Cross-resistance to Human Interferon-\(\gamma\) of Human Interferon-\(\beta\)-resistant F-IF\(^r\) Cells

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SUMMARY

F-IF\(^r\) cells, which are more resistant to the anticellular and antiviral action of human \(\alpha\) and \(\beta\) interferons (IFN-\(\alpha\) and IFN-\(\beta\)) than the parental RSa cells, were also found to be more resistant to both the anticellular and antiviral effect of IFN-\(\gamma\). A high level of 2–5A synthetase was induced by treatment with IFN-\(\alpha\) or -\(\beta\), but induction of 2–5A synthetase was not detected after IFN-\(\gamma\) treatment of the cells. F-IF\(^r\) cells had less than half the number of specific binding sites for IFN-\(\alpha\) than the parental RSa cells.

Human interferons, IFN-\(\alpha\), -\(\beta\) and -\(\gamma\), although distinct antigenically and chemically, all induce 2–5A synthetase and/or protein kinase activities (Lebleu & Content, 1982; Lengyel, 1982) resulting in the development of anticellular and antiviral effects in homologous sensitive cells. Ankel et al. (1980) reported that mouse L1210R cells, resistant to mouse IFN-\(\alpha/\beta\), were sensitive to mouse IFN-\(\gamma\) and suggested that IFN-\(\alpha/\beta\) and \(\gamma\) have different mechanisms of interaction with their target cells. We have established a cell line called RSa from human embryonic cells transformed by Rous sarcoma virus and simian virus 40 and found the cell line to be highly sensitive to the anticellular and antiviral action of IFN (Kuwata et al., 1976a). We have also reported on the characteristics of a line of IFN-\(\beta\)-resistant cells (F-IF\(^r\)), which was selected from RSa cells by growth in IFN-\(\beta\) after treatment with 100 \(\mu\)g/ml ethyl methanesulphonate for 24 h (Kuwata et al., 1979). F-IF\(^r\) cells have been treated only with IFN-\(\beta\). Another variant cell line from RSa called IF\(^r\), which was selected by treatment with IFN-\(\alpha\) and was resistant to the anticellular action of IFN-\(\alpha\), was found to be resistant to IFN-\(\beta\) and also to IFN-\(\gamma\) (Verhaegen-Lewalle et al., 1982). In the present paper, we report that the primarily IFN-\(\beta\)-resistant F-IF\(^r\) cells show a higher resistance to IFN-\(\gamma\) than do RSa cells.

The transformed cell lines, RSa and F-IF\(^r\), were grown in Eagle's MEM supplemented with 10% calf serum at 37 °C in humidified 5% CO\(_2\). IFN-\(\alpha\) with a specific activity of \(6 \times 10^6\) IU/mg protein was a generous gift from Dr K. Cantell. IFN-\(\beta\) (sp. act. \(6 \times 10^5\) IU/mg protein) was supplied by Dr A. Billiau and IFN-\(\gamma\) (sp. act. \(10^5\) U/mg protein) was supplied by the Green Cross Company, Osaka, Japan. The antiviral activity of the IFNs was measured by reduction of c.p.e. in cells challenged with vesicular stomatitis virus (VSV), and the anticellular effect of IFN was determined by counting viable cells and was expressed as percentage of the control when the cells were treated with various amounts of IFN (Kuwata et al., 1976b). Titres of IFN-\(\alpha\) and -\(\beta\) were measured by the inhibition of c.p.e. of VSV in FL cells and expressed as international reference units/ml. Titres of IFN-\(\gamma\) were measured by the inhibition of encephalomyocarditis virus cytopathogenicity and expressed as laboratory units/ml.

The effects of human IFN-\(\alpha\), -\(\beta\) and -\(\gamma\) on the growth of RSa and F-IF\(^r\) cells were examined (Fig. 1). RSa cells were highly sensitive to IFN-\(\alpha\), but even more sensitive to IFN-\(\beta\) as reported previously (Kuwata et al., 1979). RSa cells were less sensitive to IFN-\(\gamma\) than to IFN-\(\alpha\) and -\(\beta\), but 1000 U/ml IFN-\(\gamma\) suppressed their growth to 3.3% of the control. Conversely, the growth of F-IF\(^r\) cells was relatively resistant not only to IFN-\(\alpha\) and -\(\beta\) but also to IFN-\(\gamma\). Since RSa cells were sensitive to IFN-\(\alpha\) and -\(\beta\), the presence of small amounts of such IFNs in the IFN-\(\gamma\) preparation...
Fig. 1. Comparison of the anticellular effects of IFNs on the growth of RSa (●) and F-IFr (▲) cells and induction of 2-5A synthetase activity by IFNs. Cells were treated with various concentrations of IFN-α (a), IFN-β (b) and IFN-γ (c) for 6 days, detached with trypsin and viable cells were counted. Suppression of cell growth was expressed as a percentage of viable cells in control cultures. 2-5A synthetase activity in RSa (●) and F-IFr (▲) cells was expressed as radioactivity incorporated into synthesized 2-5A (Morinaga et al., 1983).

Fig. 2. Comparison of the antiviral action of IFNs in RSa (●) and F-IFr (▲) cells. The inhibition of VSV growth by various amounts of IFN-α (a), IFN-β (b) and IFN-γ (c) was measured.

