Comparative Antiproliferative Efficacies of Human α and γ Interferons

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SUMMARY

The cell growth inhibitory efficacies of preparations of human α and γ interferons (IFNs) have been tested on a variety of human cell lines. Human IFN-γ was found to be more effective at inhibiting the growth of HeLa and U-amnion cells than was human IFN-α. The Daudi cell line, on the other hand, which exhibits a strong anticytotoxic effect in response to treatment with human IFN-α, was found to be relatively insensitive to the anticytotoxic effect of human IFN-γ, as were a variety of other lymphoid cell lines. The inability of the IFN-γ to inhibit the growth of the Daudi cells is paralleled by its inability to induce the synthesis of proteins observed to be synthesized in these cells in response to IFN-α.

INTRODUCTION

Interferons (IFNs) have been divided into three categories: IFN-α (leukocyte), -β (fibroblast) and -γ (immune) (for review, see Stewart, 1979). These interferons differ in their antigenic properties and the nature of the stimuli required for their production (Johnson et al., 1977; Stewart, 1979). While the biological effects of human IFN-α and IFN-β have been extensively studied, the biological effects of IFN-γ have only recently begun to be elucidated (Dianzani et al., 1978, 1980; Blalock et al., 1980; DeLey et al., 1980; Hovanessian et al., 1980; Rubin & Gupta, 1980b). The anticytotoxic action of interferon has been shown to play a role in the antitumour capabilities of the interferons (Gresser et al., 1972; Yokota et al., 1976) and may therefore be an important parameter in evaluating the clinical usefulness of an interferon. The anticytotoxic activity of human IFN-γ has been the subject of several reports (Blalock et al., 1980; DeLey et al., 1980; Rubin & Gupta, 1980b). Blalock et al. (1980), using HeLa (cervical carcinoma) and HEp-2 (cervical carcinoma; see Lauappa, 1978) cells, and Rubin & Gupta (1980b) using HeLa, FS4 (normal human fibroblasts) and SK-OS-10 (osteogenic sarcoma) cells reported human IFN-γ to be a more potent antiproliferative agent than either IFN-α or IFN-β. DeLey et al. (1980), on the other hand, using various lymphoid cell lines (Daudi–Burkitt lymphoma, Raji–Burkitt lymphoma, etc.) found that human IFN-γ was not significantly more effective, and in some cases was less effective, in inhibiting the growth of these cells than was IFN-α or IFN-β. It has been suggested that these seemingly contradictory observations could be attributed to either the different target cells used in the studies or to the different contaminating molecules present in the different interferon preparations.

Using a monoclonal antibody developed against, and capable of neutralizing the antiviral effects of human IFN-γ, we have been able to demonstrate that it is the IFN-γ that is responsible for the anticytotoxic and protein-inducing action of partially purified preparations of human IFN-γ (Rubin et al., 1983).

In the present study we have compared the anticytotoxic activity of preparations of human IFN-γ on a variety of cell lines, including those reported to be sensitive and insensitive to the anticytotoxic action of human IFN-α.

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METHODS

Interferon. Human IFN-γ was produced by stimulation of human peripheral blood leukocytes with staphylococcal enterotoxin A (Johnson et al., 1977) (a gift from the U.S. Food and Drug Administration, Cincinnati, Ohio, U.S.A.) at 0.02 µg/ml. The interferon was partially purified on controlled pore glass bead columns to a specific activity of approx. 10^6 units/mg protein essentially as described by Wiranowska-Stewart et al. (1980) except that the column was washed with phosphate-buffered saline (PBS) containing 20% (v/v) ethylene glycol before elution of the interferon. Human IFN-α (sp. act. approx. 10^6 units/mg protein) was kindly provided by Dr M. Krim (Sloan-Kettering Institute). Interferon titres were measured by inhibition of vesicular stomatitis virus-induced cytopathic effect on GM2767 cells (Stewart, 1979) and compared with an international standard in the case of IFN-α, and compared with a laboratory standard in the case of IFN-γ.

Cell growth inhibition. Studies on the cell growth inhibitory properties of the human interferons were performed using the following cell lines: HeLa (cervical carcinoma), U-amnion (amnionic), Daudi (Burkitt lymphoma), Molt-4 (acute lymphoblastic leukaemia), GM3638 (acute lymphocytic leukaemia) and GM3639 (acute lymphocytic leukaemia). The cell growth inhibitory activity of the interferons was measured either by (i) trypsinization and counting of the HeLa and U-amnion cells treated as described in the legend to Fig. 1 or (ii) counting the lymphoid cells in an aliquot of media containing either no interferon, or IFN-α or IFN-γ (see legend to Fig. 2).

