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Interferon System in Cells from Human Tumors and from Persons Predisposed to Cancer

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In the present study, the interferon system was evaluated in fibroblasts from persons predisposed to leukemia or other cancers, in fibroblasts from persons with neoplastic disease, and in human tumor cells. Of 31 normal fibroblast strains from patients with tumors or diseases associated with a high incidence of malignancy, only one cell strain had a poor response to either of the two interferon inducers used, polyinosinic-polycytidylic acid and Chikungunya virus. On the other hand, cell cultures of five human tumors were much less sensitive to the antiviral effect of these interferon inducers and of human interferon and produced less interferon in response to Chikungunya virus than any of the nontumor tissues studied.

Viruses are known to cause naturally occurring tumors in a number of species. Although a viral etiology for human tumors has not yet been established, there is evidence that certain viruses can transform human cells in tissue culture (1, 10, 14, 23, 25), and such agents have been seen on rare occasions in human tumor cells (17, 28). The interferon system is known to be an important cellular defense against viral infection and is active against such experimental tumor viruses as simian virus 40 and the murine sarcoma-leukemia viruses, both in vitro and in vivo (20, 22, 27). Interferon is also active against transplantable tumors not deliberately induced by viruses (12). The possibility exists that the heightened susceptibility in certain diseases to leukemia and other cancers might be associated with a defect in the interferon system. The present studies were undertaken to test this possibility.

MATERIALS AND METHODS

Cell culture. Human cells were grown in Dulbecco's modification of Eagle's medium supplemented with 10% calf serum (Colorado Serum Co.). Fibroblast cultures were derived from punch biopsies of skin from clinically normal persons and from patients with a variety of diseases. The tumor cell lines used here have been described elsewhere (2). Normal fibroblasts and tumor cells were studied within the first five passages in culture in our laboratory. The diploid human cell culture (BUD-8) was provided by B. W. Uhlendorf (National Institutes of Health, Bethesda, Md.).

Viruses. The McCrumb strain of Chikungunya virus was obtained by making a 10% (w/v) suspension of infected suckling mouse brains in Eagle's medium with 10% fetal bovine serum (FBS). The pool titered $10^{9.5}$ plaque-forming units (PFU)/ml on Vero cells. Sindbis virus was grown on chick embryo tissue; it titered 10^9 PFU/ml. The Chikungunya virus was heat-inactivated by incubation at 37 C for 18 to 20 hr immediately prior to use.

Synthetic polymers. Double-stranded polyinosinic-polycytidylic ribonucleic acid (poly I:C) was prepared as described previously (9).

Human interferon. Human interferon was obtained by incubating monolayer cultures of human diploid cells (BUD-8) with live Chikungunya virus at a multiplicity of infection (MOI) of about one. The interferon-containing fluids were harvested 24 hr after infection, and residual Chikungunya virus was inactivated at *p*H 2 for 1 hr. The *p*H was then readjusted to 7.4.

Protection of human cells by interferon inducers. Human cells growing in tubes were exposed to appropriate dilutions of either poly I:C or heat-inactivated Chikungunya virus in 2% FBS in Eagle's medium for 18 to 20 hr. Cells were then washed three times and challenged with Sindbis virus (MOI, 100 PFU/cell). After incubation for 1 hr at 37 C, the cells were washed three times and incubated in 0.5 ml of Eagle's medium without serum for another 18 to 20 hr at 37 C. Virus was then harvested by freeze-thawing of cells, and the yield of Sindbis virus was determined by the hemagglutination (HA) method (8).

Interferon assays. Cultures of the BUD-8 cell strain were incubated overnight with 0.5 $\log_{10}$ dilutions of the fluid to be tested. The cells were then assayed for Sindbis virus growth as described above.

