Increased Resistance to the Anticellular Effect of Interferon in an Ultraviolet Light-resistant Human Cell Line, UVr-1

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SUMMARY

Interferon (α, β and γ) susceptibility was tested in a human cell line, UVr-1, a u.v. light-resistant variant of RSa cells; the latter have high sensitivity to both u.v. lethality and the cell proliferation inhibition (anticellular) effect of human interferon (HuIFN) preparations. UVr-1 cells were less sensitive than the parental RSa cells to the inhibitory effects of HuIFN preparations, as measured by cell proliferation and the incorporation of [3H]deoxythymidine and [3H]deoxyadenosine into acid-insoluble cellular material. Nevertheless, UVr-1 cells exposed to HuIFN showed almost the same enhanced levels of antiviral activity and pppA(2'p5'A)n synthetase activity as similarly treated RSa cells. Further, UVr-1 cells had much the same binding capacity for 125I-labelled HuIFN-αA. Thus, it seems likely that the variant has an increased resistance to the anticellular effect but not to the antiviral effect of HuIFN preparations. UVr-1 cells showed no significant difference from RSa cells in u.v.-induced DNA repair synthesis. However, when a comparison was made between the susceptibility of normal fibroblasts and fibroblasts from patients with Cockayne's syndrome, characterized by an altered u.v. sensitivity but no alteration of DNA repair replication synthesis, the Cockayne's syndrome fibroblasts, CCK-3 and CCK-4, were more susceptible to HuIFN-β as judged by cell proliferation and deoxythymidine incorporation tests.

INTRODUCTION

The cellular effects of interferons are pleiotropic (Stewart, 1979; Inglot, 1983). Inhibition of cell proliferation by human interferon (HuIFN) preparations has been intensively studied but the molecular mechanisms of the anticellular effect are not well understood (Lebleu & Content, 1982; Suzuki et al., 1982).

Recently, we found that the human cell lines RSb and RSa, both of which are unusually sensitive to the antiviral effect of HuIFN-α (Suzuki et al., 1982, 1984), are also highly sensitive to u.v. lethality (Suzuki & Kuwata, 1979; Suzuki & Fuse, 1981). In addition, a u.v.-resistant subline, UVr-10, which was established from RSb cells and has an increased capacity for u.v.-induced DNA repair synthesis, was found to have an increased resistance not only to the u.v.-mimetic agent 4-nitroquinoline-1-oxide (4NQO), but also to the antiviral effect of HuIFN-α (Suzuki et al., 1982). Such cross-sensitivity to HuIFN and u.v. is not restricted to the case of RSb and UVr-10 cells, because we have found cross-sensitivity among other human cell strains, IF7, HEC-1 and CRL1200, which were derived from HuIFN-α-exposed RSa cells, human endometrial adenocarcinoma cells and a xeroderma pigmentosum patient, respectively (Suzuki et al., 1984).

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The biological effects of u.v. have been extensively investigated (Fremuth, 1976; Harm, 1980). In particular, remarkable progress has been made in understanding u.v.-affected DNA metabolism and its related functions by using isogenic *Escherichia coli* strains differing in u.v. sensitivity (Cleaver, 1978; Radman, 1980; Hanawalt et al., 1982). Thus, if there is a close relationship between u.v. sensitivity and HuIFN susceptibility in isogenic human cell lines, then a comparison of the cellular response to u.v. and HuIFN treatments would provide greater insight into the mechanisms of susceptibility to the various effects of HuIFN.

In all the cases reported of cross-sensitivity to the two agents, an increased capacity for excision repair, as monitored by analyses of DNA repair synthesis (unscheduled DNA synthesis and repair replication synthesis), is closely associated with the acquired resistance to u.v. and HuIFN. Besides the excision repair mechanisms, other repair functions are suspected to be important in u.v.-irradiated human cells (Suzuki & Fuse, 1981; Suzuki, 1984). Thus, it is necessary to determine whether or not these suspected functions are closely related to the susceptibility to the actions of HuIFN. Recently, we established a u.v.-resistant variant from RSa cells, UVr-1, which showed no increased capacity for DNA repair synthesis. In UVr-1 cells, it is possible that repair pathways other than excision repair as detected by the assay of DNA repair synthesis are induced (Suzuki, 1984).

