Isolation of Daudi Cells with Reduced Sensitivity to Interferon.
I. Characterization

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SUMMARY

Treatment of Daudi cells with successively increasing concentrations of interferon-α resulted in the selection of a cell population which multiplied in the continued presence of $10^4$ units/ml of interferon-α. A number of clones of interferon-resistant Daudi cells were isolated from this population. Two clones, DIF₂ and DIF₃, were found to exhibit moderate and pronounced resistance, respectively, to both the antiviral and antiproliferative actions of human interferons-α and -β. These clones were also less responsive to the enhancement by interferon of Epstein-Barr virus early antigen expression. Both the surface antigens and karyotype of the interferon-resistant clones were similar to those of parental Daudi cells. After prolonged cultivation in the absence of interferon, DIF₃ cells were found to 'revert' to an intermediate interferon sensitivity. The interferon sensitivity of clone DIF₂ remained unchanged even after more than 1 year in culture.

INTRODUCTION

The isolation of cellular mutants resistant to a particular property has been of considerable importance in numerous areas of biology. A number of interferon-resistant cells have been isolated (Gresser et al., 1974; Chany & Vignal, 1970; Kuwata et al., 1976; Borecky et al., 1977; Affabris et al., 1982; Silverman et al., 1982) of which the best-characterized are clones of mouse L1210 cells (Gresser et al., 1974). These cells, which are resistant to both the antiviral and antiproliferative actions of interferon, have been shown to lack a functional receptor for interferons-α and -β (Aguet, 1980) and therefore represent a class of mutants deficient in one of the initial steps in interferon action. In an attempt to isolate other cellular mutants resistant to the action of interferon we exposed the highly interferon-sensitive Burkitt's lymphoma-derived cell line, Daudi (Adams et al., 1975), to successive treatments with increasing concentrations of interferon. We describe the isolation and characterization of two clones of Daudi cells exhibiting markedly reduced sensitivity to both the antiviral and antiproliferative actions of interferon.

METHODS

Cell culture. Daudi cells (Klein et al., 1968) were cultivated in RPMI 1640 medium with 15% foetal calf serum (Flow Laboratories, Irvine, Scotland) in static suspension culture. Cells were cloned by limiting dilution (Cooper, 1973) in RPMI 1640 medium with 15% foetal calf serum and 40% conditioned medium in Falcon microtitre plates (no. 3042).

Interferon preparations. Human interferon-α was prepared from the human lymphoid cell line Namalwa as previously described (Mogensen & Cantell, 1977) and purified by affinity chromatography (Mogensen & Cantell, 1979) to a specific activity of $2 \times 10^8$ international units/mg protein. Electrophoretically pure human interferon-β, prepared from human FS11 diploid fibroblasts (Weissenbach et al., 1979) and purified by high-pressure liquid chromatography to a specific activity of $> 2 \times 10^6$ units/mg protein, was a gift from Dr M. Rubinstein. Human interferon-β, purified by controlled-pore glass and zinc-chelate chromatography to a specific activity $\geq 2 \times 10^6$ units/mg protein, was a gift from Dr A. Billiau. All interferon titres quoted in the text are in international units.
Immunofluorescence. Epstein-Barr virus (EBV) early antigen (EA) was determined on $5 \times 10^4$ acetone-fixed cells by indirect immunofluorescence with EBV-specific [EA (+) VCA (+), or EA (−) VCA (+)] human sera and fluorescein isothiocyanate-conjugated goat anti-human IgG (Huntington Laboratories, Huntington, Ind., U.S.A.) as previously described (Tovey et al., 1982).

RESULTS

Isolation of interferon-resistant clones of Daudi cells

Treatment of Daudi cells with 10 international units/ml of human interferon-α markedly inhibited the rate of cell multiplication and resulted in some loss of cell viability after 3 to 4 days in culture (Fig. 1a). In order to select for cells resistant to the anticellular action of interferon, Daudi cells were treated with successively increasing concentrations of interferon. Using this procedure, a population of Daudi cells was obtained which multiplied in the continued presence of $10^4$ international units/ml of interferon-α. These cells were then cloned in liquid medium in the absence of interferon. Of six clones isolated one clone, DIF3, was found to be relatively resistant to the growth inhibitory action of interferon, while the other five clones exhibited intermediate sensitivity. Clones DIF2 and DIF3 were then characterized further with respect to interferon sensitivity.