Induction of proteins. The induction of proteins in cells by interferon was examined by incubating the cells with either no interferon, or with IFN-α or IFN-γ in the presence of [35S]methionine as described in the legend to Fig. 3. Preparation of cell extracts and fractionation on SDS-polyacrylamide gels was as described previously (Gupta et al., 1979; Rubin & Gupta, 1980a).

RESULTS

Growth inhibitory activity of human IFN-α and IFN-γ preparations

Duplicate sparsely seeded cultures of HeLa cells and U-amnion cells were treated with either no interferon, or with 100 or 300 units/ml of either IFN-α or IFN-γ. These cultures were allowed to incubate for either 3 or 6 days at which time the cells were trypsinized and counted. As can be seen in Fig. 1, at both interferon concentrations, both the HeLa cells and U-amnion cells show a greater sensitivity to the anticellular effects of IFN-γ than they do to the anticellular effects of IFN-α.

The effect of the interferons on the continuous growth of Daudi, Molt-4, GM3638 and GM3639 cells can be seen in Fig. 2. The growth of the Molt-4, GM3638 and GM3639 cells was found to be rather insensitive to the anticellular effects of either IFN-α or IFN-γ. Growth of the Daudi cells was inhibited dramatically at 100 units/ml IFN-α while very little, if any, inhibition of growth was observed when these cells were incubated in the presence of 300 units/ml IFN-γ. The differences in the antiproliferative capabilities of IFN-α and IFN-γ on Daudi cells cannot, apparently, be attributed to the presence of certain agents in these preparations capable of blocking or negating the anticellular effect of interferon, as Daudi cells treated with a mixture of IFN-α and IFN-γ exhibit an inhibition of growth comparable to that observed with IFN-α alone (data not shown). It would also appear that the differences in the sensitivity of the cell lines to the interferons cannot merely be the result of the different culture conditions, as HeLa cells grown in suspension culture exhibit the same sensitivities to the interferons as do HeLa cells growing in monolayers (data not shown).

Induction of proteins in Daudi cells by interferon

In order to study further the response of Daudi cells to IFN-α and IFN-γ, we tested the ability of these interferons to induce the synthesis of any novel proteins (Gupta et al., 1979; Rubin & Gupta, 1980a, b). Daudi cells at a concentration of 2 × 10^5 cells/ml were treated with either no interferon or with 100 units/ml of either IFN-α or IFN-γ in triplicate. Sets of these cultures were incubated in the presence of [35S]methionine for successive 24 h time intervals over a 72 h time period. The cultures were harvested at the end of their respective 24 h labelling periods and analysed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) (Gupta et al., 1979; Rubin & Gupta, 1980a). The autoradiograph shown in Fig. 3 shows the induction, over a 3 day period, of proteins with mol. wt. 88000, 80000, 56000 and 14000 (referred to as P88, P80, P56 and P14.
Antiproliferative effects of interferons

Fig. 1. Anticellular effects of human α and γ interferons on (a) HeLa and (b) U-amnion cells. HeLa and U-amnion cells were seeded into 25 cm² flasks at a density of 10⁵ cells/flask. After 5 h the medium was removed and duplicate sets of cultures received medium containing either no interferon (●), 100 units/ml IFN-α (▲), 300 units/ml IFN-α (●), 100 units/ml IFN-γ (□), or 300 units/ml IFN-γ (■). One set of these cultures was trypsinized on day 3, the other set on day 6, and the number of cells per flask determined.

Fig. 2. Cell growth inhibitory properties of human α and γ interferons on lymphoid cell lines. Suspension cultures of (a) GM3638, (b) Daudi (c) GM3639, and (d) Molt-4 were seeded from exponentially growing cultures at a density of 2 × 10⁵ cells/ml in media containing either no interferon (●), 100 units/ml IFN-α (▲), 300 units/ml IFN-α (●), 100 units/ml IFN-γ (□), or 300 units/ml IFN-γ (■). Samples were removed and counted at intervals as shown.

respectively) in cells treated with IFN-α, whereas treatment of the cells with IFN-γ did not induce any of these proteins. The P88, P80 and P56 proteins induced in the IFN-α-treated Daudi cells were found to co-migrate upon electrophoresis with the P88, P80 and P56 proteins induced in FS₄ cells treated with IFN-α (Gupta et al., 1979) (data not shown). The P14 protein induced in the Daudi cells may be related to the 14.5 K protein reported to be synthesized in Ehrlich ascites tumour cells (Farrell et al., 1979) and has been found to co-migrate upon electrophoresis with a protein induced by interferon treatment of several human tumour cell lines (data not shown). In addition to the synthesis of new proteins in interferon-treated Daudi cells, examination of the autoradiograph shown in Fig. 3 reveals a reduction in the synthesis of several proteins on the second and third day of IFN-α treatment.

