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Effect of Carcinogens and Analogs on Interferon Induction

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Abstract. Pretreatment of mouse embryo fibroblasts with several chemicals, including 7,12-dimethylbenz-(α)-anthracene, 2-aminofluorene, aflatoxin B_1, benzo-(α)-pyrene, styrene oxide, and the No. 4 fraction of tobacco smoke condensate, resulted in severely reduced production of interferon when the cells were challenged with Newcastle disease virus. All of the above chemicals are proven or strongly suggested carcinogens. When the analogs methyl methanesulfonate, a potent carcinogen, and ethyl methanesulfonate, a weak carcinogen, were applied to cells, interferon induction was only inhibited by the methyl methanesulfonate. Therefore, carcinogens may directly inhibit the induction of interferon by Newcastle disease virus.

Introduction

Interferon is now being used in several clinical trials for the treatment of various types of tumors, including non-Hodgkin's-type lymphomas [1]. Therefore, it is of interest to determine the type of interactions that exist between interferon and tumor-inducing substances, such as carcinogens.

Early experiments by *DeMaeyer and DeMaeyer-Guignard* [2, 3] suggested that application of known chemical carcinogens, such as benzo-(α)-pyrene, to rat embryo fibroblast cultures prior to challenge with vaccinia virus resulted in inhibition of the induction of interferon by the virus. An additional finding of significance was that benzo-(ε)-pyrene, a weak carcinogen, had no effect on the induction of interferon. These data suggested that carcinogens might have a specific inhibitory effect on the induction of interferon.

The results of the present study confirm and extend these observations. Several known and suspected potent carcinogens, including methyl methanesulfonate (MMS), markedly inhibited the induction of interferon by Newcastle disease virus (NDV) in mouse embryo fibroblasts. The rarely or weakly carcinogenic analog of MMS, ethyl methanesulfonate (EMS) [4, 5] had little or no effect on the induction of interferon by NDV. Therefore, the new data suggest that interferon induction is inhibited by pretreatment of cell cultures with an extended group of potent carcinogens.

Materials and Methods

Mouse Embryo Fibroblast Cultures. B6 H-2^k lyt2.1 mice, a gift of *Thomas Huff* and *Samuel Wellhausen*, Department of Microbiology and Immunology, University of Louisville, Ky., were bred and maintained in our laboratory. 15- to 18-day-old embryos were surgically removed from mothers, minced, trypsinized in 0.25% trypsin 1-300 (Pharmaceuticals, Cleveland, Ohio) and then suspended in Gibco minimal essential medium (MEM) with 10% fetal calf serum. Second or third passage cultures were used in all experiments and were plated in Falcon 25 cm^2 tissue culture flasks, and were used immediately upon reaching confluency.

Chemicals. Aflatoxin B_1, 2-aminofluorene, benzo-(α)-pyrene, EMS and MMS were obtained from Aldrich Chemical Co., Milwaukee, Wisc. 7,12-Dimethylbenz-(α)-anthracene and No. 4 fraction of tobacco smoke condensate were received from the Kentucky Tobacco Health and Research Institute, Lexington, Ky. Dimethylsulfoxide (DMSO) was obtained from J. T. Baker Chemical Co., Phillipsburg, N.J. Styrene oxide was kindly provided by Dr. *John Wong*, Department of Chemistry, University of Louisville, Ky.

Interferon Production. Mouse type I interferon was produced in fibroblasts with the Herts strain of NDV, as described elsewhere [6].

After inactivation of residual inducing virus by pH 2 treatment at 4°C for 4 days, the tissue culture supernatant fluid was assayed for interferon activity.

Interferon Assay. Interferon titers were determined by plaque reduction on mouse L-929 cells using Indiana strain bovine vesicular stomatitis virus. The interferon titer corresponded to the reciprocal of the highest dilution of test sample that reduced virus plaques by 50%. One interferon unit equals 0.88 NIH HG-002-904-511 reference units [6].

Results

Viability of Mouse Embryo Fibroblast Cultures

Mouse embryo fibroblasts were allowed to grow into a confluent monolayer. After reaching confluency, different cultures were treated for 24 h with several concentrations of the chemicals used in this study. These chemicals included 7,12-dimethylbenz-(α)-anthracene, 2-amin fluorene, aflatoxin B_1, No. 4 fraction of tobacco smoke condensate, benzo-(α)-pyrene, MMS, EMS, DMSO and ethanol. Immediately after removal of the chemicals, the fibroblast cultures were stained with 2× neutral red, a vital stain. All of the cultures that were treated with the dosages of chemicals that were used in the study were >95% viable by staining criteria. Additional cultures that had chemical-containing medium replaced with fresh MEM remained viable for at least another 24 h.

Effect of Known Carcinogens on Interferon Induction

The known carcinogens 2-aminofluorene and 7,12-dimethylbenz-(α)-anthracene were diluted in MEM and added to confluent fibroblast cultures. After 24 h of incubation, the culture supernatants were removed. The cultures were then challenged with NDV for 24 h and the supernatants were harvested, pH 2 treated, and then assayed for interferon activity. Reductions in interferon titers 90% were obtained when compared to non-carcinogen treated controls (table I). Several different dosages of chemicals were initially used to determine the dosage for optimum effect without affecting viability of the cultures, and the data in table I represent the optimal dosages.

Effects of Several Chemicals on Interferon Induction

Mouse embryo fibroblasts were treated with several different dosages of various chemicals before attempted induction of interferon with NDV. Aflatoxin B_1, No. 4 fraction of tobacco smoke condensate, benzo-(α)-pyrene, and styrene oxide treatments all inhibited the induction of interferon by 90% or greater (table II).

