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Effect of Environmental Carcinogens and Other Chemicals on Murine Alpha/Beta Interferon Production

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Mouse embryo fibroblast cultures were pretreated with a variety of chemicals found in the environment. After chemical treatment, polyriboinosinic–polyribocytidylic acid was added to the cultures to induce alpha/beta interferon. Pretreatment of the cell cultures with the chemical carcinogens chloroform and β-propiolactone severely inhibited the production of alpha/beta interferon, while pretreatment of the cell cultures with their poorly or noncarcinogenic analogs 1,1,1-trichloroethane and γ-butyrolactone had no effect on interferon induction. Pretreatment of the cell cultures with the possible carcinogen diethylstilbestrol had no effect on alpha/beta interferon induction. Pretreatment of the cells with the poor or noncarcinogens pyrene and ascorbic acid did not effect interferon induction; in fact, treatment with ascorbic acid may have enhanced interferon production. These results augment previous findings that most potent carcinogens can inhibit the induction of alpha/beta interferon.

INTRODUCTION

Several studies have indicated that treatment of rat or mouse fibroblast cell cultures with potent carcinogens severely inhibited the induction of alpha/beta (type I) interferon without affecting viability of the cultures (DeMaeyer and DeMaeyer-Guignard, 1964; DeMaeyer-Guignard and DeMaeyer, 1965; Hahon *et al.*, 1979; Sonnenfeld *et al.*, 1980, 1983; Barnes *et al.*, 1981; Sonnenfeld, 1983). The chemicals included chloroacetaldehyde, 7,12-dimethylbenz-(α)-anthracene, 2-naphthylamine, potent carcinogenic forms of aflatoxin, benzo-(α)-pyrene, and 3-methylcholanthrene. Treatment of cells with poorly or noncarcinogenic analogs of the chemicals, including chloroacetic acid, anthracene, 1-naphthylamine, and weakly carcinogenic forms of aflatoxin, had little or no effect on alpha/beta interferon induction (DeMaeyer-Guignard and DeMaeyer, 1965; Hahon *et al.*, 1979; Sonnenfeld *et al.*, 1980, 1983; Barnes *et al.*, 1981; Sonnenfeld, 1983). We recently have observed similar inhibitory effects of the carcinogen 7,12-dimethylbenz-(α)-anthracene on the induction of murine gamma (type II immune) interferon (Golemboski *et al.*, 1982).

Interferon may play some role in resistance to cancer, and consequently, clinical anticancer trials are in progress (Merigan *et al.*, 1979; Gutterman *et al.*, 1980). An effect of a chemical carcinogen on interferon induction may contribute to the eventual development of a tumor; therefore, it was of interest to determine if additional environmental carcinogenic and noncarcinogenic chemicals had similar effects on interferon induction.

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The results of the present study indicate that pretreatment of mouse embryo fibroblast with the carcinogens chloroform and β-propiolactone inhibited the induction of alpha/beta interferon by polyriboinosinic–polyribocytidylic acid (poly I:C)[2] in those cells. Pretreatment of the fibroblasts with their poorly or noncarcinogenic analogues 1,1,1-trichloroethane and γ-butyrolactone had no effect on interferon induction. Pretreatment of the cells with diethylstilbestrol (a possible carcinogen), pyrene, or ascorbic acid (poor or noncarcinogens) did not inhibit alpha/beta interferon production; in fact, ascorbic acid treatment may have enhanced interferon production.

MATERIALS AND METHODS

Mouse embryo fibroblast cultures. C3H/He mice were originally obtained from Laboratory Supply, Inc., Indianapolis, Indiana, and then maintained and bred in our laboratory. Fifteen to eighteen-day-old embryos were surgically removed from pregnant dams, minced, trypsinized in 0.25% trypsin 1-300 (ICN Pharmaceuticals, Cleveland, Ohio) and then suspended in Gibco (Grand Island, N.Y.) minimal essential medium (MEM) with 10% fetal bovine serum. Second or third passage cultures were used in all experiments and were plated in 25-cm^2 Falcon (Oxnard, Calif.) tissue culture flasks. Cultures were used immediately upon reaching confluency (Barnes *et al.*, 1981).