Therefore, we focused on the characterization of UVr-1 cells with respect to susceptibility to the antacellular and other effects of HuIFN, in comparison with that of the parental RSa cells. Further, we determined the HuIFN susceptibility of fibroblasts derived from patients having Cockayne's syndrome; these cells have an unusually high u.v. sensitivity but no distinct abnormality of DNA repair synthesis.

**METHODS**

**Cells and culture conditions.** The establishment and the characterization of the u.v. resistance of UVr-1 cells have been described elsewhere (Suzuki, 1984). The parental RSa cells were found to have high u.v. and HuIFN sensitivities as described (Suzuki & Fuse, 1981; Suzuki et al., 1984). Fibroblasts, named CCK-3 and CCK-4, were established from skin biopsies donated respectively by 13-year-old and 11-year-old brothers with Cockayne's syndrome (Takayanagi et al., 1981). Normal skin fibroblasts, NF-II, were obtained from a 10-year-old boy. HI normal fibroblasts were as described elsewhere (Suzuki et al., 1984). Cells were cultured in Eagle's MEM containing 10% calf serum and antibiotics (100 μg streptomycin/ml and 100 units penicillin G/ml), at 37 °C in a humidified atmosphere containing 5% CO₂. All the cell strains used here were free of mycoplasma infection, as determined by MYCOTRIM-TC (Hana Biologics Inc., U.S.A.).

**Reagents.** HuIFN-α preparations (5.0 × 10⁶ units/mg protein), produced by treating Namalwa cells with Sendai virus (Yonehara et al., 1981), were provided by Dr S. Yonehara of the Tokyo Metropolitan Institute for Medical Research, Tokyo, Japan. Recombinant HuIFN-αA (2-0 × 10⁶ units/mg protein) (Staehehlin et al., 1981) was provided by the Nippon Roche Co. Ltd., Kamakura, Japan. HuIFN-β preparations (2-0 × 10⁶ units/mg protein), produced by treating diploid fibroblast cells from human embryos with poly(rI :rC) (Heine et al., 1980), were provided by Dr A. Billiau and Dr J. van Damme of the Rega Institute, University of Leuven, Belgium. HuIFN-γ preparations (1-0 × 10⁷ units/mg protein), produced by treating human peripheral blood lymphocytes with phytohaemagglutinin, were provided by The Green Cross Corporation, Osaka, Japan. The u.v. lamp and irradiation conditions were as described elsewhere (Suzuki & Fuse, 1981). Other chemical agents and isotopes were purchased from the Nakarai Co. Ltd. (Japan) and the Japan Radioisotope Association, respectively, unless otherwise stated.

**Measurement of cell survival.** HuIFN sensitivity tests determined by cell proliferation studies and colony formation assays were done as described elsewhere (Suzuki et al., 1982). Survival percentage ratios (counts of viable cells in test dishes divided by counts of viable cells in control dishes) × 100, and the D₀ value (the u.v. dosage required to reduce the colony-forming activity from any point on the exponential portion of the surviving fraction curve to 37% of that point) were obtained as described (Suzuki et al., 1982).

**Measurements of [³H]deoxythymidine ([³H]dThd) and [³H]deoxyadenosine ([³H]AdR) incorporation.** Incorporation of [Me-³H]deoxythymidine (60 Ci/mmol, New England Nuclear) and [¹²⁵I,²',³',⁵'-³H]deoxyadenosine (70 Ci/mmol, Amersham) into acid-insoluble cellular material was measured according to the method described by Suzuki et al. (1982). For assays of [³H]AdR incorporation, labelled cells were incubated with 0.1 M-NaOH at 37 °C for 1 h before precipitation with TCA to ensure hydrolysis of the labelled RNA materials.

**Flow cytometric analysis.** Cells were stained using a single-step staining technique (Taylor, 1980) followed by maintenance at 4 °C for over 2 h and then were analysed with a Cytofluorograph model 4800A (Ortho Diagnostic
Assay of \( dT\delta d \) kinase activity. The \( dT\delta d \) kinase assay was performed with an interface reaction method developed and described by Lin & Munyon (1974).

