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USE OF INTERFERON SYSTEMS IN IMMUNOTOXICOLOGY

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INTRODUCTION

Interferons are a group of proteins that were originally described as being produced in response to virus infections and acting by making virgin cells refractory to virus infection^(1,2). It has recently become apparent that interferons are much more complex than originally as described^(3,4). There are now several types of interferons⁽⁵⁾. Alpha interferon is primarily produced by leukocytes in response to virus or double-stranded RNA-(polyriboinosinic-polyribocytidylic acid=poly I:C). Beta interferon is primarily produced by fibroblasts in response to virus or poly I:C. Therefore, unless purified, interferon produced by fibroblasts after poly I:C challenge contains a minor alpha component and a major beta component. Gamma (type II immune) interferon is produced as a lymphokine as part of an immune response of lymphoid cells to antigen or mitogen. Alpha and beta interferons appear to have some structural and functional similarities but gamma interferon appears to be a distinct entity⁽⁶⁾.

Interferons have also been shown to have several additional activities in addition to the originally described antiviral activity^(3,4). These include regulation of cell growth and division, regulation of immune responses, and cytotoxicity to tumors and tumor cells^(3,4). As a result of these activities, the use of interferons in clinical anti-cancer trials is now being actively pursued⁽⁷⁾.

Since interferons may be involved in defenses against tumors, it was of interest to study the interactions between interferons and carcinogens. Several workers had suggested that carcinogens could inhibit the induction of interferon, while closely matched, poorly, or non-carcinogenic analogues had no effect on interferon induction⁽⁸⁻¹²⁾. We began a survey of the effects of a wide

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variety of types of carcinogens and analogues on interferon induction⁽¹³⁻²⁰⁾. Our results to date suggest a strong correlation between carcinogenic potential of a chemical and the effects of the chemical on interferon induction⁽¹³⁻²⁰⁾.

MATERIALS AND METHODS

MOUSE EMBRYO FIBROBLAST CULTURES

C3H/He mice were originally obtained from Laboratory Supply, Indianapolis, Indiana and then maintained and bred in our laboratory. Fifteen to 18 day-old embryos were surgically removed from pregnant dams, and then trypsinized to single cells. The cells were next suspended in Gibco (Grand Island, New York) minimal essential medium with 10% fetal bovine serum. Second or third passage cultures were used in all experiments and were plated in 25 cm² plastic tissue culture flasks. Cultures were immediately used after reaching confluency⁽¹³⁻¹⁹⁾.

MOUSE SPLEEN CELL CULTURES

Female 6-8 week old Swiss/Webster mice were obtained from Laboratory Supply Company, Indianapolis, Indiana. Cultures of 5×10^6 spleen cells were prepared in 1 ml of RPMI-1640 medium (Gibco, Grand Island, New York) supplemented with 1% fetal bovine serum and 5×10^{-5} M 2-mercaptoethanol⁽²⁰⁾.

CELL VIABILITY DETERMINATION

Cell viabilities were determined by trypan blue dye exclusion⁽¹³⁻²⁰⁾.

PRODUCTION OF ALPHA/BETA INTERFERON

Alpha/Beta interferon was induced in mouse embryo fibroblast cultures by stimulating with 50 µg of poly I:C for 60-90 minutes, and then adding additional fresh tissue culture medium. In some cases, Newcastle disease virus was the inducer. DEAE-dextran was included to insure maximum interferon production. Tissue culture supernatants were harvested at 24 hours and assayed for interferon antiviral activity⁽¹³⁻¹⁹⁾.

PRODUCTION OF GAMMA INTERFERON

Mouse spleen cell cultures were stimulated with 34 µg of phytohemagglutinin-P and incubated for 3 days at 37°C in 5% CO₂. Culture supernatants were then harvested and assayed for interferon activity⁽²⁰⁾.

INTERFERON ASSAY

Antiviral titers were measured by performing a plaque reduction assay on mouse L-929 cells with the Indiana strain of vesicular stomatitis virus as the test virus⁽²¹⁾. The antiviral titer corresponded to the reciprocal of the furthest dilution of test sample that reduced virus plaques by 50%. In this assay, one interferon antiviral unit is equivalent to 0.88 NIH G-002-904-511 reference units.