(Wiranowska-Stewart, 1981; de Ley et al., 1980) might lead to imprecise results. Therefore, 1000 U/ml of the IFN-γ preparation was pretreated with 1000 U/ml of anti-IFN-α or -β serum or medium for 1 h at 37°C to exclude the effect of contamination by other types of IFN, and the
residual anticellular activity of IFN-γ preparation was measured. In these tests, we could not detect any significant decrease of anticellular activity of IFN-γ in both cell types after treatment with these antisera (results not shown). These results indicate that contamination of IFN-γ preparation with the other types of IFN was negligible. The antiviral effects of IFNs in these cells were also examined (Fig. 2). We compared the VSV yield in RSa cells with that in F-IFc cells after the cells were treated with various concentrations of IFN. Treatment with IFN-α inhibited VSV replication by 5-3 log10 units in RSa cells, whereas it reduced VSV titres by only 2-0 log10 units in F-IFc cells (Fig. 2a). This indicated that F-IFc cells were more resistant than RSa cells. F-IFc cells were as sensitive to the antiviral activity of IFN-α as their parental cells at early passage (Kuwata et al., 1979), but after continuous passages, the cells appear to have decreased sensitivity. IFN-β showed a much higher antiviral activity than IFN-α, but F-IFc cells were also found to be relatively less sensitive to the antiviral activity of IFN-β than RSa cells (Fig. 2b). In contrast, IFN-γ had much less antiviral activity in RSa cells and it was able to reduce VSV titres only up to 1-7 log10 units (Fig. 2c), whereas we could not detect any significant effect of IFN-γ on the multiplication of VSV in F-IFc cells. Thus, F-IFc cells are less sensitive than RSa cells to the antiviral activity of all three types of IFN.

According to Ankel et al. (1980), mouse leukaemia L1210R cells, which are resistant to IFN-α/β, are sensitive to mouse IFN-γ. Likewise, murine sarcoma virus-transformed mouse cells which were selected for their resistance to the antiviral action of IFN-α/β, were reported to be sensitive to mouse IFN-γ (Bourgeade et al., 1980). In contrast to their results, our data showed that the human transformed F-IFc cells, which were resistant to IFN-β, were also resistant to IFN-α and IFN-γ. In the case of L1210R cells, they are completely resistant to IFN-β and neither 2-5A synthetase nor protein kinase is induced after IFN treatment (Hovanessian et al., 1980). In F-IFc cells, the same level of 2-5A synthetase was induced as in RSa cells by treatment.
with IFN-\( \beta \) (Fig. 1b), and in the case of IFN-\( \alpha \), the level of 2-5A synthetase in F-IF\(^r\) cells was about 40% of that in RSa cells (Fig. 1a). However, when RSa cells were treated with IFN-\( \gamma \) only a low level of 2-5A synthetase was induced as reported by Verhaegen-Lewalle et al. (1982) and in F-IF\(^r\) cells no significant 2-5A synthetase was induced (Fig. 1c). These results indicate that the anticytotoxic effect of IFN does not necessarily parallel the level of 2-5A synthetase.

The initial step of IFN action involves its binding to a specific high-affinity cell surface receptor (Aguet, 1980; Aguet & Blanchard, 1981). In an analysis of the binding of labelled IFN, Agu et al. (1982) showed that mouse IFN-\( \alpha/\beta \) and IFN-\( \gamma \) do not share a common receptor, and in human Daudi cells, Branca & Baglioni (1981) showed that IFN-\( \beta \), but not IFN-\( \gamma \), inhibits the binding of labelled IFN-\( \alpha \) to the cells. These data suggest that two types of IFN receptor are present, namely one for IFN-\( \alpha/\beta \) and the other for IFN-\( \gamma \). On the other hand, Anderson et al. (1982) examined the binding of labelled IFN-\( \gamma \) to human fibroblast cells and found that the binding is completely inhibited by the presence of unlabelled IFN-\( \gamma \) or IFN-\( \beta \), although to a lesser degree, but not by the presence of IFN-\( \alpha \). This indicates that a specific receptor for IFN-\( \gamma \) exists on human fibroblasts and that IFN-\( \beta \) can also bind to this receptor. Our results are consistent with this report. The specific binding of IFN-\( \alpha \) to the F-IF\(^r\) cells was examined by using \(^{3}H\)leucine-labelled IFN-\( \alpha \) (Yonehara et al., 1983a, b). From Fig. 3, the \( K_D \) value for RSa cells was calculated to be \( 7.7 \times 10^{-12} \) and there were no significant differences between the \( K_D \) values of RSa and F-IF\(^r\) cells. The number of receptor sites of F-IF\(^r\) cells was estimated to be 290 (Fig. 3b); less than half of those of RSa cells, which were reported to be 780 (Fig. 3a) (Morinaga et al., 1983). By using \(^{125}I\)-labelled IFN-\( \alpha A \), the number of binding sites on F-IF\(^r\) cells has been found to be 16.6% and that on IF\(^r\) cells to be 32.1% of RSa cells respectively (Fuse et al., 1984). However, numbers of IFN-specific binding sites do not always correlate with the sensitivity of cells to IFNs (Yonehara et al., 1983a), and the exact mechanism of IFN resistance in F-IF\(^r\) cells awaits further studies.

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Short communication


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