Chemicals. Chloroform and 1,1,1-trichloroethane were obtained from Fisher Scientific, Inc., Fair Lawn, New Jersey. L-Ascorbic acid, γ-butyrolactone, DES, β-propiolactone, and pyrene were all obtained from Sigma Chemical Company, St. Louis, Missouri. Dimethyl sulfoxide was also obtained from Sigma.

Interferon production. Mouse alpha/beta interferon was produced in mouse embryo fibroblast cultures as previously described (Dianzani *et al.*, 1968). Poly (I:C) (P-L Biochemicals, Milwaukee, Wisc.) was the inducer with DEAE-dextran added to ensure maximum induction. Tissue culture supernatants were harvested at 24 hr postinduction and assayed for antiviral activity.

Interferon assay. Antiviral titers were determined by means of a plaque reduction assay on mouse L-929 cells using the Indiana strain of vesicular stomatitis virus (Brodeur and Merigan, 1974). The interferon titer corresponded to the reciprocal of the greatest dilution of test sample that reduced virus plaques by 50%. One interferon unit in this assay was equivalent to 0.88 NIH G-002-904-511 reference units.

Statistical analysis. Statistical analysis of the data was carried out by means of a Student *t* test.

RESULTS

Effects of Chemical Treatment on Cell Viability

All chemicals were dissolved in DMSO. DMSO was an acceptable solvent because it did not affect the viability of the mouse embryo fibroblast as determined by trypan blue dye exclusion (data not shown). The highest dosage of each

[2] Abbreviations used: DES, diethylstilbestrol; DMSO, dimethylsulfoxide; MEM, minimal essential medium; poly(I:C), polyriboinosinic–polyribocytidylic acid.

chemical used in this study was the highest dosage that did not affect the viability of the cell cultures (≥93% of cells viable) as determined by trypan blue dye exclusion.

Effects of Chemical Treatment on Alpha/Beta Interferon Production

Various concentrations of chemicals were added to different fibroblast cell cultures. The cells were incubated at 37°C for 24 hr, and then the chemicals were removed. The cells were washed with MEM with 10% fetal bovine serum. Poly (I:C) was added to induce alpha/beta interferon, and culture supernatants were harvested and assayed for antiviral activity.

All interferon titers from cells treated with chemicals dissolved in DMSO were compared to interferon titers from cells treated with an equivalent volume of DMSO only. The interferon titers from DMSO-treated cells did not differ significantly from titers obtained from cells that received only poly (I:C) (data not shown). Since the interferon assay is a titration, a statistically significant decrease of 50% or greater (or a 100% increase) in antiviral titer was required for the chemical treatment to be judged to have a reliable effect on interferon induction (Barnes *et al.*, 1981).

Pretreatment of cells with the carcinogen chloroform (100 μM) significantly decreased alpha/beta interferon induction, while pretreatment of cells with its analogue 1,1,1-trichloroethane had no effect (Table 1). The carcinogen β-propiolactone also inhibited interferon induction, while its analog γ-butyrolactone had no effect (Table 2). Treatment of cells with DES (Table 3) or pyrene (Table 4) also