Assay of the antiviral effect of HuIFN. The antiviral effect of HuIFN preparations was measured in a yield reduction assay with a strain of the Indiana serotype of vesicular stomatitis virus (VSV) as described (Suzuki et al., 1982).

Assay of \( pppA(2'p5'A) \), (\( 2\ldots5A \)) synthetase activity. The procedure for the assay of cellular levels of \( 2\ldots5A \) synthetase activity was a slight modification of that already reported (Suzuki et al., 1982; Verhaegen-Lewalle et al., 1982). For estimating the levels in cell nuclei, nuclei were prepared by a modification of the method of Hershey et al. (1973). Briefly, cells with and without HuIFN treatment were suspended at 0 °C in buffer containing 10 mM-Tris-HCl pH 7.4, 1 mM-EDTA, 4 mM-MgCl\(_2\) and 6 mM-2-mercaptoethanol, and homogenized in a Wheaton Dounce Tissue Homogenizer. The homogenates were centrifuged at 0 °C for 3 min at 800 g. The pelleted nuclei were free of cytoplasmic contaminants, as determined by phase and electron microscopy.

Protein determination. Protein concentrations in the enzyme sources were quantified by a method described elsewhere (Suzuki et al., 1982), using bovine serum albumin as a standard.

Studies on binding of \( ^{125}\text{I} \)-labelled HuIFN to cells. Iodination of HuIFN and binding assays were done principally according to the method described by Fuse et al. (1984).

Other methods. To detect possible incorporation of radioactivity into virus particles released into the medium, the sucrose gradient method described by Tomita et al. (1979) was applied 24 h after cultivation of about 1 \( \times 10^6 \) cells in medium containing 200 \( \mu \)Ci \( [3\text{H}]d\text{Tdh} \) or \([3\text{H}]\text{uridine} \) (28 Ci/mmol) per 10 ml. Searches for viral particles, measurement of reverse transcriptase activity in material obtained from HuIFN- or u.v.-treated cells and electron microscopic observations were done as described (Suzuki et al., 1982). Assay of u.v.-induced repair replication with a 3.5 h post-irradiation incubation in \([3\text{H}]d\text{Tdh} \) (10 \( \mu \)Ci/ml), was done according to a previously described method (Suzuki et al., 1982). Almost all the experiments were done under a dim light or a yellow lamp. In each test, UV\( r-1 \) and RSa cell lines were examined concurrently, and the results are expressed as the mean of values obtained from more than three independent experiments, unless otherwise stated.

RESULTS

HUIFN-\( \alpha \) sensitivity of UV\( r-1 \) cells

The susceptibility of UV\( r-1 \) cells to the antiviral effect of HuIFN-\( \alpha \) was first examined by comparing it with that of the parent RSa cells, using the cell proliferation method. As depicted in Fig. 1 (a), RSa cells receiving 100 units/ml or 500 units/ml HuIFN-\( \alpha \) never entered the state of exponential growth; the proliferation of UV\( r-1 \) cells was not so drastically inhibited, but the antiviral effect in these cells increased at higher doses of HuIFN-\( \alpha \). Control cell cultures of both lines had much the same exponential growth curves, as also shown in Fig. 1 (a). Thus, by plotting the survival percentage ratios on the 6th day after treating the cells at various doses of HuIFN-\( \alpha \), it was clear that the surviving fraction \( \text{of UV}\( r-1 \) cells was greater than that of RSa cells (Fig. 1 b).

On the 3rd day after mock treatment in the cell proliferation study, the cell cycle distributions in the controls were determined by flow cytometry. In the case of UV\( r-1 \) cells, 53\%, 39\% and 8\% of the cells had G1, S and G2 plus M DNA contents, respectively. RSa cells had similar fractions of the population in G1, S and G2 plus M: 51\%, 41\% and 8\%. The HuIFN-\( \alpha \) sensitivity of exponentially growing UV\( r-1 \) and RSa cell populations having these compositions was further compared by colony formation assays. As shown in Fig. 2, the colony-forming ability of RSa cells previously exposed to HuIFN-\( \alpha \) preparations for 3 days was depressed, whereas that of exposed UV\( r-1 \) cells was not. Moreover, Fig. 2 shows that, when the cells were added to the plates as a single-cell suspension (\( 2 \times 10^3 \) cells/100 mm dish) and then exposed to HuIFN-\( \alpha \) and -\( \alpha \), exposure of RSa cells for only 12 h led to a more marked decrease of colony formation activity than was seen for UV\( r-1 \) cells. Natural and recombinant HuIFN-\( \alpha \) preparations had similar effects, as is also shown in Fig. 2. In these colony formation assays, cloning efficiencies were 10 to 15\% for UV\( r-1 \) cells and 6 to 10\% for RSa cells.