Table I. Effect of carcinogens on interferon production

Treatment of fibroblasts	Antiviral titer	% decrease from control
Newcastle disease virus	1,000	–
7,12-Dimethylbenz-(α)-anthracene + NDV		
39.0 μM	100	90
3.9 μM	86	91
2-Aminofluorene + NDV		
0.050 μM	21	98
0.005 μM	247	81

Table II. Blind study of the effect of various chemicals on interferon induction

Chemical treatment[1]	Interferon titer ± SE	% decrease from control	p_{NDV}[2]
None (NDV control)	1,456 ± 455	–	–
Aflatoxin B_1 0.05 μM	105 ± 82	93	<0.05
No. 4 fraction, tobacco smoke[3] condensate, 10×	89 ± 20	95	<0.05
Benzo-α-pyrene, 0.05 μM	122 ± 74	92	<0.05
Styrene oxide, 0.05 μM	149 ± 60	90	<0.05

[1] Mouse embryo fibroblasts were pretreated with the appropriate chemical for 24 h. Supernatants were removed, cells washed and NDV applied for 24 h. Cell supernatants were then harvested and assayed for antiviral activity.

[2] Statistical analysis was performed by means of a Student's T analysis.

[3] Arbitrary dilutions of the No. 4 fraction of tobacco smoke condensate were used.

Table III. Blind study of the effect of a weak and a potent carcinogen on interferon production

Chemical treatment[1]	Interferon titer ± SE	% decrease[2] from control	p
None (NDV control)	1,456 ± 455	–	–
Ethyl methanesulfonate 0.05 μM	1,680 ± 294	–	>0.5
Methyl methanesulfonate 0.05 μM	137 ± 82	91	<0.05

[1] Experimental protocol as in table II.

[2] EMS and MMS were dissolved in ethanol only.

Table II represents the effects of optimum dosages of the chemicals used. DMSO was used as a solvent for all of the above chemicals. Pretreatment of fibroblast cultures with DMSO resulted in a small (0–40%), not

statistically significant decrease in the titers of interferon induced by NDV.

EMS and MMS were solubilized in ethanol. Pretreatment of mouse fibroblasts with ethanol had no effect on the titers of interferon induced by NDV. Pretreatment of the cell cultures with EMS had a minimal effect on the induction of interferon by NDV, while MMS pretreatment severely decreased the titer of interferon induced by NDV (table III).

Discussion

The results of the present study indicate that several well-known carcinogens, including 7,12-dimethylbenz-(α)-anthracene, benzo-(α)-pyrene, 2-aminofluorene, aflatoxin B_1, and the No. 4 fraction of tobacco smoke condensate, all inhibited the induction of interferon by NDV. Styrene oxide, an important industrial chemical, which was positive in the Ames assay, but negative in all *Bacillus* assays done to date, also inhibited the induction of interferon by NDV. Further studies of the carcinogenic potential of styrene oxide may be required.

DMSO has been used in clinical trials as a drug for the treatment of arthritis. However, these trials have been suspended due to the teratogenic potential of the chemical [7, 8]. It is of interest to note that pretreatment of cells with DMSO did not result in the significant inhibition of interferon induction by NDV.

EMS and MMS are closely related analogs which differ with respect to carcinogenic potential as determined by tumor induction [4, 5]. MMS is a highly carcinogenic mutagen, while EMS is a rare or weakly carcinogenic mutagen. In the present study, MMS strongly inhibited the induction of interferon by NDV, while EMS had no significant effect. In a recent study, fibroblasts were pretreated with four different forms of aflatoxin [9]. The degree of inhibition of interferon induction correlated with the proven carcinogenic potential of the aflatoxin forms, i.e. the more carcinogenic the form of aflatoxin, the greater the inhibition of interferon induction. In view of these findings and the previously reported discrimination between benzo-(α)-pyrene and benzo-(ε)-pyrene [3] and the presently observed discrimination between EMS and MMS, carcinogens may directly inhibit the induction of interferon.

The mechanism by which carcinogens inhibit the induction of interferon is not known. A toxic effect of the carcinogens to the cell cultures does not seem likely, since the results of this study indicate that the target cells are alive when they fail to produce interferon. However, a general effect of the carcinogens on DNA, RNA, or protein synthesis and differential dosage effects of potent and weak carcinogens cannot be ruled out and are the subject of future studies.

Other possible mechanisms include alteration of the cell membrane by the carcinogen resulting in reduced interaction of virus with the cells. Recent studies showing that pretreatment of cells with carcinogens inhibited the induction of interferon by poly-inosinic–poly-cytidylic acid, a nonviral inducer of interferon [10]. In view of those studies and the earlier finding that carcinogen pretreatment does not inhibit the replication of the viruses used to induce interferon [2], it is unlikely that virus-membrane interactions are severely disrupted by carcinogen treatment of cells.

Another possible mechanism could involve induction of a protease by the carcinogen in a manner analogous to bacterial 'SOS' repair systems [11]. This protease could degrade induced interferon, or interfere with the control mechanisms for interferon induction. The true nature of the proposed effect of carcinogens on interferon induction is the subject of extended studies.

The implications of these studies toward an understanding of the mechanisms of carcinogenesis are still obscure. It is possible to speculate that there may be a balance between interferon levels and oncogenesis and that balance may be upset by the action of a carcinogen. In addition, if further studies continue to indicate a differential effect on interferon induction by strong and weak carcinogens, the effect of a chemical on interferon induction may have some use in determination of the carcinogenic potential of that chemical.

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