Isolation of Daudi Cells with Reduced Sensitivity to Interferon. II. On The Mechanisms of Resistance

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SUMMARY

The mechanism of interferon resistance was studied in two clones of Daudi cells, DIF 2 and DIF 3, which exhibit respectively moderate and pronounced resistance to both the antiviral and antiproliferative actions of human interferons-α and -β. Clones DIF 2 and DIF 3 were found to possess specific high affinity interferon receptors similar to those of parental Daudi cells. However, DIF 2 cells, which have a tetraploid karyotype, had approximately twice as many interferon-binding sites as either DIF 3 or parental Daudi cells. One of the first detectable changes in Daudi cells following interferon treatment is a rapid increase in the intracellular concentration of cyclic GMP. No increase in cyclic GMP was observed in DIF 2 or DIF 3 cells treated with interferon-α. However, neither DIF 2 nor DIF 3 cells respond to sodium azide, a non-physiological inducer of cyclic GMP. Interferon treatment was found to induce the production of 2'-5'-oligo-isoadenylate synthetase in DIF 2 and DIF 3 cells in a manner similar to parental Daudi cells, indicating that these cells possess functional interferon receptors. The levels of 2'-5'-oligo-isoadenylate synthetase and 2'-5' A phosphodiesterase activity were similar in all three cell lines, suggesting that the interferon resistance of clones DIF 2 and DIF 3 was not due to a deficiency of pp(A2' p)nA.

INTRODUCTION

In the previous article (Dron & Tovey, 1983) we have described the isolation and characterization of two clones of Daudi cells with markedly reduced sensitivity to both the antiviral and antiproliferative actions of interferon-α and -β. Clone DIF 3 exhibits a slight decrease in growth rate in the presence of 105 international units/ml of interferon-α, whereas the multiplication of parental Daudi cells is completely inhibited by only 10 units/ml of interferon-α. Clone DIF 2 is of intermediate interferon sensitivity. In this article we describe experiments designed to elucidate the mechanisms of interferon resistance of these cells.

METHODS

Cells and cell culture. The isolation and characterization of clones DIF 2 and DIF 3 were described by Dron & Tovey (1983). All cells were cultivated in RPMI 1640 medium with 15% foetal calf serum (Flow Laboratories, Irvine, Scotland).

Interferon preparations. The interferon preparations used were the same as those described in the previous article. Purification and radiolabelling of human interferon-α from Namalwa cells were carried out as described by Mogensen et al. (1981). 125I-IFN-α had a titre of 16000 international units/ml and a radioactive content of 75 μCi/μg IFN protein at the time of the experiment. Exponentially multiplying cells were incubated for 2 h with different concentrations of 125I-IFN-α at 37 °C and then immediately placed on ice. Two ml of cell suspension per point was centrifuged at 4 °C and washed twice with 4 ml of RPMI 1640 medium containing 1% foetal calf serum. Cell pellets were transferred for counting (LKB Rackgamma 1270, 80% efficiency). Bound radioactivity is expressed per 106 cells.
Assay of 2'-5'-oligo-isoadenylate synthetase. Cell extracts were mixed with 50 µl of poly(rI)·(rC) agarose (P-L Biochemicals) for 15 min at 30°C and the non-adsorbed material was removed by centrifugation. The poly(rI)·(rC)-adsorbed material was incubated with 2.5 mM[α-32P]ATP, 400 Ci/mmol (Amersham International) for 20 h at 30°C, treated with bacterial alkaline phosphatase (Sigma) and then eluted from a column of acid alumina (Sigma) (300 µl) with 3.0 ml of 1 M-glycine-HCl buffer pH 2.0 as previously described (Merlin et al., 1981). Results are expressed as pmol ATP incorporated per h per 10⁵ cells.

Cyclic nucleotide determination. One ml of cell suspension was treated with 1 ml of 12% trichloroacetic acid for 15 min at 0°C, centrifuged (2000 g, 10 min) and the supernatant was extracted with ether, purified on a column of Dowex AG1X8 (Murad et al., 1971) and assayed for cAMP content using a protein-binding assay as previously described (Rochette-Egly & Castagna, 1977), and for cyclic GMP content using a radioimmune assay (Amersham International). Recovery (70 to 80%) was monitored with 1 nCi of [3H]cyclic AMP or [3H]cyclic GMP. Results are expressed as the mean of four replicates in pmol per 10⁶ cells.