\([3\text{H}]d\text{Tdh} \) and \([3\text{H}]\text{AdR} \) incorporation in HuIFN-\( \alpha \)-treated UV\( r-1 \) cells

As shown in Fig. 3 (a), incorporation of radioactivity in RSa cells was inhibited even 1 h after exposure to 250 units/ml HuIFN-\( \alpha \), and after about 6 h exposure it was suppressed to about one-
Fig. 1. Effects of continuous exposure to HuIFN-α on cell growth (a) and the survival percentage ratio 6 days after exposure (b). (a) period of HuIFN-α treatment. UV-1 cells: ■, control; ▲, 100 units/ml; ■, 500 units/ml. RSa cells: ○, control; △, 100 units/ml; ●, 500 units/ml. (b) ■, UV-1 cells; ●, RSa cells.

Fig. 2. Effects of HuIFN-α and HuIFN-αA exposure on the colony formation ability of UV-1 (■, ▲, △) and RSa (●, ○, △) cells. After exposure to HuIFN-α for 3 days as shown in Fig. 1, cells were singly dispersed by treatment with 0.05% trypsin in phosphate-buffered saline and then replated (2 x 10^3 cells/100 mm dish) (■, ●); 20 h after seeding 2 x 10^3 exponentially growing cells of each strain per 100 mm plastic dish, the cells were exposed to HuIFN-α (■, ○) and HuIFN-αA (▲, △) for 12 h. Control cells were treated by the same methods except for the exposure to HuIFN.

Half of the level seen in control cells. The incorporation inhibition in UV-1 cells was less marked than that in RSa cells (Fig. 3a). Levels of dThd kinase activity in each cell line were also examined. The specific activities in control cultures of UV-1 and RSa cells differed, being about 10 to 12 c.p.m./min/mg protein and 18 to 20 c.p.m./min/mg protein, respectively. However, as also depicted in Fig. 3(a), the dThd kinase activities in each cell line after HuIFN-α treatment did not change drastically during the time period examined, although a slight decrease was observed in HuIFN-αA-treated cells of each line.

When the degree of [3H]dThd incorporation inhibition was compared 24 h after treatment with various doses of HuIFN-α, it was evident that UV-1 cells were less susceptible to the inhibitory effect of the agent than were RSa cells, as summarized in Fig. 3(b). As is also shown in Fig. 3(b), [3H]AdR incorporation 24 h after HuIFN-α treatment was also markedly depressed in RSa cells but not in UV-1 cells.

HuIFN-β and -γ sensitivity of UV-1 cells

Fig. 4(a) shows that the survival percentage ratios for UV-1 cells 4 days after continuous exposure to HuIFN-β preparations proved to be higher than those for RSa cells. The incorporation of [3H]dThd and [3H]AdR in HuIFN-β-treated UV-1 cells was less depressed than that of treated RSa cells (Fig. 4b).
IFN resistance of a u.v.-resistant cell line

UVr-1 cells were also less susceptible to the inhibitory effects of HuIFN-γ preparations, as assessed by cell proliferation (Fig. 4a) and [3H]dThd incorporation (Fig. 4b), than were RSa cells.

Induction of 2–5A synthetase and antiviral activities by HuIFN in UVr-1 cells

To determine whether other cellular effects of HuIFN on UVr-1 cells were affected to the same extent, HuIFN-α-induced 2–5A synthetase activities were compared. Table 1 shows that exposure to HuIFN-α (100 or 500 units/ml) for 6 h resulted in an induction of the enzyme activity, and that the induced levels in UVr-1 cells were much the same as those in RSa cells treated with the corresponding dose. A similarity in the levels of 2–5A synthetase activity was also noted in nuclear preparations from UVr-1 and RSa cells treated with HuIFN-α (Table 1). Irrespective of the time of exposure, UVr-1 cells showed approximately the same level of induced enzyme activity as did RSa cells (data not shown).