TABLE 1
EFFECTS OF CHLOROFORM AND 1,1,1-TRICHLOROETHANE ON MURINE ALPHA/BETA INTERFERON PRODUCTION

Treatment of cells	No. of experiments	Antiviral titer	Percentage change	*P*
0.02% DMSO + poly (I:C)	13	1,782 ± 168	—	—
0.02% DMSO + 1 μM Chloroform + poly (I:C)	3	908 ± 52	−49	NS
0.02% DMSO + 1 μM 1,1,1-trichloroethane + poly (I:C)	3	1,152 ± 267	−35	NS
0.2% DMSO + poly (I:C)	12	1,729 ± 237	—	—
0.2% DMSO + 10 μM chloroform + poly (I:C)	3	1,321 ± 687	−23	NS
0.2% DMSO + 10 μM 1,1,1-trichloroethane + poly (I:C)	3	1,269 ± 351	−27	NS
2% DMSO + poly (I:C)	14	1,851 ± 259	—	—
2% DMSO + 100 μM chloroform + poly (I:C)	3	852 ± 161	−54	<0.05
2% DMSO + 100 μM 1,1,1-trichloroethane + poly (I:C)	3	1,494 ± 759	−19	NS

TABLE 2
EFFECTS OF β-PROPIOLACTONE AND γ-BUTRYOLACTONE ON MURINE ALPHA/BETA INTERFERON PRODUCTION

Treatment of cells	No. of experiments	Antiviral titer	Percentage change	*P*
0.02% DMSO + poly (I:C)	13	1782 ± 168	—	—
0.02% DMSO + 1 μM β-propiolactone + poly (I:C)	5	1740 ± 336	−2	NS
0.02% DMSO + 1 μM γ-butyrolactone + poly (I:C)	6	1673 ± 185	−6	NS
0.2% DMSO + poly (I:C)	12	1729 ± 237	—	—
0.2% DMSO + 10 μM β-propiolactone + poly (I:C)	5	1769 ± 168	+2	NS
0.2% DMSO + 10 μM γ-butyrolactone + poly (I:C)	6	1949 ± 135	+13	NS
2% DMSO + poly (I:C)	14	1851 ± 259	—	—
2% DMSO + 100 μM β-propiolactone + poly (I:C)	5	878 ± 94	−52	<0.05
2% DMSO + 100 μM γ-butyrolactone + poly (I:C)	6	1791 ± 135	−3	NS

had no effect on interferon production by those cells. Treatment of cells with ascorbic acid may have slightly increased interferon production (Table 5).

DISCUSSION

Several studies have indicated that pretreatment of cell cultures with potent carcinogens, such as chloroacetaldehyde, 7,12-dimethylbenz-(α)-anthracene, 2-naphthylamine, and aflatoxin, resulted in inhibition of alpha/beta interferon induction in those cultures (DeMaeyer and DeMaeyer-Guignard, 1964; DeMaeyer-Guignard and DeMaeyer, 1965; Hahon *et al.*, 1979; Sonnenfeld *et al.*, 1980, 1983; Barnes *et al.*, 1981; Sonnenfeld, 1983). Weakly or noncarcinogenic analog of the

TABLE 3
EFFECT OF DIETHYLSTILBESTROL (DES) ON MURINE ALPHA/BETA INTERFERON PRODUCTION

Treatment of cells	No. of experiments	Antiviral titer	Percentage change	*P*
0.2% DMSO + poly (I:C)	12	1729 ± 237	—	—
0.2% DMSO 10 μM DES + poly (I:C)	3	1296 ± 381	−25	NS
2% DMSO + poly (I:C)	14	1851 ± 259	—	—
2% DMSO + 25 μM DES + poly (I:C)	10	2101 ± 193	+14	NS

TABLE 4
EFFECT OF PYRENE ON MURINE ALPHA/BETA INTERFERON PRODUCTION

Treatment of cells	No. of experiments	Antiviral titer	Percentage change	*P*
0.02% DMSO + poly (I:C)	13	1782 ± 168	—	—
0.02% DMSO + 1 μM pyrene + poly (I:C)	6	2159 ± 234	+21	NS
0.2% DMSO + poly (I:C)	12	1729 ± 237	—	—
0.2% DMSO + 10 μM pyrene + poly (I:C)	6	1849 ± 87	+7	NS
2% DMSO + poly (I:C)	14	1851 ± 259	—	—
2% DMSO + 100 μM pyrene + poly (I:C)	6	2268 ± 168	+23	NS

chemicals had no effect on interferon induction. In the present study, we wished to extend the range of chemicals tested to include several common chemicals. The chemicals were chosen from the Report of the International Collaborative Program for Evaluation of Short-Term Tests for Carcinogens (de Serres and Ashby, 1981).