RESULTS

Binding of ¹²⁵I-interferon-α to Daudi cells and interferon-resistant clones

To determine whether the interferon resistance of clones DIF₂ and DIF₃ was due to the absence of functional interferon receptors the direct binding at 37°C of radiolabelled IFN-α at equilibrium to clones DIF₂ and DIF₃ was compared with that to parental Daudi cells (Fig. 1).

Binding of ¹²⁵I-IFN-α to all three cell lines was saturable and was inhibited by an excess of unlabelled interferon. Scatchard analysis of the binding of labelled interferon-α gave linear plots and the binding constants for site saturation and affinity accorded well with the previously published values for parental Daudi cells (Mogensen et al., 1981). The binding curves for clone DIF₃ and wild-type Daudi cells were indistinguishable. Clone DIF₂ however, bound

![Graph](image-url)  

Fig. 1. Direct binding of ¹²⁵I-interferon-α to Daudi, DIF₂ and DIF₃ cells. The curves show the binding of human IFN-α (Namalwa, sp. act. ≥ 2 x 10⁸ reference units/mg protein) at 2 h equilibrium to parental Daudi cells (○), clone DIF₂ (△), and clone DIF₃ (○). The symbols indicate the actual experimental values and the curves were calculated from the linear regressions of the Scatchard plots (Scatchard, 1949). The background of non-specific binding (< 5% of specific binding) has been subtracted from the values presented.
Table 1. The effect of human interferon-α on the intracellular concentration of cyclic GMP in cloned Daudi cells*

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Treatment</th>
<th>Cyclic GMP (pmol/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>2.13 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>Interferon</td>
<td>3.04 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Sodium azide</td>
<td>3.76 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>1.92 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>Interferon</td>
<td>2.16 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>Sodium azide</td>
<td>2.23 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>2.00 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Interferon</td>
<td>1.08 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Sodium azide</td>
<td>1.10 ± 0.17</td>
</tr>
</tbody>
</table>

* Each cell type (2 × 10^6 cells/ml) was treated with either 10^4 units/ml of electrophoretically pure human interferon-α (sp. act. 2 × 10^8 reference units/mg protein) in phosphate-buffered saline (PBS), or with an equal volume of PBS alone, or with 10 mM-sodium azide. Samples (1.0 ml) of cell suspension were then taken at various times after treatment and assayed for cyclic GMP content as described in Methods. The cyclic GMP concentrations shown in the table are the values obtained at 5 min after treatment.

† Determined by Student’s t-test.
‡ NS, Not significant.

approximately twofold more interferon than either clone DIF3 or parental Daudi cells. The increased binding on DIF2 cells may be related to the tetraploid karyotype of these cells (Mogensen et al., 1982; Epstein et al., 1982).

Determination of the influence of soluble factor(s) produced by DIF3 cells on the interferon sensitivity of Daudi cells

To determine whether the phenotype of interferon resistance was due to the production by DIF3 cells of a soluble factor capable of either inactivating interferon at the cell surface or inhibiting its action, Daudi cells were treated with interferon in the presence of increasing concentrations of nutrient medium in which DIF3 cells had been cultivated. This treatment had no effect on the sensitivity of Daudi cells to the antiproliferative action of interferon (data not shown).

Induction of cyclic nucleotides

The binding of interferon to its specific cell surface receptor is followed by a rapid increase in the intracellular concentration of cyclic GMP (Tovey et al., 1979; Tovey & Rochette-Egly, 1981; Tovey, 1982). Treatment of Daudi cells with electrophoretically pure IFN-α caused a rapid increase in the intracellular concentration of cyclic GMP within 1 to 5 min (Table 1). A similar increase in the intracellular concentration of cyclic GMP was observed when Daudi cells were treated with sodium azide, a non-physiological activator of guanylate cyclase (Table 1). No increase in cyclic GMP was observed when clones DIF2 and DIF3 were treated with either IFN or sodium azide (Table 1). Indeed, some decrease in the intracellular concentration of cyclic GMP was observed in DIF3 cells following treatment with either interferon or sodium azide (Table 1).