STATISTICAL ANALYSIS

The data were analyzed by means of Student's t-test. P values of <0.05 were required for statistical significance.

RESULTS

EFFECTS OF CHEMICALS ON ALPHA/BETA INTERFERON

Mouse embryo fibroblasts were pretreated with chemicals and then challenged to induce alpha/beta interferon as described in the protocol in Figure 1. Several pairs of different types of probable carcinogens and poor or non-carcinogens were included. In all cases except two, benzidine and diethylstilbestrol, carcinogen pretreatment significantly decreased alpha/beta interferon production (Table 1)⁽¹³⁻¹⁹⁾. Since the interferon assay is a titration, a statistically significant decrease of 50% or greater as compared to a solvent-only control was required. Poor or non-carcinogens had no effect on alpha/beta interferon induction (Table 1)⁽¹³⁻¹⁹⁾. No effect on viability was observed after any of the chemical treatments.

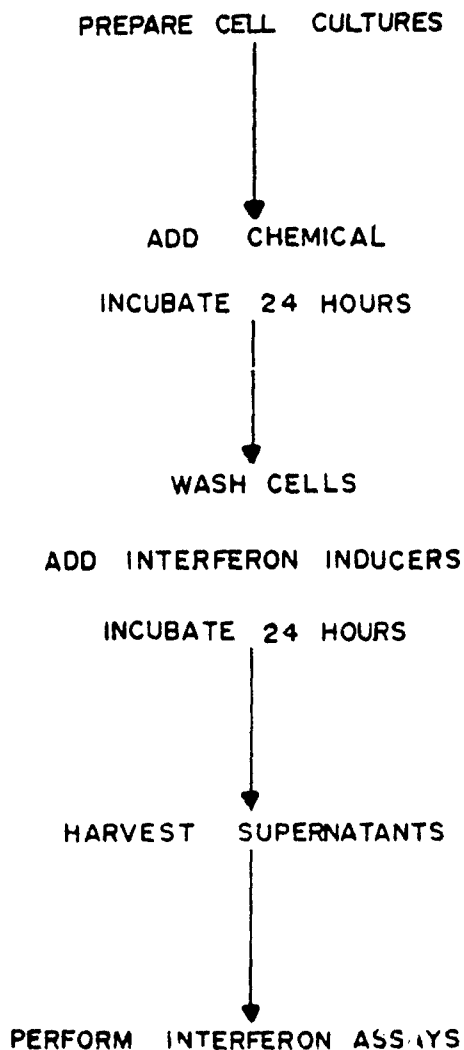


Figure 1. Protocol for determination of the effects of carcinogens on interferon induction.

EFFECTS OF CHEMICALS ON GAMMA INTERFERON

7,12-dimethylbenz-(a) anthracene (DMBA) was added to spleen cell cultures prior to induction of gamma interferon. Gamma interferon production was significantly inhibited, but in this case viability was slightly reduced (data not shown)(20). When DMBA was added to target L-929 cells together with exogenous gamma interferon, no effect on the antiviral activity of the gamma interferon was observed.