The levels of antiviral activity of HuIFN-α in UVr-1 cells, estimated by suppression of the yield of VSV, were not significantly different from those in RSa cells (Table 1).

Treatment of UVr-1 cells with HuIFN-β and -γ preparations resulted in almost the same levels of 2–5A synthetase and antiviral activity as seen in treated RSa cells (data not shown).

Other studies of UVr-1 cells

From an analysis of the binding of 125I-labelled HuIFN-α, the total number of binding sites per cell and the dissociation constant for UVr-1 cells were the same as those for RSa cells, 1100 and 8.7 × 10⁻¹⁰ M, respectively.

In another series of experiments, we examined virus production in UVr-1 and RSa cells. However, in neither cell line was antiviral, anticellular or reverse transcriptase activity detected.

Fig. 3. [3H]dThd incorporation and dThd kinase (TK) activity (a) during exposure for 48 h to HuIFN-α (250 units/ml), and (b) effects of various concentrations of HuIFN-α on [3H]dThd and [3H]AdR incorporation and dThd kinase activity after HuIFN-α treatment. Values for HuIFN-α-treated cells are expressed as a percentage of those measured at the same time in the controls. Controls for each cell line were about 5 × 10⁴ to 6 × 10⁴ c.p.m. of [3H]dThd and 3 × 10² c.p.m. of [3H]AdR in the acid-insoluble material obtained from 5 × 10⁴ cells. [3H]dThd incorporation: ■, UVr-1 cells; ○, RSa cells; dThd kinase: □, UVr-1 cells; ◯, RSa cells; [3H]AdR incorporation: ▲, UVr-1 cells; △, RSa cells.
Fig. 4. Comparisons of the survival percentage ratio (a) and [3H]dThd or [3H]AdR incorporation (b) after exposure of UV^-1 and RSa cells to HuIFN-β and -γ. (a) Four days after culture in medium with or without HuIFN, the ratio of the number of the exposed and control cells was calculated. ■, HuIFN-β-treated UV^-1 cells; ●, HuIFN-β-treated RSa cells; □, HuIFN-γ-treated UV^-1 cells; ○, HuIFN-γ-treated RSa cells. (b) Twenty-four h after HuIFN treatment [3H]dThd (■, ●, ▲, △) and [3H]AdR (□, ○) incorporation was examined. Values for HuIFN-treated cells are expressed as a percentage of those measured at the same time in the controls without HuIFN treatment. Control values (c.p.m. per 5 × 10^4 cells) were about 5 × 10^3 c.p.m. of [3H]dThd and 3 × 10^2 c.p.m. of [3H]AdR. ■, □, HuIFN-β-treated UV^-1 cells; ●, ○, HuIFN-β-treated RSa cells; ▲, HuIFN-γ-treated UV^-1 cells; △, HuIFN-γ-treated RSa cells.

Table 1. 2-5A synthetase and antiviral activity* in HuIFN-α-treated UV^-1 and RSa cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>HuIFN-α (units/ml)</th>
<th>2-5A synthetase activity</th>
<th>Antiviral Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole cells</td>
<td>Nuclei</td>
</tr>
<tr>
<td>UV^-1</td>
<td>10</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.2</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>500</td>
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<tr>
<td></td>
<td>500</td>
<td>4.0</td>
<td>4.7</td>
</tr>
</tbody>
</table>

* Activities in HuIFN-α-exposed cells are expressed as relative ratios, by setting the levels of specific activities as 1.0 for unexposed control cells. 2-5A synthetase activity was assayed 6 h after HuIFN-α exposure. The 2-5A synthetase activities of control UV^-1 and RSa cells were 1.2 × 10^5 to 1.3 × 10^5 c.p.m./mg protein, irrespective of the enzyme sources. The VSV yield in control UV^-1 and RSa cells was 7·0 (log_{10} TCID_{50}/0.2 ml).
IFN resistance of a u.v.-resistant cell line

