Human Interferons Alpha and Beta Have More Potent Priming Activities than Interferon Gamma

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SUMMARY

The priming activities of human IFN-α, IFN-β and IFN-γ were compared on the same antiviral basis using human buffy coat leukocytes stimulated with Sendai virus or concanavalin A (Con A) to produce IFN-α or IFN-γ, respectively. Pretreatment of leukocytes with any type of IFN enhanced their IFN-α and IFN-γ production, but IFN-α and IFN-β had more potent priming activities than IFN-γ. IFN-α and IFN-γ did not potentiate the priming activity of each other in either the IFN-α- or the IFN-γ-producing system. Pretreatment of leukocytes with relatively high doses of IFN-α or IFN-β (1000 to 3000 IU/ml) resulted in a 40- to 50-fold increase in the IFN-γ production of Con A-stimulated leukocytes. This observation will be of use in producing IFN-γ with a high titre.

Human interferons (IFNs) can be classified into three groups: leukocyte (α), fibroblast (β) and immune (γ) IFNs (Stewart et al., 1980). IFN-α and IFN-β show similarities in protein composition as well as in physicochemical and biological properties (Burke, 1981/82). Although IFN-γ differs markedly in its chemical structure, it shares many of the pleiotropic activities of IFN-α and IFN-β, but displays a higher anti-tumour activity in vivo (Rubin & Gupta, 1980) and a higher cytostatic activity in vitro (Blalock et al., 1980). On the other hand, IFN-γ enhances the expression of class I and class II major histocompatibility complex products in neoplastic cells more effectively than IFN-α and IFN-β (Dolei et al., 1983). Furthermore, in a two-dimensional gel electrophoresis system, striking qualitative and quantitative differences were demonstrated in the intracellular response of fibroblasts to IFN-γ as compared to IFN-α and IFN-β (Weil et al., 1983).

In addition to the biological effects mentioned above, the IFNs have priming activity. Cells pretreated with IFN generally yield more IFN and produce it sooner than unprimed cells when exposed to an inducer able to induce IFN in unprimed cells (Stewart et al., 1971, 1972). For example, human leukocytes pretreated with IFN-α before induction with Sendai virus produce more IFN-α than unprimed cells (Cantell et al., 1974). Similarly, the IFN-γ production of phytohaemagglutinin-stimulated human leukocytes is doubled by pretreatment with 100 IU/ml IFN-α (Wiranowska-Stewart et al., 1980). To our knowledge, the priming activity of IFN-γ has not been characterized in detail.

In the present studies we have investigated the priming activities of IFN-α, IFN-β and IFN-γ and have considered the following questions. (i) Is there any difference in the priming activities of the IFN types? (ii) Do the IFNs potentiate each other in terms of their priming activities? (iii) Do the priming activities of the IFNs depend on the IFN-producing system (IFN-α or IFN-γ) they are used in?

IFN production was performed in duplicate cultures of 1 ml, each in wells in a plastic plate (24-well tissue culture cluster, Costar). Prior to stimulation, cells were treated with purified IFN-α, IFN-β or IFN-γ for 2 to 4 h. Sendai virus-induced leukocyte IFN-α was purified to a sp. act. of...
Short communication

Fig. 1. Effects of human IFN-α (●), IFN-β (■) and IFN-γ (▲) on the production of IFN-α (a) and IFN-γ (b). Leukocytes were suspended in Eagle's MEM containing 2 mg/ml human gamma-globulin-free human serum. Cell concentrations for IFN-α and IFN-γ production were 1 × 10⁷/ml and 2.5 × 10⁷/ml, respectively. Leukocytes were pretreated with IFNs for 2 h (a) or 4 h (b) prior to stimulation with 400 HAU/ml Sendai virus (a) or 15 μg/ml Con A (b). Antiviral activities of the supernatants were determined on WISH cells 16 h after induction. Values are averages of four independent experiments. In two cases, IFN titres in the supernatants of the IFN-α-treated leukocytes were measured in MDBK cells to determine the trace of priming IFN remaining after three washing cycles (□).

1 × 10⁶ IU/mg protein by chromatography on Controlled Pore Glass (CPG-75) beads (Electronucleons, Fairfield, N.J., U.S.A.) and molecular sieving on Sephacryl S-200 (Pharmacia). In some cases these preparations were further purified by immunoaffinity chromatography to a sp. act. of 3 × 10⁷ IU/mg protein. The IFN-γ was produced in concanavalin A (Con A)-stimulated leukocyte cultures and purified to a sp. act. of 1 × 10⁵ U/mg protein by chromatography on CPG-75 beads.

