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Brief Communication

Enhancement of X-ray-induced Transformation in C3H/10T$^1/_2$ Cells by Interferon[1]

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SUMMARY

Interferon may enhance malignant transformation induced by X-rays in a C3H mouse embryo-derived cell line. The inhibitory effect of interferon on cell division during the proliferative phase of the expression of the transformational damage may be of importance in the understanding of this finding.

INTRODUCTION

It has been clearly demonstrated that, in addition to its antiviral activity, interferon has a wide variety of effects on noninfected cells (6) and can under certain conditions suppress cell proliferation (4). Recently, it has been shown that reproducible oncogenic transformation can be induced by X-irradiation in a line of mouse embryo cells (11). The frequency of this non-viral-induced transformation was highly dependent upon the kinetics of cell proliferation after irradiation (10, 11). To our knowledge, the influence of interferon on non-viral-induced transformation has not heretofore been investigated. We report here that interferon can significantly enhance X-ray transformation *in vitro*.

MATERIALS AND METHODS

We used the mouse embryo fibroblast cell line designated 10T$^1/_2$ CL8 isolated by Reznikoff *et al.* (9). The techniques for cultivation and maintenance of these cells, as well as for performing transformation experiments, have been described in detail (8, 9, 11).

Semipurified mouse interferon (generously provided by Dr. I. Gresser) was prepared from suspension cultures of mouse C243 cells induced with Newcastle disease virus (12). Control preparations were prepared in a manner identical with that used in the preparation of interferon except that the interferon inducer was omitted. The specific activity of the mouse C243 cell interferon ranged between 2.8×10^6 and 1.2×10^7 reference units/mg of protein. Partially purified human interferon prepared in leukocyte suspension inoculated with Sendai virus was kindly provided by Dr. E. Mogensen. The specific activity was 3.5×10^5 units/mg of protein.

Inactivation of mouse interferon (see Table 2) was performed either by incubating interferon with 500 μg of trypsin per ml for 1 hr at 37° (trypsin activity was neutralized by 100,000 units of iniprol; Choay, France) or by dialyzing interferon first against sodium periodate (0.02 M) for 2 hr at 37°, then against 5% glucose solution for 2 hr, at 4°, and finally against 0.1 M phosphate-buffered saline overnight at 4°. The residual antiviral activity as assayed on 10T$^1/_2$ cells with vesicular stomatitis virus was reduced 100-fold, and no antiviral activity was detected in the preparation at the dilution used in the experiments.

The experimental procedures were as follows. Mouse interferon was added to exponentially growing cells 24 hr prior to irradiation with 400 rads at a concentration of 200 units/ml. The cultures were subsequently maintained for 6 to 7 weeks, and the dense colonies of transformed cells (type III foci) that developed overlying the confluent monolayer of normal cells were scored (8, 11). Upon reinjection into syngeneic hosts, 80 to 100% of type III foci induced by X-rays lead to large nonregressing tumors (11). During the 6- to 7-week incubation period, fresh interferon was included in each medium change.

RESULTS

The effect of interferon on the proliferation of 10T$^1/_2$ cells is shown in Chart 1. Whereas incubation with interferon led to a moderate reduction in the initial rate of proliferation, the saturation density of confluency was suppressed by a factor of 2 to 3. This is consistent with its effects in other cell lines (4).

The results of an experiment to examine the influence of interferon on X-ray-induced transformation are shown in Table 1. In several such experiments, the transformation frequency following irradiation alone was 0.01 to 0.06%. This frequency is somewhat lower than that previously reported (11) but has been a consistent finding with the serum lot used in these experiments. As can be seen in Table 1, however, a marked increase in the number of transformed foci occurred when the cells were continuously maintained in the presence of interferon. The transformation frequency

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(ranging between 0.7 and 2.9% in 4 experiments) is considerably higher than that ever observed with radiation alone, even in doses up to 1500 rads (11). The slight increase in transformation seen with 24 hr of pretreatment with interferon is not statistically significant. Furthermore, continuous interferon treatment of nonirradiated cells did not lead to any transformation.

In order to determine whether interferon itself was the factor responsible for this effect, experiments were performed with several control and inactivated interferon preparations. The results are shown in Table 2. Both the control preparation and human interferon were ineffective in enhancing transformation, and furthermore inactivation of mouse interferon by trypsin or periodate treatment abolished its effect.

Mouse interferon produced a slight but not significant reduction in the plating efficiency of both irradiated and control cells. Because of the growth-inhibitory effect produced by the continuous presence of interferon, the colonies of interferon-treated cells are smaller and less dense than those of nontreated cells.