2′-5′-Oligo-isoadenylate synthetase activity in Daudi cells and interferon-resistant clones

In agreement with previous reports (Krishnan & Baglioni, 1980; Tomita et al., 1982; Silverman et al., 1982) Daudi cells were found to contain high levels of endogenous 2′-5′-oligo-isoadenylate synthetase activity (Fig. 2), which was enhanced several-fold following treatment with highly purified human interferon-α (Fig. 2). The level of endogenous 2′-5′-oligo-isoadenylate synthetase activity in DIF2 and DIF3 cells was similar to that found in parental Daudi cells although in all three cell lines the basal level of the enzyme varied considerably from one experiment to another. An increase in 2′-5′-oligo-isoadenylate synthetase activity was also observed in both DIF2 and DIF3 cells following interferon treatment (Table 2). The level of 2′-5′ phosphodiesterase activity was similar in all three cell lines and did not change significantly following interferon treatment (data not shown).
Fig. 2. The effect of interferon-α on the level of 2′-5′-oligo-isoadenylate synthetase in Daudi and DIF3 cells. Daudi or DIF3 cells were seeded at $2 \times 10^5$ cells/ml in RPMI 1640 medium with 10% foetal calf serum containing the concentration of electrophoretically pure human interferon-α (sp. act. $2 \times 10^8$ reference units/mg protein) indicated. After 24 h treatment, $5 \times 10^6$ cells per sample were centrifuged (9000 rev/min, 2 min) and resuspended in 500 μl of cold lysis buffer (20 mM-HEPES pH 7.5, 5 mM- MgCl₂, 120 mM-KCl, 7 mM-dithiothreitol, 10% glycerol, 0.5% Nonidet P40) and incubated for 2 min on ice. The samples were then centrifuged (9000 rev/min, 8 min, 4 °C) and the supernatant was recovered and assayed for 2′-5′-oligo-isoadenylate synthetase activity as described in Methods. (a) Parental Daudi cells, (b) clone DIF2, (c) clone DIF3; □, untreated, ■, interferon-treated.

**DISCUSSION**

Cells have been isolated in which the phenotype of interferon resistance has been shown to be due to the lack of a functional receptor for interferons-α and -β (Aguet, 1980; Aguet & Blanchard, 1981). Receptor binding studies carried out at 37 °C, where binding is a function of the activity of the interferon–receptor complex (Mogensen & Bandu, 1983), suggest that the interferon-carrying capacity of the cellular receptors is unimpaired in the interferon-resistant Daudi cell clones, DIF2 and DIF3.

The interferon-resistant clones, in contrast to parental Daudi cells, do not exhibit elevated levels of cyclic GMP following interferon treatment. However, these cells do not respond either to sodium azide a non-physiological inducer of cyclic GMP, and it remains to be established whether these changes are related to the phenotype of interferon resistance.

Interferon induces a number of cellular proteins including 2′-5′-oligo-isoadenylate synthetase, a double-stranded RNA-activated enzyme that synthesizes a series of oligonucleotides of general structure ppp(A₂pₙ)ₐ. The products of the synthetase activate at subnanomolar concentrations an endoribonuclease leading to inhibition of protein synthesis. This system has been implicated in both the antiviral and antiproliferative actions of interferon (Kerr et al., 1981; Lengyel, 1981; Lebleu & Content, 1982). The levels of both endogenous and interferon-induced 2′-5′-oligo-isoadenylate synthetase activity, and ppp(A₂pₙ)ₐ degradation are similar in Daudi cells and the interferon-resistant clones, suggesting that the decreased interferon sensitivity of DIF2 and DIF3 cells is not due to a lack of ppp(A₂pₙ)ₐ. However, this remains to be established by direct measurement of intracellular ppp(A₂pₙ)ₐ in virus-infected or growth-arrested cells. Silverman et al. (1982) were unable to detect ppp(A₂pₙ)ₐ in interferon-treated wild-type or resistant Daudi cells isolated by a different procedure, again arguing against an
involvement of the ppp(A2'p)nA system in the interferon resistance of Daudi cells. We are currently investigating whether any correlation exists between the production of other interferon-induced proteins and the loss of interferon sensitivity of DIF3 cells.

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