RESULTS

Antiviral response in human fibroblasts. The ability of the synthetic polymer poly I:C to induce resistance to Sindbis virus was tested in a large

TABLE 1. *Sensitivity of normal human skin fibroblasts to the antiviral effect of poly I:C*

Patient origin of fibroblasts[a]	No. examined	Minimal effective concn of poly I:C (μg/ml)[b]
Normal	7	<0.1, 0.1, 0.1, 0.2, 0.3, 1.0, 1.0
Leukemia	7	<0.1, <0.1, <0.1, 0.1, 0.2, 0.2, 1.0
Sarcoma	4	<0.1, 0.3, 0.2, 10
Down's syndrome	4	<0.1, <0.1, <0.1, 0.1
Fanconi's anemia	4	<0.1, <0.1, <0.1, 0.1
Bloom's syndrome	1	<0.1
Xeroderma pigmentosum	2	0.3, 0.3
Wiskott-Aldrich syndrome	2	0.3, 0.3
Trisomy E	2	0.3, 0.3
Other diseases[c]	5	<0.1, <0.1, <0.1, <0.1, 0.3

[a] Normal fibroblasts were established as diploid cultures from patients with various diseases or from normal individuals.

[b] Minimum concentration of poly I:C which reduced the yield of Sindbis virus hemagglutination fourfold in a one-step growth cycle.

[c] One each of the following disorders: myeloid metaplasia, ataxia telangiectasia, Turner's syndrome, Gardner's syndrome, basal cell nevus syndrome.

number of normal skin fibroblast strains from patients with diseases which predispose to malignancies and from normal controls. The minimal effective concentration (MEC) of poly I:C was 1 μg/ml or less for all but one of the skin fibroblasts tested (Table 1). Of note, no striking insensitivity to poly I:C was demonstrated by skin fibroblasts from persons with a variety of inborn or acquired diseases associated with an increased incidence of tumors. Only one culture, obtained from an individual with a sarcoma, showed a relative insensitivity to poly I:C. It required 10 μg/ml for protection.

Heat-inactivated Chikungunya virus was also tested for its ability to stimulate the antiviral system of the cells. Cultures were incubated overnight with 10-fold dilutions (10^{-2} to 10^{-5}) of heat-inactivated Chikungunya virus and then assayed for reduction of Sindbis virus growth. Table 2 shows the effect of the 10^{-4} dilution on formation of Sindbis virus HA. Each of the normal fibroblast cultures showed complete suppression of Sindbis HA at this concentration of Chikungunya virus. This included the strain of fibroblasts which was resistant to poly I:C. The

TABLE 2. *Sensitivity of human fibroblasts to the antiviral effect of heat-inactivated Chikungunya virus*

Patient	Tissue	Respond to 10^{-4} dilution of inactivated Chikungunya virus[a]
Normal	Normal skin	3/3
Normal	Synovium, muscle, kidney, and pleura (fibroblasts)	4/4
Sarcoma	Normal skin	2/2
Xeroderma pigmentosum	Normal skin	1/1
Fanconi's anemia	Normal skin	1/1
Human embryo	Embryo fibroblast	1/1
Rheumatoid arthritis	Synovial fibroblast	1/1

[a] Respond = total suppression of measurable Sindbis virus hemagglutination in a one-step growth cycle. Dilution of Chikungunya virus of 10^{-4} = concentration of $10^{5.5}$ PFU/ml prior to heat inactivation.

TABLE 3. *Sensitivity of human fibroblasts and human tumors to two interferon stimulators*

Cell type	Origin	Minimal effective concn of poly I:C (μg/ml)[a]	Respond to 10^{-4} dilution of Chikungunya virus[b]
Fibroblasts from normal patients	Normal skin, kidney, pleura, muscle, synovium	<0.1–1.0	Positive
Tumor cells			
MC-3	Sarcoma	>10	No response
8387L	Sarcoma	10	Positive
SA-4	Sarcoma	>10	No response
C584	Sarcoma	>10	No response
9812	Bronchogenic CA	>10	No response

[a] Minimum concentration of poly I:C which reduced the yield of Sindbis virus hemagglutination fourfold in a one-step growth cycle.