TABLE 1. EFFECTS OF CARCINOGENS AND ANALOGUES ON INTERFERON INDUCTION

Chemical	In vivo ^a Carcinogenic Potential	Effect on Interferon Induction	Minimal Effective Concentration
8-Propiolacetone	+	NS ^b	--
γ-Butyrolacetone	-	-52%	100 μM
Pyrene	-	NS	--
Benzo-(a)-pyrene	+	-90%	0.05 μM
1-naphthylamine	-	NS	--
2-naphthylamine	+	-56%	100 μM
Anthracene	-?	NS	--
7,12-dimethylbenz (a) anthracene	+	-86%	4 μM
9,10-dimethylanthracene	+?	-50%	100 μM
Chloroethanol	-	NS	--
Chloroacetic acid	-	NS	--
Chloroacetaldehyde	+	-77%	0.005 μM
Ethyl methanesulfonate	-?	NS	--
Methyl methanesulfonate	+	-91%	0.05 μM
1,1,1-Trichloroethane	-	NS	--
Chloroform	+	-54%	100 μM
Amorphous Nickel Sulfide	-?	NS	--
Crystalline Nickel Sulfide	+?	-75%	2 μg/ml
3,3'-5,5'-tetramethylbenzidine	-	NS	--
Benzidine	+	NS	--
2-aminofluorine	+	-81%	0.005 μM
Aflatoxin B ₁	+	-93%	0.05 μM
Number 4 fraction tobacco smoke condensate	+	89%	10x ^c
Sytrene oxide	+?	-90%	0.05 μM
2-methylquinoline	+?	-79%	0.01 μM
4-aminobiphenyl	+	-73%	0.01 μM
Hydrazine sulfate	+	-72%	0.01 μM
Aniline-HCl	+	-75%	0.01 μM
Diethylstilbestrol	+?	NS	--
Isobutyl Nitrite	+?	61%	0.01%
"RUSH" (Recreational isobutyl nitrite)	+?	62%	0.01%
Ascorbic Acid	-	NS	--
Glycine v Leucine	-?	NS	--
Glycine v Isoleucine	-?	NS	--

^a Data from references 13, 14, and 22.

^b NS = Not a significant decrease.

^c Arbitrary laboratory concentrations.

DISCUSSION

Pretreatment of mouse embryo fibroblasts with a variety of carcinogens resulted in significant depression of alpha/beta interferon induction by poly I:C or Newcastle disease virus⁽¹³⁻¹⁹⁾. Treatment of the cells with poorly or non-carcinogenic analogues had no significant effect on interferon induction.

Of the 34 chemicals tested, only the following were exceptions: Benzidine, a carcinogen, had no effect on interferon induction. This may be due to insufficient activation of benzidine by the mouse embryo fibroblasts⁽¹³⁾. Diethylstilbestrol (DES) also had no effect on interferon induction. The lack of effect of DES may have been due to the apparent unique mechanism of carcinogenic action of this chemical. DES appears to act through an hormonal action and is not usually mutagenic to bacteria^(17,23).

Gamma interferon induction was also inhibited by carcinogens⁽²⁰⁾. Since many different types of carcinogens apparently affect the production of different types of interferons, it is possible that multiple mechanisms may have been involved. Viability of the cell cultures was not dramatically affected, and virus replication was not depressed by carcinogen-treatment⁽⁹⁾. Therefore, it is unlikely that carcinogen treatment resulted in a non-specific general toxic shutdown of cellular metabolism. Other induced proteins in addition to interferon may also be affected.

Since carcinogen treatment had no effect on preformed exogenous interferon⁽²⁰⁾, it is unlikely that the carcinogens were bound to the interferon inactivating it. Rather, it is likely the carcinogens affected the actual interferon production mechanism. The carcinogens may be binding to cellular nucleic acids, preventing the production of interferon.

The results of the present study suggest a high correlation between carcinogenic potential of a chemical and suppression of interferon induction. After extensive further testing, inhibition of interferon induction may prove useful as part of a battery of screening tests for carcinogenic potential of chemicals. This system has the advantages of using fibroblasts, which may be primary target cells for carcinogens in vivo, and being relatively straightforward to interpret. As assays for interferon improve with the development of radioimmune assays and enzyme linked-immunoassays, inhibition of interferon assays may be readily performed in a number of laboratories.

