Enhanced Production of Human Interferon by u.v. Irradiated Cells

(Accepted 24 June 1974)

SUMMARY

U.v. irradiation of human diploid cells enhanced interferon production after induction with poly I.C. Moreover, interferon production was even further increased by DEAE-dextran in cultures already enhanced by u.v.

A number of previous investigations have demonstrated that irradiation decreases interferon production in vivo and in vitro. Burke (1966) summarized studies dealing with the effects of u.v. irradiation, and Coppey & Markovits (1969) reported on the kinetics of interferon production of tissue culture cells after u.v. irradiation. In these earlier experiments interferon induction was performed with virus. U.v. irradiation seems to decrease virus induced interferon production in both animals and tissue culture cells. Only in a few experiments where X-rays were used did irradiation not affect, or even enhance, interferon production in mice induced with endotoxin (Nagano, Maehara & Nakamura, 1970; Glasgow, 1972) or poly I.C (Talas et al. 1973).

The experiments showing the effect of u.v. irradiation in poly I.C induced cells were carried out in human diploid fibroblasts which were derived in our laboratory from tissue fragments of human foetal lung or of foreskin. For primary cultures, Eagle’s minimum essential medium (EMEM) supplemented with 10% foetal bovine serum and 300 µg/ml neomycin sulphate was used. Serial passages were performed by the trypsin technique. The cells were grown as monolayers in EMEM with 10% foetal bovine serum without antibiotics. Mycoplasma checks were negative and chromosome counts showed the cells to stay diploid in the course of the passages. For irradiation experiments, five day old monolayer cultures (lung fibroblasts passages 13 to 21, foreskin fibroblasts passages 8 to 29) in 3 cm plastic Petri dishes, containing EMEM with 10% foetal bovine serum and 300 µg/ml neomycin sulphate per ml, were used.

The confluent cell sheets were irradiated with a Philips TUV 30 W lamp (maximal emission at 254 nm) or a Mineralight UVS-11-lamp (Ultraviolet Products, Inc., with a short wavelength filter for maximal transmission at 254 nm) at varying distances for 10 s. The radiation was measured by use of a short wavelength u.v. meter (Ultraviolet Products, Inc., Model J-225). Immediately before irradiation the media were withdrawn and after irradiation the cells were washed and induced with 1 ml EMEM containing 50 µg poly I.C (Miles Labs., Inc.) and 300 µg neomycin sulphate and, if stated, DEAE-dextran. One hour after induction the cells were washed three times and incubated with 1 ml of EMEM containing 300 µg neomycin sulphate without serum. Eight hours after induction the media were withdrawn in order to be tested for antiviral activity and incubation of the cultures was continued after replenishing them with fresh EMEM + neomycin sulphate. Withdrawal and replacement of media was repeated at 24 and 48 h after induction.

Production of interferon was assayed by plaque inhibition with VSV (bovine vesicular stomatitis virus, Indiana serotype) as challenge in human skin fibroblasts. One interferon unit corresponded to 0.55 reference research units as measured with the 69/19 standard
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Fig. 1. Interferon production in human diploid fibroblasts (lung) induced by poly I.C 8, 24, 48 and 72 h after induction, cumulative yields. ●—●, cells not irradiated; ▲—▲, cells irradiated with 10000 erg/cm²; ■—■, cells irradiated with 20000 erg/cm²; ○—○, cells irradiated with 40000 erg/cm²; △—△, cells irradiated with 80000 erg/cm².

Table 1. Comparison of interferon production in human diploid fibroblasts (foreskin) induced with poly I.C (50 µg/ml) or poly I.C (50 µg/ml) and DEAE-dextran (5 µg/ml), respectively. Influence of antimetabolites and u.v.-irradiation

<table>
<thead>
<tr>
<th>Interferon units/culture*</th>
<th>DEAE-dextran (0 µg/ml)</th>
<th>DEAE-dextran (5 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>410</td>
<td>2250</td>
</tr>
<tr>
<td>U.v.-irradiation (40000 erg/cm²)</td>
<td>8375</td>
<td>41600</td>
</tr>
<tr>
<td>Actinomycin D + cycloheximide†</td>
<td>32600</td>
<td>49370</td>
</tr>
</tbody>
</table>

* Cumulative interferon units per culture from four harvests (5½ h, 24 h, 48 h + 72 h after induction), mean titre of five cultures.
† Unirradiated cells were induced as described in the text. After removal of inducer, 10 µg/cycloheximide in 1 ml EMEM with neomycin sulphate was added to each culture. 5 h after induction, 1 µg actinomycin D per culture was added and both agents were withdrawn at 5½ h after induction (1st harvest) and replaced by EMEM.

preparation (Medical Research Council). In order to characterize the interfering agent as interferon, it was shown to be destroyed by trypsin, resistant to ribonuclease, not sedimented at 100000 g for 90 min, not dialysable, stable at pH 2, and to induce cell mediated resistance to virus growth. It could be purified tenfold by chromatography on SP-Sephadex C-25 (Sulphopropyl Sephadex, Pharmacia Uppsala) with 80% recovery (Jungwirth et al. 1972). Interferon production in human fibroblasts was strongly enhanced and protracted after an irradiation dose as small as 10000 erg/cm² (Fig. 1). Eight hours after induction, when control cells had almost ceased their interferon production, u.v. irradiated cells were producing interferon, and this process continued up to 72 h after induction. Increase of the radiation dose up to 40000 erg/cm² resulted in further augmentation of interferon production, whereas a radiation dose of 80000 erg/cm² was less efficient. Possibly, the enhancement of interferon production by u.v. irradiation and the previously described enhancement...
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by antimetabolites (Tan et al. 1970; Vilček, 1970; Myers & Friedman, 1971) are based on a similar mechanism, i.e. on the inhibition of a hypothetical repressor protein.*

In human diploid fibroblasts augmentation of interferon production by u.v. irradiation was further enhanced by inducing the cells in the presence of DEAE-dextran (Table 1). Interferon production in irradiated cells was increased fivefold by addition of DEAE-dextran to the inducer. In cells treated with antimetabolites (cycloheximide and actinomycin D) interferon production was only slightly increased (1.5-fold) by aid of DEAE-dextran. It has been shown that induction with a poly I.C/DEAE-dextran complex did not enhance, and even suppressed, interferon production in rabbit kidney cells treated with antimetabolites (Vilček, Barmak & Havell, 1972). In u.v. irradiated or drug treated human fibroblasts under the conditions shown above, nearly equivalent amounts of interferon were obtained by the addition of DEAE-dextran to the inducer.

In view of the demand of larger amounts of clinically applicable interferon, improved methods for the production of human interferon are strongly needed (Havell & Vilček, 1972; Billiau, Joniau & De Somer, 1973). The u.v. irradiation technique has the advantage of being inexpensive, easy to handle and of furnishing interferon preparations free of antimetabolites.

I thank Professor H. Tuppy from the Institute of Biochemistry of the University of Vienna and Dr P. Swetly for helpful discussion. Mrs A. Pytelka, Mrs I. Bonfert, and Mrs E. Willburger, I thank for excellent technical assistance.

REFERENCES


* Note added in proof: After this manuscript was submitted, similar data, concerning the enhancement of interferon production by u.v. irradiation in rabbit cells were published (Mozes & Vilček, 1974).
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