Fig. 1. Effect of interferon on the multiplication of parental and interferon-resistant clones of Daudi cells. Cells were cultivated either in RPMI 1640 medium with 15% foetal calf serum alone or in medium containing electrophoretically pure human interferon-α at the concentrations indicated. Cell concentration was determined at the times indicated with a model ZB-1 Coulter counter. Results are expressed as the mean of three replicates. ○, Untreated; ●, 1 unit/ml of interferon; □, 10 units/ml; ■, $10^2$ units/ml; △, $10^3$ units/ml; ▲, $10^4$ units/ml; ▼, $10^5$ units/ml. (a) Daudi cells, (b) clone DIF2, (c) clone DIF3.
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Fig. 2. Effect of interferon on the replication of VSV and EMC virus in parental and interferon-resistant clones of Daudi cells. Cells were treated for 24 h with the concentration of electrophoretically pure interferon-α indicated. The cells were then centrifuged (800 g, 10 min) and resuspended in medium without interferon but containing VSV or EMC virus at a m.o.i. of 0.1 for a 1 h adsorption period at 37 °C. The cells were then centrifuged, washed three times and resuspended in nutrient medium. Virus replication was determined 18 h later. Cultures of Daudi cells were then frozen and thawed three times, centrifuged (2000 rev/min, 10 min) and the supernatant was titrated on L929 cells. Virus titres are expressed as tissue culture infectious dose (TCID_{50}) per 200 μl. (a) Challenged with VSV: O, Daudi cells; △, clone DIF2; ●, clone DIF3. (b) Challenged with EMC virus: O, Daudi cells; Δ, clone DIF2; ●, clone DIF3.

Sensitivity of cloned Daudi cell variants to the antiproliferative action of interferon

Treatment of clone DIF2 with from 1 to 10^5 international units/ml of interferon-α caused a dose-dependent decrease in cell growth rate (Fig. 1b). However, a 100- to 1000-fold greater concentration of interferon-α was required to inhibit the multiplication of clone DIF2 to the same extent as the parental cells treated with 1 international unit/ml of interferon-α (Fig. 1a). Furthermore, in marked contrast to Daudi cells, the viability of DIF2 cells was unaffected even in the presence of 10^5 international units/ml of interferon-α (Fig. 1b).

Treatment of clone DIF3 with human interferon-α at concentrations as high as 10^5 international units/ml reduced cell growth rate only slightly, resulting in a 10 to 15% decrease in cell number at 96 h (Fig. 1c).

Sensitivity of cloned Daudi cell variants to the antiviral action of interferon

The sensitivity of clones DIF2 and DIF3 to the antiviral action of interferon was compared to that of parental Daudi cells using vesicular stomatitis virus (VSV, Indiana strain) as challenge virus. Parental Daudi cells were found to be highly sensitive to the antiviral activity of interferon when challenged with VSV at a m.o.i. of 0-1 or 3. Thus, at the highest concentration of interferon used (10^4 international units/ml), a 4·8 \log_{10} reduction in virus yield was observed (Fig. 2a). This
compared with a 2.9 and a 1.2 log₁₀ reduction in virus yield for clones DIF₂ and DIF₃, respectively (Fig. 2a). VSV multiplied to a similar extent in all three cell lines (Fig. 2a). Many viruses replicate poorly or not at all in Daudi cells (Hilfenhaus, 1976; M. Dron & M. G. Tovey, unpublished results) and, of the viruses tested other than VSV, only encephalomyocarditis virus (EMC) multiplied to a sufficient extent in Daudi cells to permit the interferon sensitivity of these cells to be determined. Although the yield of EMC virus in all three cell lines was modest (2.4 to 2.7 log₁₀ virus yield) the results obtained were essentially similar to those using VSV. Thus parental Daudi cells were highly sensitive to the protective effect of interferon when challenged with EMC at a m.o.i. of 0.1, clone DIF₂ was moderately sensitive, and clone DIF₃ was relatively insensitive (Fig. 2b).

In agreement with our previous work (Tovey et al., 1982), treatment of Daudi cells with human interferon-α enhanced the number of cells expressing Epstein–Barr virus (EBV) early antigen (Fig. 3). Treatment of clone DIF₂ with human interferon-α also increased the number of EA-positive cells, although to a lower extent than for the parental cells (Fig. 3). A small increase in the number of EA-positive cells was observed when clone DIF₃ was treated with high concentrations of interferon-α (Fig. 3).

Fig. 3. Effect of interferon on the expression of EBV early antigen in parental and interferon resistant clones of Daudi cells. Cells were treated for 72 h with highly purified human interferon-α (sp. act. 1 × 10⁸ units/mg protein) at the concentrations indicated. The number of EA-positive cells was then determined as described in Methods. ○, Daudi cells; Δ, clone DIF₂; ●, clone DIF₃.
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Sensitivity of cloned Daudi cell variants to different interferons

Wild-type Daudi cells were found to be equally sensitive to human interferon-β or cloned human interferon-α2 as to human interferon-α (data not shown). Clones DIF2 and DIF3 were also found to be as resistant to highly purified human interferons β or α2 as to preparations of interferon-α of comparable purity when determined either in terms of antiviral or anti-proliferative activity (data not shown). In accord with a previous report (Tomita et al., 1982), Daudi cells were found to be resistant to human interferon-γ. DIF3 cells were similarly insensitive to the antiviral action of human interferon-γ (data not shown).