[b] Respond = total suppression of measurable Sindbis virus hemagglutination.

TABLE 4. *Sensitivity of human fibroblasts and human tumors to interferon*

Patient origin of cultures	No. tested	Tissue	Minimal effective concn of interferon (units/ml)[a]
Normal	3	Normal skin	1, 1, 1
Normal	1	Synovium	1
Human embryo	1	Embryo fibroblast	1
Fanconi's anemia	3	Normal skin	1, 5, 5
Xeroderma pigmentosum	1	Normal skin	5
Down's syndrome	1	Normal skin	0.5
Rheumatoid arthritis	1	Synovium	5
Congenital hemihypertrophy	3	Normal skin	1, 1, 1
Carcinoma	1	Carcinoma	50
Sarcoma	4	Sarcoma	5, 50, 50, 50

[a] Minimum concentration of human interferon which reduced the yield of Sindbis virus hemagglutination fourfold in a one-step growth cycle.

10^{-5} dilution of Chikungunya virus was inactive as an inducer in any of the fibroblast cultures tested. A number of fibroblast cultures derived from other tissues, such as lung, kidney, muscle, and synovium, were also tested for responsiveness to Chikungunya virus (Table 2). These cells were as sensitive as skin fibroblasts to the antiviral effect of Chikungunya virus.

Antiviral system in human tumors. The interferon system of human cell lines derived from four sarcomas and one bronchogenic carcinoma was studied. These tumor cell lines demonstrated morphological and growth characteristics of tumor cells (2). Each of the tumor cell lines was relatively resistant to the antiviral effect of the two interferon stimulators (Table 3). A poly I:C concentration of 10 μg/ml, which protected all of the fibroblast cell strains, was able to protect only one of the tumor lines (8387L). Similarly a concentration of inactivated Chikungunya virus (10^{-4} dilution) that effectively protected each normal human fibroblast strain was only able to protect completely 8387L. In the absence of inducers, Sindbis virus grew in the tumor cells to HA titers comparable to those noted with the normal fibroblast cells. The tumor cells were not absolutely insensitive to the interferon stimulators, as evidenced by the ability of a 10- to 100-fold larger inoculum of Chikungunya virus to protect each one.

Response to human interferon. The insensitivity of the human tumor lines to two interferon stimulators could be related to an inability to produce or to respond (or both) to interferon. The sensitivity of tumor and normal cells to interferon was tested by pretreating cell cultures overnight with human interferon at 0.1, 1.0, 10, or 100 units/ml. The induced resistance to Sindbis virus growth was then measured.

Table 4 lists the MEC of human interferon necessary for protection of the cells. The MEC for each of the nontumor cells was 5 units/ml or less, whereas four of the five tumors required an interferon concentration of 50 units/ml. The tumor which was the most responsive to interferon, 8387L, was also the most sensitive to the protective effects of both poly I:C and heat-inactivated Chikungunya virus. These results indicate that the tumor cells were relatively resistant to the antiviral effect of human interferon.

Normal and tumor cell cultures were exposed to either 10 or 100 units/ml of interferon for 18 to 20 hr. The remaining amount of interferon in supernatants from each was not found to differ significantly. These findings argue against the possibility that increased degradation of interferon is responsible for the insensitivity of tumor cells to interferon or interferon inducers.

Production of interferon. The ability of tumor cells and normal fibroblasts to produce interferon after stimulation by heat-inactivated Chikungunya was also studied (Table 5). The majority of the tumors produced 30 units/ml or less of interferon in response to a 10^{-2} dilution of heat-inactivated virus, whereas each of the nontumor cells tested produced at least 100 units/ml. Production of interferon by tumor line 8387L was borderline (100 units/ml), as it was for the other aspects of the interferon system.

