Inhibition of Spontaneous Mammary Carcinoma of Mice by Treatment with Interferon and Poly I:C[1] (35565)

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A variety of virus-induced neoplasias have been reported to be retarded by prophylactic or therapeutic treatment with inducers of interferon or passively administered interferon (1–5). Recently, Gresser and colleagues (6) have shown that spontaneously occurring leukemia in AKR mice responds to repeated doses of mouse brain interferon. Treatment with material obtained from "normal" mouse brain did not retard the leukemia. These studies (1–6) indicate a potent antitumor activity of endogenous and exogenous interferon and prompted this preliminary study to determine the influence of similar therapy upon the development of a solid tumor in mice. The system employed RIII mice, a strain in which approximately 90% of the females spontaneously develop mammary adenocarcinomas. The development of the mammary tumors is governed by the presence of the mammary tumor virus (MTV) as well as other factors (7, 8). It is not yet clear whether the tumor-inhibiting activity of interferon resides in its virus inhibitory activity, or if interferon may inhibit transformed cells directly by some presently unknown mechanism. In this study mice were observed for the appearance of MTV in milk and for the rate and incidence of mammary tumor development.

Materials and Methods. Mice. RIII mice were distributed into four groups of 15 each. One group was not treated and served as a control. One group was treated with 100 μg of poly I:C in 0.5 ml of phosphate buffered saline (PBS). "Normal" mouse serum or 0.5 ml of serum containing 1000–2500 units of interferon was administered to the other two groups. Interferon was assayed by 50% plaque inhibition method described by Wagner (9). Treatments of 1 injection/week began when mice were 6 weeks (38–44 days) of age and continued for 35–42 weeks.

Interferon. Serum interferon was prepared by inoculation of 0.2 ml of Newcastle disease virus (NDV), containing approximately 2×10^8 plaque-forming units prior to ultraviolet irradiation sufficient to reduce viability to 0.001%, iv into CD-1 mice purchased from Chas. River Co., Cambridge, Mass. Mice were exsanguinated 6–8 hr after injection; the serum was separated, acidified to pH 2.0 for five days, returned to neutral pH (approx 7.0) and passed through 0.45-μ filters, prior to storing at —20°. Serum obtained similarly from mice not injected with NDV served as control. *Poly I:C.* The double-stranded synthetic RNA consisting of polyinosinic acid and polycytidilic acid (poly I:C) (purchased from P.L. Biochemicals, Milwaukee, Wisconsin) and was solubilized in PBS. This preparation of poly I:C had been shown to act as an interferon inducer in mice and as being capable of inducing resistance to virus challenge in mice on numerous occasions. *Assay for MTV in milk.* Samples of milk taken from each mouse on about day 14 after each parturition were tested for mammary tumor virus (MTV) antigen by immunodiffusion employing rabbit antiserum (10–12). Although the test is highly specific it is not highly sensitive in quantitating MTV tumorigenesis. Samples after 6–8 parturitions were tested for each mouse.

Results. Figures 1 and 2 show the kinetics of development of mammary tumors in the four groups. As shown, normal mouse serum did not reduce the number of mice developing tumors nor did it change the rate of development from that seen in the untreated

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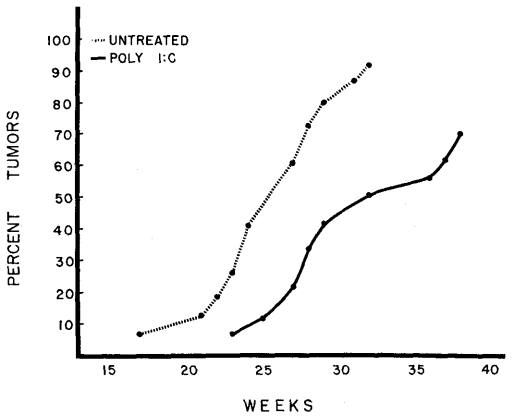

FIG. 1. Comparison of mammary tumor appearance in untreated mice and those injected weekly (ip) with poly I:C. Injections started at 6 weeks of age.

control group. Poly I:C and serum interferon both inhibited the rate of development of tumors and at the end of the 10-month period of observation 6 mice treated with serum interferon were free of palpable tumors, whereas all of those inoculated with the normal serum had developed tumors. The poly I:C-treated mice developed tumors at a significantly slower rate than did the control group.

In spite of the delay in tumor development in treated mice, all milk samples were positive for MTV antigen except for one at the first lactation in each of four groups of mice; they all became positive at the second; and later lactations.

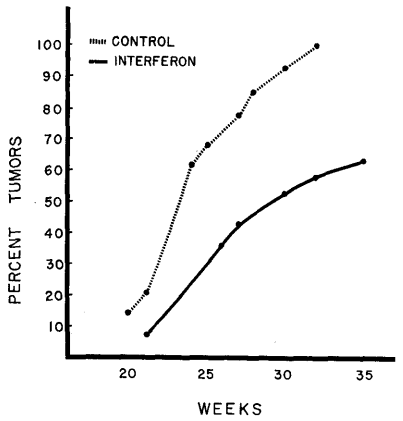

FIG. 2. Comparison of mammary tumor appearance in mice injected weekly (ip) with normal serum and with serum containing interferon. Injections started at 6 weeks of age.

Discussion. The present data could suggest that interferon (endogenous or exogenous) can act directly on transformed cells and not necessarily through a mechanism which interferes with virus multiplication. It was observed that no great difference could been seen in the appearance of MTV antigen in the milk, which is an index of virus multiplication; but, in contrast, that the rate of tumor development was suppressed by treatment with poly I:C or serum interferon. If the interferon were suppressing the malignancies indirectly by inhibiting MTV multiplication, one could expect that the appearance of MTV in the milk would have been suppressed by therapy. Thus, the data demonstrates that tumors were inhibited while concomitant suppression of MTV in milk may not have occurred.

The data are insufficient to elucidate the mode of action of the therapy, but they do demonstrate the inhibitory effect of poly I:C and exogenous interferon on a spontaneously occurring solid tumor.

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