Pretreatment of mouse embryo fibroblasts with the carcinogens (Purchase *et al.*, 1981) chloroform and β-propiolactone resulted in a significant decrease in alpha/beta interferon production. No dose response effect of the treatments was observed; i.e., a threshold effect of the chemicals on interferon induction was observed. Such threshold effects of carcinogenic chemicals on interferon induction have been previously reported (Sonnenfeld *et al.*, 1983), and the significance of such a threshold effect requires further study and perhaps the development of more precise methods for the titration of interferon. Treatment of the cell cultures with non carcinogenic analogs of the chemicals, 1,1,1-trichloroethane and γ-butyrolactone, had no effect on interferon induction.

Treatment of the cell cultures with these chemicals at the dosages used did not result in apparent toxicity to the cultures as determined by trypan blue dye exclusion. This is further reinforced by earlier findings that virus replication was

TABLE 5
EFFECT OF ASCORBIC ACID ON ALPHA/BETA INTERFERON PRODUCTION

Treatment of cells	No. of experiments	Antiviral titer	Percentage change	*P*
0.02% DMSO + poly (I:C)	3	164 ± 23	—	—
0.02% DMSO + 1 μM ascorbic acid + poly (I:C)	3	407 ± 166	+148	NS
0.2% DMSO + poly (I:C)	3	164 ± 4	—	—
0.2% DMSO + 10 μM ascorbic acid + poly (I:C)	3	389 ± 130	+137	NS
2% DMSO + poly (I:C)	3	309 ± 128	—	—
2% DMSO + 100 μM ascorbic acid + poly (I:C)	3	453 ± 103	+47	NS

not inhibited or affected in any fashion in cell cultures treated with carcinogens (DeMaeyer-Guignard and DeMaeyer, 1965). Therefore, the effects of carcinogens on interferon induction may be specific to the interferon molecule, but more generalized effects on induced proteins can not be ruled out. Experiments are in progress in our laboratory to study this possibility.

DES has long been identified as an oncogenic agent for female offspring from women who have been treated with it (Herbst *et al.*, 1971; Herbst, 1981; Purchase *et al.*, 1981). DES appears to act through an hormonal mechanism and is not mutagenic to bacteria (Bridges, 1981). Therefore, DES may not have an effect on interferon induction because of its unique mechanism of carcinogenic action. An alternative explanation for the lack of effect of DES on interferon induction may be related to the toxicity of DES for mouse embryo fibroblasts. The highest dosage of DES that was nontoxic was 25μM, whereas other chemicals could be used at dosages of 100 μM. It may not be possible to separate the toxic and interferon induction inhibition effects of DES.

The chemical pyrene had no effect on interferon induction. Pyrene is still unclassified with regard to its carcinogenic potential (Purchase *et al.*, 1981). The structurally related chemicals benzo-(α)-pyrene and benzo-(ε)-pyrene have previously been tested for their effects on interferon induction (DeMaeyer-Guignard and DeMaeyer, 1965; Barnes *et al.*, 1981). Benzo-(α)-pyrene, a potent carcinogen, inhibited interferon induction, while benzo-(ε)-pyrene, a poor carcinogen, had no effect.

Treatment of the cell cultures with ascorbic acid, an unclassified but unlikely carcinogen (Purchase *et al.*, 1981), resulted in a slight enhancement in interferon production. This is in agreement with previous findings (Siegel, 1975).

The results of the present study extend previous studies suggesting that treatment of cell cultures with carcinogenic chemical inhibits the induction of alpha/beta interferon, while treatment with noncarcinogenic or poorly carcinogenic chemicals does not decrease interferon production. Extensive further studies are required before generalizations can be made and before the physiological significance of this phenomenon can be determined.

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