Detection of mycoplasma. Infection of cells in tissue culture with mycoplasma has been reported to decrease, increase, or to have no effect on interferon production and sensitivity (3, 24, 29). To determine whether mycoplasma might have contributed to our results, the cells used in the present studies were tested for contamination by these agents. Three of the tumor cell lines and one skin fibroblast strain were found to contain mycoplasma. Of note, the fibroblast strain was normally responsive to the interferon inducers and to interferon. In additional studies to rule out an effect of mycoplasma, three of the myco-

TABLE 5. *Production of interferon by human fibroblasts and human tumors*

Patient origin of cell culture	Tissue	Interferon produced (units/ml)[a]
Normal	Normal skin	1,000
		600
		100
		100
Fanconi's anemia	Normal skin	200
Sarcoma	Normal skin	300
		100
Carcinoma	Carcinoma (9812)	<3
Sarcoma	Sarcoma (8387L)	100
	(MC-3)	30
	(C584)	<3
	(SA4)	<3

[a] The cells studied were incubated overnight with a 10^{-2} dilution of heat-inactivated Chikungunya virus ($10^{7.5}$ PFU/ml prior to heat inactivation); the culture fluid was then harvested and assayed for interferon.

plasma-free fibroblast strains were infected with mycoplasma isolated from the tumor lines and were then tested for their sensitivity to poly I:C and human interferon. In no case did mycoplasma infection cause these cells to become resistant to the antiviral effects of either poly I:C or interferon. These results indicate that the relative insensitivity of the tumor cell lines to the two interferon stimulators and to interferon itself was not related to the presence of mycoplasma.

DISCUSSION

In the present study, the interferon system was evaluated in skin fibroblasts from persons predisposed to leukemia or other cancers and in skin fibroblasts from persons with leukemia or sarcomas. Down's syndrome and Fanconi's aplastic anemia are two inborn disorders which predispose to leukemia (15). Fibroblasts from persons with these disorders have been shown to be more sensitive to transformation in tissue culture by simian virus 40 than are skin fibroblasts from clinically normal persons (16). Further, simian virus 40 is known to be sensitive to the action of interferon (27), and the growth cycle of the virus in human fibroblasts is relatively slow (23). It was reasoned that a defect in the interferon system in cells from these patients might contribute to an increased simian virus 40 transformation susceptibility in tissue culture. In the present studies, we found no defect in either production of or sensitivity to interferon in normal fibroblasts from persons with Down's syndrome, Fanconi's anemia, or any of seven other syndromes studied which predispose to cancer (18). The results of recent studies on the production of interferon by lymphocytes from patients with Down's syndrome or Fanconi's anemia were similar to the present findings (26). These results argue against decreased production of or sensitivity to interferon being responsible for the enhanced susceptibility of certain cells to simian virus 40 transformation. From the present studies, it is concluded that the interferon system functions well in normal fibroblast cells derived from the large majority of tumor-susceptible patients.

In contrast to the results with normal fibroblasts, the human tumors studied were in general much less sensitive to the antiviral effect of poly I:C and Chikungunya virus, produced less interferon in response to Chikungunya, and were less sensitive to human interferon than any of the nontumor tissues studied. Lack of responsiveness to interferon has been reported in hamster, mouse, and rat cell cultures transformed spontaneously by methyl-cholanthrene or by a variety of deoxyribonucleic acid and ribonucleic acid oncogenic viruses (4, 19, 21). Other studies have indicated no obvious correlation between viral- or carcinogen-induced transformation and decreased sensitivity to interferon in rat cells (11). Certain human tumor lines, such as HeLa and KB, have been reported to have a decreased ability to produce and respond to interferon (6, 13), but this has also not been a constant finding (5). The reasons for the insensitivity of tumor cells to interferon reported here remain to be clarified. However, Chany and his colleagues have recently reported that extracts of human sarcoma cells contain a substance which antagonizes the antiviral effect of interferon (7). Studies are now in progress to determine whether similar anti-interferon activity can be extracted from our human tumor cells.

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