Isolation of Daudi Cells with Reduced Sensitivity to Interferon. IV. Characterization of Clones with Altered Binding of Human Interferon α Subspecies

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SUMMARY

Three clones of Daudi cells, DIF3, DIF8 and DIF9, have been isolated which possess specific interferon (IFN) receptors even though these cells are resistant to both the antiviral and antiproliferative actions of human IFN-α. Studies with 125I-labelled cloned human IFN-α subspecies showed that clone DIF3 bound IFN in a manner identical to that of the IFN-sensitive parental cells. The initial peak of binding observed 30 min after treatment of either Daudi or DIF3 cells at 37 °C with IFN-α2 or IFN-α6 was, however, either absent or much reduced when using DIF8 cells which are resistant to the antiproliferative action of IFN-α2 and -α8. Similarly, no initial peak of binding was observed after treatment of DIF9 cells with IFN-α2 at 37 °C. In accord with its reduced biological activity, the binding of 125I-IFN-α1 to either Daudi or DIF3 cells was considerably lower than that of either of the other two IFN-α subspecies and followed the form of a hyperbola without an initial transitory peak of binding. It is suggested that the phenotype of IFN resistance of DIF8 and DIF9 cells may be related to a defect in receptor activation whereas the same phenotype of DIF3 cells would appear to be unrelated to IFN binding.

INTRODUCTION

Interferons (IFNs) are a group of closely related proteins which interact with specific cell surface receptors to exert a wide range of biological effects on cells. IFNs comprise three types, of which two (α and β) bind to a common site on the cell surface, but the third (γ) binds to a separate receptor (Branca & Baglioni, 1981; Raziuddin et al., 1984). Individual IFN-α subspecies share a common binding site, but exhibit different binding affinities which correlate with their respective specific biological activities (Aguet et al., 1984; Uzé et al., 1985). Very little is known, however, about the events which lead from the initial interaction of IFNs with their cell surface receptors to the subsequent activation of certain genes involved in the development of IFN action, although recent findings suggest that IFNs may induce at least some of their various effects via receptor activation (Mogensen & Bandu, 1983; Yonehara et al., 1983).

We have recently described the isolation of clones of the highly IFN-sensitive cell line Daudi, selected in the presence of progressively increasing concentrations of human IFN-α, which are resistant to both the antiviral and antiproliferative actions of human IFN-α and -β (Dron & Tovey, 1983; Dron et al., 1985). Although one clone (DIF3) of IFN-resistant Daudi cells has been shown to possess specific IFN receptors (Tovey et al., 1983) and to exhibit enhanced levels of 2′-5′-oligo-isoadenylate synthetase activity in response to IFN treatment, a number of proteins induced by IFN in parental Daudi cells are not induced in this or other IFN-resistant clones (Dron et al., 1985). It was of interest, therefore, to determine whether the phenotype of IFN resistance of these clones was related to differences in the binding of IFN-α subspecies. We report here that of three clones of Daudi cells with a similar phenotype of IFN resistance, one clone bound IFN in a manner similar to the IFN-sensitive parental Daudi cells whereas the other two clones exhibited differences in the binding of certain IFN-α subspecies.
Methods

Cells and cell culture. The isolation and characterization of IFN-resistant Daudi cells have been described previously (Dron et al., 1985). All cells were cultivated in RPMI 1640 medium with 15% foetal calf serum (Flow Laboratories).

IFN preparations. Cloned human IFN-α1 and -α2 of specific activity 1·1 × 10⁸ and 2·0 × 10⁸ International Units/mg protein respectively, were a gift from Professor C. Weissman (Zürich, Switzerland). Cloned human IFN-β (α9) of specific activity 2·0 × 10⁸ International Units/mg protein was a gift from Professor J. Nüesch (Ciba-Geigy, Basel, Switzerland). IFN-α1, -α2 and -α8 were labelled with 125I and purified as previously described (Mogensen & Uzé, 1985); they had titres of 5 × 10⁴, 1 × 10⁵ and 2 × 10⁴ International Units/ml and a radioactive content of 110, 110 and 55 μCi per μg IFN protein respectively at the time of use.