Characteristics of cloned Daudi cell variants

In agreement with the known characteristics of Daudi cells, the parental cells and clones DIF2 and DIF3 were all found to carry surface μ chain immunoglobulin and to lack both membrane-bound HLA antigens and β2-microglobulin. The parental cells possessed an approximately diploid karyotype with certain chromosomal abnormalities characteristic of Daudi cells. These comprised a translocation of chromosomes 8:14 characteristic of Burkitt’s lymphoma cells, a deletion of the long arm of chromosome 15, and a trisomy of chromosome 7 (Arce-Gomez et al., 1978; Rosa et al., 1982). The majority of cells also exhibited a deletion of the short arm of chromosome 5 not previously described for Daudi cells. Both clones DIF2 and DIF3 exhibited the same chromosomal abnormalities as the parental cells, although in addition 95% of DIF2 cells were tetraploid.

Interferon sensitivity of clones on long-term culture

Clone DIF2 exhibited a sensitivity to the antiproliferative action of interferon after 1 year in culture in the absence of interferon similar to that shown shortly after its initial isolation. On three separate occasions after some 80 to 100 days in culture in the absence of interferon, clone DIF3 was found to ‘revert’ to an intermediate sensitivity. When these ‘revertant’ cells were subcloned, of 16 clones isolated 14 were found to be of intermediate sensitivity and two were found to be relatively insensitive to the antiproliferative action of interferon. Two clones of intermediate and one clone of low sensitivity to the antiproliferative action of interferon were also tested for sensitivity to the antiviral activity of interferon using VSV as challenge virus. Each clone was found to exhibit the same degree of sensitivity to both of these actions of interferon (data not shown).

DISCUSSION

The interferon-resistant clones DIF2 and DIF3 were isolated from a population of Daudi cells selected in the presence of stepwise increasing concentrations of interferon. When a similar procedure is used for the isolation of cells resistant to a drug such as methotrexate, resistant cells arise as a result of an increase in the number of genes coding for the target enzyme of the drug: dihydrofolate reductase in the case of methotrexate. The amplified genes may be either stable or unstable when cells are subsequently cultivated in the absence of the drug (Brown et al., 1982; Bostock & Tyler-Smith, 1982; Bertino et al., 1982; Masters et al., 1982). We do not know whether such a mechanism of acquired drug resistance is involved in the genesis of interferon resistance. However, clones of Daudi cells have been isolated in which the phenotype of reduced interferon sensitivity is stable as in the case of clone DIF2 or is unstable, as in the case of certain subclones of DIF3.

Although Daudi cells with reduced interferon sensitivity have been described previously (Silverman et al., 1982), these cells were not characterized for sensitivity to the antiviral action of interferon and were an uncloned population which may represent a mixture of cells of differing interferon sensitivity. Clones DIF2 and DIF3, however, exhibit moderate and high resistance respectively, to the antiviral action of interferon when challenged with either VSV or EMC virus. These cells are also resistant to the enhancement of EBV early antigen expression which occurs in interferon-treated parental Daudi cells latently infected with Epstein–Barr virus (Tovey et al., 1982).
Although clones DIF\textsubscript{2} and DIF\textsubscript{3} are several orders of magnitude less sensitive to the action of interferon than the highly interferon-sensitive parental Daudi cells, and unlike Daudi cells are not killed by interferon, these cells are nevertheless partially sensitive to interferon. Spontaneously occurring interferon-resistant Burkitt's lymphoma-derived cell lines such as Raji or Namalwa also retain a similar partial interferon sensitivity (Adams et al., 1975). These cells (Mogensen et al., 1981) in common with clones DIF\textsubscript{2} and DIF\textsubscript{3} possess functional interferon receptors (Tovey et al., 1983), suggesting that the mechanism determining interferon resistance is initiated after binding of interferon to its receptor. It is interesting to note that to date the only interferon-resistant cells that have been described, which are completely refractory to all the effects of interferon tested, are mouse L1210 R cells which lack a functional receptor for interferons-\(\alpha\) and -\(\beta\) (Aguet, 1980; Aguet & Blanchard, 1981), and human HEC-1 cells (Chen et al., 1981) which have also been shown to exhibit reduced binding of interferons-\(\alpha\) and -\(\beta\) (Verhaegen-Lewalle et al., 1982).

The interferon-resistant clones of Daudi cells which we have described provide a valuable addition to those interferon-resistant cells isolated previously. In the accompanying article we describe experiments designed to elucidate the mechanism of interferon resistance in these cells (Tovey et al., 1983).

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