REFERENCES

1. Issacs, A. and J. Lindenmann (1957), Virus interference I. The interferon, Proc. Roy. Soc. Ser. B 147-258.
2. Sonnenfeld, G and T. C. Merigan (1979), The role of interferon in viral infections, Springer. Sem. Immunopath., 2:311.
3. Gresser, I. (1977), Commentary on the varied biological effects of interferon, Cell. Immunol., 34:406.
4. Sonnenfeld, G. (1980), Modulation of immunity by interferon, Lymphokine Rep., 1:113.
5. Stewart, W. E., II, J. E. Blalok, D. C. Burke, C. Chany, J. K. Dunnick, E. Falcoff, R. M. Friedman, G. J. Galasso, W. J. Joklik, J. T. Vilcek, J. S. Youngner, and K. C. Zoon (1980), Interferon nomenclature, Nature, 286:110.
6. Sonnenfeld, G. (1983), Effects of interferon on antibody formation, In: Interferons, N. B. Finter, series ed. Volume IV Interferon and the Immune Systems, S. Vilcek and E. DeMaeyer, (eds.), Elsevier-North Holland, Incorporated, Amsterdam, In Press.
7. Merigan, T. C. (1982), Interferon therapy in human viral infections and malignant disease, In: Interferon: Immunobiology and Clinical Significance, E. R. Stiehm, moderator, Ann. Intern. Med., 96:80.
8. DeMaeyer, E. and J. DeMaeyer-Guignard (1964), Inhibition by 3-methylcholanthrene of interferon formation in rat embryo cells infected with Sindbis virus, J. Natl. Cancer Inst., 32:1317.
9. DeMaeyer-Guignard, J. and E. DeMaeyer (1965), Effect of carcinogenic and noncarcinogenic hydrocarbons on interferon synthesis and virus plaque development, J. Natl. Cancer Inst., 34:265.
10. Hahon, N., J. A. Booth, and J. D. Stewart (1979), Aflatoxin inhibition of viral interferon induction, Antimicrob. Agents Chemother., 16:277.
11. Treagen, L. and A. Furst (1970), Inhibition of interferon synthesis in mammalian cell cultures after nickel treatment, Res. Commun. Chem. Path. Pharmacol., 1:395.
12. Hahon, N., J. A. Booth, and D. J. Pearson (1980), Inhibition of viral interferon induction in mammalian cell monolayers by metallic copper, aluminum, and nickel particles, In: The In Vitro Effects of Mineral Dust, R. C. Brown et al. (eds.), Academic Press, London, p. 219.

13. Sonnenfeld, G., M. C. Barnes, J. Schooler, and U. N. Streips (1980), Inhibition of interferon induction as a screen for the carcinogenic potential of chemicals, In: Interferon: Properties and Clinical Uses, A. Khan, N. O. Hill, and G. L. Dorn (eds.), Wadley Inst. Molec. Med., Dallas, p. 589.
14. Barnes, M. C., U. N. Streips, and G. Sonnenfeld (1981), Effects of carcinogens and analog on interferon induction, Oncology, 38:98.
15. Sonnenfeld, G., R. W. Hudgens, and U. N. Streips (1982), Effect of aromatic carcinogens and non-carcinogenic analogues on the induction of murine alpha/beta interferon, Int. J. Environ. Risk Assess., In Press.
16. Sonnenfeld, G. (1982), Effects of sidestream smoke components on alpha/beta interferon production, Oncology, In Press.
17. Sonnenfeld, G., R. W. Hudgens, and U. N. Streips (1982), Effect of environmental carcinogens on murine alpha/beta interferon production, Environ. Res., In Press.
18. Hersh, E. M., J. M. Reuben, H. Bogerd, M. Bielski, P. W. A. Mansell, A. Rios, G. R. Newell, and G. Sonnenfeld (1982), Effect of the recreational agent isobutyl nitrite on human peripheral blood leukocytes and on in vitro interferon production, Cancer Res., In Press.
19. Sonnenfeld, G., U. N. Streips, and M. Costa (1982), Differential effects of amorphous and crystalline nickel sulfide on murine alpha/beta interferon production, Submitted for publication.
20. Golemboski, K. A., D. K. D. Delor, U. N. Streips, and G. Sonnenfeld (1982), Effect of 7,12-dimethylbenz-(a)anthracene on production and action of gamma interferon, J. Natl. Cancer Inst., 68:993.
21. Brodeur, B. R. and T. C. Merigan (1974), Suppressive effect of interferon on the humoral response to sheep red blood cells, J. Immunol., 113:1319.
22. de Serres, F. J. and J. Ashby (1981), Evaluation of Short-Term Tests for Carcinogens, F. J. de Serres and J. Ashby (eds.), Elsevier-North Holland, Incorporated, New York.
23. Bridges, B. A. (1981), Summary report on the performance of bacterial mutation assays, In: Evaluation of Short-Term Tests for Carcinogens, F. J. de Serres and J. Ashby (eds.), Elsevier-North Holland, Incorporated, New York, p. 49.

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