory mechanisms involved in the interferon-induced activities will help to improve the clinical usage of interferons, as well as to gain insights into the modes of interferon action. The results presented here suggest that when used in combinations, (i) lower interferon concentrations will suffice to provide levels of the antiviral state attainable only when using much higher levels of interferons separately, (ii) the stability profile of the antiviral state is determined by the dominant ingredient in the mixtures, and (iii) the regulation of the antiviral states induced by MulFN-α/β and by MulFN-γ are independent of each other.

Thus, by incorporating small relative amounts of MulFN-α/β, it is possible not only to amplify the induction level of the antiviral state caused by MulFN-γ, but also to maintain the enhancement profile of the antiviral state observable after the removal of a higher dose of MulFN-γ. Similarly, by incorporating small relative amounts of MulFN-γ, it is possible not only to amplify the antiviral state induction level of MulFN-α/β, but also to moderate the regulatory effect of MulFN-α/β observable after the removal of a higher dose of MulFN-α/β.

REFERENCES


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