Fig. 5. Fraction of colony formers plotted against doses of u.v. (a) and HuIFN-β (b) in Cockayne's syndrome fibroblasts CCK-3 (■) and CCK-4 (□), and normal fibroblasts HI (○) and NF-11 (●). Twenty h after seeding $1 \times 10^3$ cells of each strain in one 100 mm plastic dish, the cells were irradiated with u.v. or exposed to HuIFN-β for 24 h. Control cells were treated by the same methods except for u.v. irradiation and HuIFN-β exposure.

either in the medium or in sonically treated cell materials. When cells (over 70) were randomly examined under the electron microscope, no virus-like particles were detected. Furthermore, sucrose gradient analyses of the medium obtained after cell culture with $[^3]$HdTd and $[^3]$H]uridine showed no peak of radioactivity.

_HuIFN susceptibility of Cockayne's syndrome fibroblasts_

CCK-3 and CCK-4 fibroblasts, derived from Cockayne's syndrome patients, had a much lower colony formation ability after u.v. irradiation than did irradiated HI and NF-11 cells, as shown in Fig. 5(a). The $D_0$ values for CCK-3 and CCK-4 cells were 1.5 J/m$^2$ and 1.4 J/m$^2$, both of which were much lower than those for HI and NF-11 cells, 23.4 J/m$^2$ and 10.3 J/m$^2$, respectively. Cloning efficiencies for each strain were much the same, 20 to 25%. Despite such high u.v. sensitivity, CCK-3 and CCK-4 cells had the same or higher levels of u.v.-induced repair replication, when compared with those in normal HI and NF-11 cells (Table 2).

Using colony formation assays, HuIFN-β treatment produced a greater inhibition of colony formation in both CCK-3 and CCK-4 cells than it did in normal fibroblasts (Fig. 5b). $[^3]$HdTd incorporation into CCK-3 and CCK-4 cells was also inhibited to a great extent by HuIFN-β compared to a slight inhibition in normal fibroblasts, as illustrated in Fig. 6. But levels of antiviral activity of HuIFN-β in Cockayne's syndrome fibroblasts were not significantly different from those in normal fibroblasts (Fig. 7).
Fig. 6. Effects of HuIFN-β on [3H]dThd incorporation in Cockayne's syndrome fibroblasts CCK-3 (■) and CCK-4 (□), and normal fibroblasts HI (○) and NF-11 (●). All of the control values for each cell strain were 2.0 × 10³ to 2.5 × 10³ c.p.m./1 × 10⁴ cells.

Fig. 7. Comparison of the induction of antiviral activities by HuIFN-β in Cockayne's syndrome fibroblasts CCK-3 (■) and CCK-4 (□), and normal fibroblasts HI (○) and NF-11 (●). The VSV yield is expressed as percentage of virus yield in control cells without HuIFN-β treatment, 5.0 (log₁₀ TCID₅₀/0.2 ml).

Table 2. Repair replication* in Cockayne's syndrome and normal fibroblasts irradiated with u.v.

<table>
<thead>
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<th>U.v. (J/m²)</th>
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<th>32</th>
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<tr>
<td>HI</td>
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<tr>
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</tr>
<tr>
<td>CCK-4</td>
<td>0</td>
<td>75</td>
<td>110</td>
</tr>
</tbody>
</table>

* Values, as c.p.m. [3H]dThd/µg DNA, are the means of those obtained in two independent experiments.
UVr-1 cells were established by treating RSa cells with ethyl methanesulphonate as a mutagen, and subsequently with u.v. (Suzuki, 1984). In the present study, we found that regardless of the HuIFN preparations used (α, αA, β or γ), this u.v.-resistant variant strain did have an increased resistance to the anticytotoxic effect of HuIFN.

There has been some discussion as to whether the degree of the anticytotoxic effect seen when HuIFN-α treatment is applied is dependent on the cell proliferation rate (Creasey et al., 1980; Leanderson & Lundgren, 1982). At the time of HuIFN addition, the UVr-1 cells were exponentially proliferating, at much the same rate and with similar proportions of cell cycle phases as those seen in the RSa cells. Thus, the main cause of the increased HuIFN resistance of UVr-1 cells may not reside in their proliferation rates. Although there has also been much discussion regarding the chromosomes which contain genes controlling the actions of IFN (De Clercq et al., 1975; Epstein & Epstein, 1976; Revel et al., 1976; Tan & Greene, 1976; Wiranowska-Stewart & Stewart, 1977; Lubiniecki et al., 1979; Zhang et al., 1982; Razuiddin et al., 1984), we were not able to carry out an accurate karyotyping due to the presence of numerous undefinable or abnormal chromosomes in the cell lines we used.