Following the priming, leukocytes were washed three times with Hanks' balanced salt solution and stimulated with 400 haemagglutination units (HAU) per ml Sendai virus (Béládi et al., 1980) or with 15 μg/ml Con A (Calbiochem) to produce IFN-α or IFN-γ, respectively. Antiviral activity was assayed on human amnion (WISH and WK), human diploid fibroblast, monkey kidney (CV-1), or bovine kidney (MDBK) cells. Titres of IFN-α and IFN-γ preparations were expressed in IU/ml and U/ml; they were calibrated with NIH human leukocyte IFN reference reagent G-023-901-527. At the end of this work a human IFN-γ reference reagent (NIH Gg23-901-530) became available. In our test 1 U of IFN-γ correspond to 0.43 reference U.

To compare the three known IFN types for their effectiveness in the enhancement of IFN-α production, we treated leukocyte cultures with IFNs at various concentrations and stimulated them with Sendai virus (Fig. 1 a). IFN-α and IFN-β caused dose-dependent increases in IFN-α production, which reached a maximum of twice the basal level after treatment with 100 to 300 IU/ml. A similar effect was achieved only with 10- to 15-fold times more IFN-γ. There was no significant difference in the IFN titres using 2, 4 or 6 h IFN pretreatment periods (data not shown).

We also compared the efficacies of the three IFN types in the enhancement of IFN-γ production of Con A-stimulated leukocytes (Fig. 1 b). Similarly IFN-γ production was enhanced in a dose-dependent manner by IFN pretreatment, and IFN-α and IFN-β showed more potent priming activities than IFN-γ. The IFN-γ production was doubled by very low doses of IFN-α or IFN-β (3 to 7 IU/ml). Pretreatment of leukocytes with relatively high doses of IFN-α or IFN-β (1000 to 3000 IU/ml) caused a very efficient enhancement (more than 40-fold) of IFN-γ production, revealing the possibility of producing IFN-γ with a high titre. The length of the priming period (between 2 and 6 h) had no effect on the priming efficiency of the IFNs. Using
longer priming periods the titres were decreased (data not shown). Since a similar priming effect was achieved with IFN-α purified to a sp. act. of $1 \times 10^6$ IU/mg protein and IFN-α purified by immunoaffinity chromatography to a sp. act. of $3 \times 10^7$ IU/mg protein, it seems that the IFN itself, and not other contaminants, is responsible for the priming effect. The high antiviral titres in the supernatants of the primed cultures were not due to the potentiation of the IFN-γ which was produced by the trace of IFN used for priming (see Fig. 1b) since addition of a specific antiserum to IFN-α in excess to a culture producing IFN-γ in IFN-α-pretreated leukocytes did not decrease the IFN titre produced (data not shown). The properties of the IFN produced by primed and Con A-induced leukocytes were identical to those of IFN-γ (Langford et al., 1979; Blalock et al., 1980; Rubin & Gupta, 1980; Van Damme et al., 1983), since 80 to 90% of its antiviral activity was sensitive at pH 2, and it protected only cell lines of human origin against vesicular stomatitis virus (VSV). Development of its antiviral activity on WISH cells was much slower than that of IFN-α. It possessed a higher antinuclear activity than those of IFN-α and IFN-β, and it had a mol. wt. of 51000 (data not shown).

It has recently been shown that IFN-α or IFN-β and IFN-γ can potentiate each other in terms of their antiviral (Fleischmann et al., 1979), anti-tumour (Fleischmann et al., 1980) and immunoregulatory (Weigent et al., 1983) activities. We therefore investigated whether mixed IFN preparations (IFN-α + IFN-γ) enhanced IFN production more effectively than a single type (IFN-α or IFN-γ). Since the priming potency of IFN-α was much more pronounced than that of IFN-γ, the question was whether IFN-γ can enhance the priming activity of IFN-α. Our results showed that the IFN-α and IFN-γ production of leukocytes pretreated with a mixture of IFN-α and IFN-γ did not exceed the level for IFN-α-treated cells with the same antiviral activity (Fig. 2a, b); so in our systems we could not detect any potentiation. However, in WISH cells the antiviral activities of these preparations were potentiated by a factor of 5 to 10 at a 1:1 ratio (100 units each) in the VSV yield reduction test (data not shown).

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