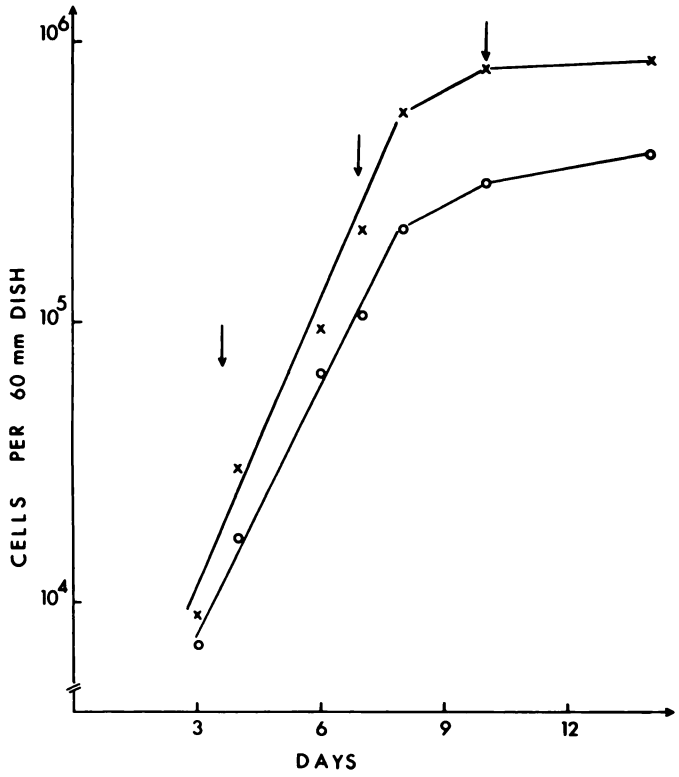

Chart 1. Effect of continuous incubation with interferon on the proliferation of mouse 10T½ cells *in vitro*. ×, control cells; ○, interferon-treated cells. Cells were seeded at an initial density of 5 × 10^3 cells/60-mm Petri dish. Mouse interferon was added 24 hr after seeding at a concentration of 200 units/ml and included in each medium change as indicated by *arrows*.

DISCUSSION

The mechanism for this stimulatory effect of interferon on X-ray transformation is not clear at the present time. We may suggest 3 possible explanations.

1. There may be a direct effect of interferon on cellular DNA. However, preliminary experiments showed that the pretreatment with interferon did not increase cell mortality due to X-irradiation or affect DNA repair (D. Brouty-Boyé, unpublished work).

2. It is possible that the effect of interferon on X-ray transformation could involve a virus-mediated mechanism. Although murine type C viral particles are present in 10T½ cells, there has been no evidence for their spontaneous expression or for their induction by carcinogen treatment (7) or X-irradiation. Furthermore, there are no experimental results showing the activation by interferon of the expression of endogenous virus particles.

3. Because cell proliferation is critical to the expression of malignant transformation *in vitro* (2, 3, 5, 10, 11), it is tempting to speculate that the effect of interferon on cell division may be involved. The continuous presence of interferon not only decreased the initial rate of cell multiplication but also maintained the final cell density at confluency at a lower level (Chart 1). If this inhibitory effect was selective for normal cells as opposed to potentially transformed cells, the proliferation of transformed cells and their development into recognizable foci might be facilitated under conditions in which the normal cell density was suppressed. Evidence in favor of this hypothesis comes from the recent work of Bertram (1). He found that lowering the serum concentration in 10T½ cell cultures 7 to 10 days after treatment with chemical carcinogens enhanced the ultimate transformation frequency by a factor of 2. This treatment also had the effect of reducing the cell density at confluency. Therefore, it is possible on the basis of the present results to relate the

Table 1

Enhancement of X-ray-induced transformation in C3H/10T½ C1 8 cells by interferon

Treatment of cells[a]	Plating efficiency (%)	Total type III foci/total dishes	Transformation frequency (%)[b]
No irradiation or interferon	14	0/15	<0.01
Irradiation only plus control preparation	3.6	1/19	0.02
Interferon preirradiation only (24 hr)	2.8	4/18	0.11
Interferon pre- and postirradiation	2.8	61/14	2.22

[a] C3H/10T½ cells were seeded into 100-mm dishes at concentrations of 2000 cells/dish for nonirradiated cultures and 7000 cells/dish for irradiated cultures.

[b] Percentage of transformed type III foci based on the number of viable (colony-forming) cells. The total number of viable cells was obtained by multiplying the number of cells seeded by the plating efficiency.

Table 2
Effect of control preparations and interferon on X-ray-induced transformation

	Plating efficiency (%)	Total type III foci/total dishes	Transformation frequency (%)
Nonirradiated cells	12.0	0/15	<0.01
Irradiated cells (400 rads)	3.3	2/20	0.06
Pre- and postirradiation treatment with			
Control preparation	3.0	2/20	0.06
Trypsin-inactivated mouse interferon	3.2	2/20	0.06
Periodate-inactivated mouse interferon	2.7	2/20	0.06
Human interferon	2.4	2/20	0.08
Mouse interferon	2.0	14/20	0.70

effect of interferon on X-ray transformation to its suppressive effect on cell division during the proliferative phase of the expression of the transformational damage. Experiments to examine further this hypothesis are currently underway.

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