Binding experiments were carried out as described previously (Mogensen et al., 1981). Briefly, exponentially growing cells were incubated for the times indicated with different concentrations of 125I-labelled IFN-α2 at either 4 or 37 °C and then immediately placed in ice. A 4·5 ml amount of cell suspension (2·0 × 10⁵ cells/ml) per point was centrifuged at 4 °C and washed twice with 4 ml RPMI 1640 medium containing 1% foetal calf serum. Non-specific binding determined at 37 °C in the presence of a 100-fold excess of unlabelled IFN was less than 5% for IFN-α2 and -α8 and about 35% for IFN-α1. Cell pellets were used for counting (LKB Rackgamma 1270, 80% efficiency). Bound radioactive IFN was expressed either as pg per 10⁶ cells taking a mean mol. wt. of 20000 for all the IFN subspecies, or as a concentration (pM) determined as described previously (Uzé et al., 1985).

Results

Effect of different human IFN-α subspecies on the multiplication of cloned Daudi cell variants

The multiplication of parental Daudi cells exhibited a sensitivity to cloned human IFN subspecies α2 or -α8 (Fig. 1) similar to multiplication in the presence of human leukocyte IFN (a mixture of IFN-α subspecies) or human IFN-β (Dron & Tovey, 1983). In agreement with previous reports (Aguet et al., 1984; Uzé et al., 1985) cloned IFN-α1 was found to be at least 20-fold less active on Daudi cells than the other IFN-α subspecies (Fig. 1).

Clones DIF3, DIF4 and DIF9 are resistant to both the antiviral and antiproliferative actions of human IFNs (Dron et al., 1985). Clone DIF8 has also been shown to be resistant to the antiproliferative action of cloned human IFN subspecies α1, -α2 and α8 (Fig. 1). The phenotype of IFN resistance of DIF8 cells was maintained for at least 7 months following cultivation of the cells in the absence of IFN (unpublished results).

Binding of 125I-labelled human IFN-α subspecies to Daudi cells and IFN-resistant clones

The binding of 125I-labelled IFN-α subspecies was studied at a concentration at which the multiplication of Daudi cells was completely inhibited and that of IFN-resistant cells hardly affected (25 pM for IFN-α2 and IFN-α8 and 220 pM for IFN-α1). At 37 °C both IFN-α2 and -α8 bound specifically to Daudi cells with an initial peak of binding at 30 to 60 min, followed by a gradual reduction in the amount of bound material in the ensuing 2 to 3 h (Fig. 2a).

IFN-α2 and -α8 were also found to bind specifically to IFN-resistant DIF8 cells although an initial peak of binding was observed only for IFN-α8, and then in a much reduced form (Fig. 2b).

IFN-α1 was found to bind to both Daudi and DIF8 cells in a manner markedly different to that of either IFN-α2 or IFN-α8 (Fig. 2). Thus, even in the presence of a concentration of free IFN-α1, tenfold greater than that of either IFN-α2 or IFN-α8, the amount of IFN-α1 bound to either Daudi or DIF8 cells was only 30 to 50% of that of either the other two IFN-α subspecies (Fig. 2). Furthermore, binding of 125I-labelled IFN-α1, to both Daudi and DIF8 cells followed the form of a hyperbola without the initial transitory peak of binding observed with the other IFN-α subspecies (Fig. 2).

In order to determine how the phenotype of IFN resistance was associated with altered binding of an IFN subspecies the kinetics of binding of 125I-labelled IFN-α was studied in detail for both parental and IFN-resistant Daudi cells.

Time course of binding of 125I-labelled IFN-α2 to parental and IFN-resistant Daudi cells

Parental Daudi cells exhibited specific binding of 125I-labelled IFN-α2, both at physiological (37 °C) and low temperatures (4 °C). Similar kinetics of binding of 125I-labelled IFN-α2 at 37 °C were observed for five IFN-sensitive clones of Daudi cells isolated in the absence of any
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Fig. 1. Effect of different human IFN-α subspecies on the multiplication of cloned Daudi cell variants. Cells were seeded at $1 \times 10^5$ cells/ml in RPMI 1640 medium with 15% foetal calf serum in the presence of the indicated concentration of a particular purified cloned IFN-α subspecies. (The specific activities of the cloned IFNs were the same as those of the radiolabelled IFN preparations quoted in Methods.) Twenty-five pm of human IFN-α2 or -α8 is equivalent to a concentration of 100 International Units/ml. Cell concentration was determined in triplicate with a model ZB-1 Coulter counter after 4 days in culture. Results are expressed as a percentage of the cell concentration of the untreated control culture. Parental Daudi cells were treated with IFN-α1 (△), IFN-α2 (○) or IFN-α8 (□). IFN-resistant DIF_8 cells were also treated with IFN-α1 (△), IFN-α2 (○) or IFN-α8 (□).