DISCUSSION

Data presented in this report demonstrate that preparations of human IFN-γ capable of inhibiting the growth of certain cell lines are incapable of inhibiting the growth of other cell lines. These observations clarify the seemingly contradictory observations reported on the anticellular capabilities of human IFN-γ (Blalock et al., 1980; DeLey et al., 1980; Rubin & Gupta, 1980b). The inability of DeLey et al. (1980) to observe the strong anticellular activity of IFN-γ observed by others (Blalock et al., 1980; Rubin & Gupta, 1980b) was apparently due to their use of target cells resistant to, or relatively resistant to, the antiproliferative effects of IFN-γ.
Fig. 3. Induction of proteins in Daudi cells by human α or γ interferons. Cultures of Daudi cells seeded at a density of $2 \times 10^5$ cells/ml were treated, in triplicate, with either no interferon (control, C), 100 units/ml IFN-α or 100 units/ml of IFN-γ. One set of these cultures was labelled with $^{35}$S]methionine for the first 24 h period (day 1) while the second and third sets of these cultures were incubated in the presence of $^{35}$S]methionine for the second and third 24 h periods (day 2 and day 3) respectively. Following the 24 h labelling periods, the cells were harvested and cell extracts prepared and fractionated as described previously (Gupta et al., 1979; Rubin & Gupta, 1980a). The mol. wt. (shown $\times 10^{-3}$) markers run in parallel are: myosin (200K), phosphorylase b (94K), bovine serum albumin (67K), ovalalbumin (43K), α-chymotrypsinogen (25.7K), β-lactoglobulin (18.4K) and cytochrome c (12.3K). The protein bands induced by interferon are indicated by arrows.
The results presented here allow us to classify cell lines according to their ability to respond to the antiproliferative effects of interferons as follows. (i) Those which respond more strongly to the antiproliferative effect of IFN-α than to the antiproliferative effect of IFN-γ (Daudi); (ii) those which respond more strongly to the antiproliferative effect of IFN-γ than to IFN-α (HeLa, U-amnion); (iii) those which respond poorly to the antiproliferative effects of either IFN-α or IFN-γ (Molt-4, GM3638 and GM3639).

The observation that the Daudi cells are sensitive to the growth inhibitory effect of IFN-α but insensitive to the effect of IFN-γ suggested to us that the Daudi cells may be incapable of responding to IFN-γ. Because we were unable to study the antiviral responsiveness of the Daudi cells, we examined whether these cells were capable of synthesizing the interferon-induced proteins, which can be used as a measure of cellular responsiveness to interferons α, β and γ (Gupta et al., 1979; Rubin & Gupta, 1980a, b). We found that Daudi cells treated with IFN-α synthesized the interferon-induced proteins, while no such induction was found when these cells were treated with IFN-γ. In addition to the synthesis of the interferon-induced proteins, we also observed in response to IFN-α, but not IFN-γ, a reduction in the apparent synthesis of other proteins. The significance of the reduction in the synthesis of certain proteins and the role this plays in the antiproliferative action of IFN-α is currently under investigation.

The inability of IFN-γ to inhibit growth of Daudi cells, which are sensitive to the growth inhibitory capabilities of IFN-α, and the inability of IFN-γ to induce, in Daudi cells, the synthesis of proteins that are synthesized in response to IFN-α show that the cells differ in some way in their response to the two interferons. The reason for the difference is at present unclear but it should be noted that it has been demonstrated that IFN-α and IFN-γ have different receptors (Aguet & Blanchard, 1981; Branca & Baglioni, 1981), so that it is possible that the lack of responsiveness of the Daudi cells to IFN-γ might be the result of these cells not having receptors to IFN-γ. Resolution of this question must await the availability of purified radiolabelled human IFN-γ.

Differences in the responsiveness of cells to different interferons has also been reported with L1210 mouse leukaemia cells which had been selected for their resistance to mouse IFN-β (Gresser et al., 1974). This cell line, which is resistant to the antiviral and antiproliferative effects of IFN-β, and lacks a receptor for IFN-β (Aguet, 1980), has been found to be sensitive to the antiviral and antiproliferative action of IFN-γ (Ankel et al., 1980; Hovanessian et al., 1980). Studies of such cell lines should give a better understanding of the mechanisms by which the different interferons act.

The differential sensitivities of cell lines to the interferons suggest that there may be differences in the therapeutic value of interferons against different tumours. A screening of the sensitivity of different tumour cell lines and freshly derived tumours to the effects of the different human interferons may allow one to predict which tumours will respond clinically to a particular type of interferon.

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REFERENCES


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