The resistance to u.v. killing of UVr-1 cells was also associated with an increased resistance to the inhibitory effect of HuIFN on [3H]dThd incorporation into acid-insoluble material (Fig. 3 and 4b). This inhibition of incorporation seems to be unrelated to the level of dThd kinase activity, which has been suggested to play an important role in cellular nucleotide uptake (Plagemann & Erbe, 1972; Schäer & Maurer, 1982), because the levels of dThd kinase activity in HuIFN-α-exposed UVr-1 and RSa cells were unchanged in comparison with the levels in control cells (Fig. 3). In human lymphoblastoid (Daudi) cells the absence of any clear relationship between the effects of IFN on the utilization of exogenous [3H]dThd and the inhibition of cell growth has also been suggested (Gewert et al., 1983). However, there is some cross-sensitivity between dThd incorporation inhibition and cell proliferation inhibition by HuIFN in all the sets of human cells that show cross-sensitivity to HuIFN and u.v. (Suzuki et al., 1982, 1984), including the present pair UVr-1 and RSa. UVr-1 cells were also refractory to [3H]AdR incorporation inhibition by HuIFN (α or β) (Fig. 3b and 4b). The resistance of UVr-1 cells to inhibition of nucleotide incorporation by HuIFN, irrespective of the labelled precursor, may reflect an increased resistance of the cells to HuIFN-affected DNA synthesis inhibition. Interestingly, recent reports by Moore et al. (1984) and Suhadolnik et al. (1984) have suggested that HuIFN inhibits the processing of newly synthesized low molecular weight DNA into larger DNA size classes.

The induction of 2-5A synthetase activities in whole cell extracts after HuIFN treatment does not always correlate with the effect of HuIFN (Lebleu & Content, 1982, Suzuki et al., 1982). Recently, the presence in cell nuclei of an activity which synthesizes 2-5A has been reported (Nilsen et al., 1982; Laurent et al., 1983), but even in enzyme preparations from nuclei of UVr-1 cells, the induced levels of 2-5A synthetase activity did not differ from those of RSa cells (Table 1). Thus, the level of 2-5A synthetase activity does not seem to play an important role in the increased resistance of UVr-1 to HuIFN.

Brouty-Boye et al. (1979) reported that increased IFN resistance was associated with X-ray-induced transformation in mouse cells. Irradiation with X-rays or u.v. might modify the surface of UVr-1 cells so as to cause a decrease in IFN-binding capacity, leading to their resistance to the effects of IFN. However, UVr-1 and RSa cells had the same capacity to bind 125I-labelled HuIFN-αA. Thus, the HuIFN resistance of UVr-1 cells is not due to changes in the numbers of HuIFN-binding sites. The marked induction of 2-5A synthetase and antiviral activities observed in HuIFN-exposed UVr-1 cells implies that the intracellular effects of HuIFN are exerted in these cells.

Fibroblasts derived from Cockayne’s syndrome patients are known to have normal so-called excision repair mechanisms (Wade & Chu, 1979). M. Ikenaga (Radiation Biology Center, Kyoto University, Kyoto, Japan) has also confirmed the normal DNA repair capacity of the u.v.-sensitive CCK fibroblasts (personal communication). Although the main cause of the high u.v. sensitivity in Cockayne cells is unclear, a reduced level of host cell reactivation of u.v.- and γ-ray-
irradiated adenovirus (Rainbow & Howes, 1982) and defects in the recovery of DNA and RNA synthesis after u.v. treatment (Mayne & Lehmann, 1982; Lehmann et al., 1979) have been reported. If DNA repair pathways other than excision repair are induced in u.v.-irradiated UV-sensitive cells, these pathways or related functions may be responsible for the cross-sensitivity to u.v. and HuIFN.

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REFERENCES


IFN resistance of a u.v.-resistant cell line


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