Fig. 2. Binding of $^{125}$I-labelled human IFN-α subspecies to Daudi and DIF_8 cells. Exponentially growing Daudi cells (a) or DIF_8 cells (b) were treated with 25 pm $^{125}$I-labelled IFN-α2 (△) or IFN-α8 (■) or 220 pm $^{125}$I-labelled IFN-α1 (○). IFN binding was then determined as described in Methods at the times indicated.
selective pressure (Fig. 3a). However, both the kinetics of binding and the amount of IFN bound varied with temperature. At 4 °C, the amount of IFN bound to the cells increased progressively for the first 3 to 4 h of incubation in the presence of 25 pM of free IFN to attain approximately 20% of that bound at 37 °C (Fig. 3b). At 37 °C binding was maximal after 30 to 60 min of incubation in the presence of the same concentration of free IFN (Fig. 3b). The amount of bound IFN then decreased progressively in the ensuing hours (Fig. 3b).

Human 125I-labelled IFN-α2 also bound in a specific manner to the IFN-resistant clones DIF3, DIF8, and DIF9. DIF3 cells appeared to bind IFN-α2 in a manner identical to that of parental Daudi cells (Fig. 3c). However, clones DIF8 and DIF9 did not exhibit the initial peak of IFN binding observed when either parental Daudi cells or DIF3 cells were treated with 125I-labelled IFN-α2 at 37 °C (Fig. 3b, c). At the lower temperature (4 °C) the form of the time course of specific binding for all three IFN-resistant clones was very similar to that of the parental cells incubated in the presence of the same concentration of free IFN (25 pM), although the amount of IFN bound by the resistant cells at a given time was approximately 1.5-fold less than that bound by the IFN-sensitive cells as shown for DIF8 in Fig. 3. At 37 °C, however, the time course of binding of 125I-IFN-α2 to DIF8 and DIF9 cells was markedly different to that of parental Daudi cells. The parental cells exhibited an initial peak of binding at 30 min followed by a reduction of approximately 40% of bound material in the ensuing 2 h whereas no such initial peak of interferon binding was seen in those two resistant clones in which binding of 125I-IFN-α2 followed the form of a hyperbola (Fig. 3b, c). In common with the phenotype of IFN resistance of DIF8 cells, the curve of IFN binding remained unchanged when tested after prolonged cultivation of the cells in the absence of IFN (data not shown).

The characteristic time course of binding of IFN to parental Daudi cells was observed in five independent experiments in the presence of 25 pM of free IFN. In the presence of 250 pM of free IFN, both Daudi and DIF8 cells retained their characteristic curves of IFN binding even though the amount of IFN bound by each of these cell types increased two- to threefold (data not shown).

**Analysis of the binding of 125I-IFN-α2 to Daudi and DIF8 cells**

Parental Daudi cells incubated at 37 °C in the presence of increasing concentrations of 125I-IFN-α2 exhibited specific binding (Fig. 4). At low concentrations of free IFN, more IFN was
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Fig. 4. Saturation isotherms of the binding of 125I-labelled IFN-α2 to Daudi cells. Parental Daudi cells were treated for either 30 min (○), 4 h (●) or 24 h (□) at 37 °C with the concentration of 125I-labelled IFN-α2 indicated in the figure (32 pM human IFN-α2 is equivalent to a concentration of 128 International Units/ml).

Fig. 5. Saturation isotherms of the binding of 125I-labelled IFN-α2 to IFN-resistant DIF8 cells. Cells were treated for either 30 min (○), 4 h (●) or 24 h (□) at 37 °C with the concentration of 125I-labelled IFN-α2 indicated in the figure (32 pM human IFN-α2 is equivalent to a concentration of 128 International Units/ml).

bound to Daudi cells at 4 h than at 30 min, but at high IFN concentrations less IFN remained bound to the cells at 4 h than at 30 min. Furthermore, at 24 h the amount of bound IFN was only 30 to 40% of that bound at 4 h (Fig. 4).

IFN-resistant DIF8 cells incubated at 37 °C in the presence of increasing concentrations of 125I-IFN-α2 exhibited specific binding curves of the same overall form as those of the IFN-sensitive parental cells (Fig. 5).

The amount of specific binding exhibited by the IFN-resistant cells at 30 min was, however, consistently lower (1.5- to 2.5-fold) than that exhibited by parental Daudi cells at all the IFN concentrations tested (Fig. 4, 5). In contrast, at 4 and 24 h, DIF8 cells showed higher specific binding than parental Daudi cells (Fig. 4, 5).

DISCUSSION

Cells have been described for which the phenotype of IFN resistance has been shown to be due to a lack of functional IFN receptors (Aguet & Blanchard, 1981); other cells have been characterized which are resistant to certain actions of IFN even though they possess IFN receptors (Affabris et al., 1983; Fuse et al., 1984; Aguet et al., 1981; Mogensen et al., 1981). The binding of IFN to its cell surface receptor is, however, a complex process which may involve an activation step (Mogensen & Bandu, 1983; Sarkar & Gupta, 1984). Thus, whether or not cells bind IFN may not be a sufficient indication of the presence of a functional and complete IFN receptor complex.

We have recently described the isolation of clones of Daudi cells which are resistant to both the antiviral and antiproliferative actions of human IFN-α/β even though these cells possess specific IFN receptors and respond to IFN treatment by the production of elevated levels of 2'–5' oligo-isoadenylate synthetase (Tovey et al., 1983). In this study, we have confirmed and extended these observations to show that in common with parental Daudi cells the IFN-resistant clones exhibit specific binding of individual cloned IFN-α subspecies even though these cells are resistant to the action of these IFNs. Indeed, clone DIF3 appeared to bind individual IFN-α subspecies in a manner identical to that of parental Daudi cells. However, the initial transient peak of binding of IFN-α2 and -α4 to Daudi cells observed at physiological temperature was either absent or much reduced in two other resistant clones, DIF8 and DIF9.
Mogensen & Bandu (1983) have presented evidence to suggest that the initial transient peak of binding of IFN-α₂ to Daudi cells may reflect an initial binding of IFN to surface receptors followed by its transfer to an activation complex on the cell membrane. An initial transient peak of binding at 37 °C could also reflect internalization and receptor down-regulation (Faltynek et al., 1985) although this would seem unlikely since no internalization or degradation of radiolabelled IFN was detected in extracts of Daudi cells 30 min after IFN treatment and only minor degradation was detected at later times (Eid & Mogensen, 1983).

It is tempting to speculate, therefore, that the phenotype of IFN resistance of DIF₈ and DIF₉ cells may be related to a defect in receptor activation, although such a defect would not appear to involve a major change in the structure of the IFN receptor since the IFN receptor complex of the IFN-resistant cells exhibited the same molecular weight as that of parental Daudi cells when analysed by Sephacryl S-400 chromatography. The IFN receptor complex of the IFN-resistant cells also exhibited protein kinase activity in common with that of parental Daudi cells (M. Dron & P. Eid, unpublished results). It is possible, however, that the apparent differences in IFN binding observed 24 h after IFN treatment may be due at least in part to differences in internalization and receptor down-regulation between parental Daudi cells and the IFN-resistant clones.

Hannigan et al. (1984) have also reported differences in the binding of IFN-α₂ in an independently isolated line of IFN-resistant Daudi cells which possess specific IFN receptors and respond to IFN treatment by the production of elevated levels of 2′-5′-oligo-isoadenylate synthetase (Silverman et al., 1982). The authors suggested that the phenotype of IFN resistance of these cells involved the loss of a high affinity interaction between cellular receptors and IFN-α₂. It should be emphasized, however, that impaired receptor activation cannot account for all cases of IFN resistance, since DIF₃ cells exhibit an initial peak of IFN binding similar to that of parental Daudi cells and yet are resistant to both the antiviral and antiproliferative actions of IFNs.

Our results suggest that the treatment of cells with progressively increasing concentrations of IFN can give rise both to cells in which IFN resistance is associated with altered binding of IFN and also cells with unimpaired IFN binding in which IFN resistance would appear to be situated at another level.

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