Inhibition of Radiogenic Lymphoma Development in Mice by Interferon (35944)

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In some strains of mice, lymphoid tumors are elicited with great effectiveness by X-irradiation. From such tumors, viruses have been extracted which, on inoculation into susceptible hosts, induce new, morphologically identical neoplasms from which they can again be recovered (1, 2). The assumption that these viruses are in fact the causative agent of the radiation-induced disease would be strengthened if it could be shown that procedures which inhibit the replication of viruses decrease the incidence of radiogenic lymphomas.

Interferon, which inhibits the replication of most animal viruses, has been shown to delay the neoplastic process resulting from inoculation of several leukemogenic viruses (3, 4), as well as the evolution of the "spontaneous," presumably Gross virus-induced lymphoma of strain AKR mice (5). Therefore we undertook a study of the effect of interferon and of the interferon inducer, polyinosinic acid–polycytidylic acid (poly I:C) on the development of radiation-induced lymphosarcoma. The host animal was the C57BL mouse, which is highly susceptible to the leukemogenic effect of X-rays (6), and from which the radiation leukemia virus (RadLV) has been obtained (2). The effect of interferon on the development of RadLV-induced lymphoma was also examined.

Materials and Methods. X-Irradiation was begun when the mice were 6 weeks old; it was administered in four weekly whole-body exposures, at dose rates as indicated in individual experiments. The derivation and preparation of RadLV have been described (2, 7); the virus was inoculated intrathymically into mice aged 2 weeks.

Interferon was obtained from L cell monolayers infected with Newcastle disease virus, as described earlier (8), and used at a concentration of 1200 units/ml. Control fluid was obtained from uninfected cultures. The fluids were injected intraperitoneally in 0.2 to 0.3 ml volumes, starting immediately after the inoculation of RadLV or the first exposure to X-irradiation, and continued three times weekly for the duration indicated in the individual experiments.

Poly I:C was prepared by annealing the constituent homopolymers (Miles Laboratories) in Eagle's minimal essential medium. With this process, the typical hypochromic effect was observed, and the complex had a high degree of antiviral activity in a human skin cell-vesicular stomatitis virus assay. Poly I:C was used at a concentration of 0.15 mg/mouse/injection; the treatment schedule was the same as for interferon.

Results are recorded as percentage of mice dying of lymphoma; latent periods are the time interval between first treatment and death.

Results. Viral leukemogenesis. In a first attempt, in which the animals received a highly virulent preparation of RadLV, treatment with interferon was entirely without effect. Therefore less active virus preparations were used in subsequent work. Injections of interferon and of control fluid were carried out for 9 weeks. The results of two such experiments are shown in Fig. 1a and b. In the first experiment, it is apparent that interferon caused a significant delay in the induction and/or growth of lymphomas, represented by a shift to the right of the cumulative incidence curve. In the second experiment, there was in addition a decrease in the final incidence.

X-Ray leukemogenesis. The dose commonly used in our laboratory to produce 90 to

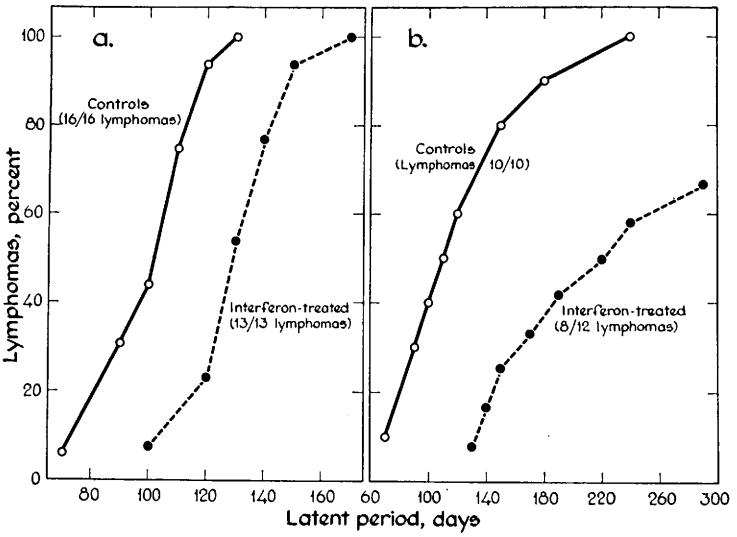

FIG. 1. Effect of interferon on RadLV-induced lymphoma development: (a and b) two preparations of RadLV.

100% lymphomas is 168 R/exposure. Under these conditions, injections of interferon did not change the course of the disease, although they were carried out until death of the recipients (Fig. 2a). Therefore, and in view of our findings with viral leukemogenesis, X-ray dosage was lowered to 130 R/exposure; it seemed possible that a less than maximal lymphoma response might be more susceptible to modification by interferon. The results (Fig. 2b) support this idea. Irradiation alone or with control fluid injection produced 63 and 61% lymphomas, respectively, with similar latent periods. By contrast, the group receiving interferon (for a period of 3 months) had only 31% lymphomas, and a 50 day increase in average latent period relative to that of the control groups. The difference in incidence is significant ($p<.05$).

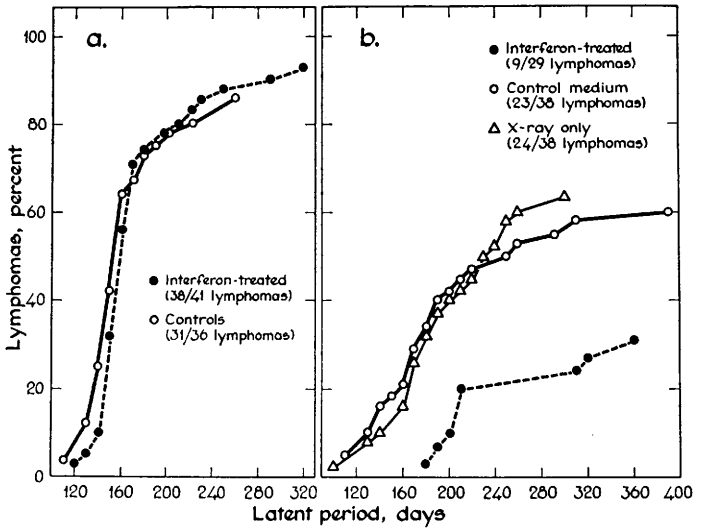

FIG. 2. Effect of interferon on X-ray induced lymphoma development: (a) X-ray dose 168 R × 4; (b) X-ray dose 130 R × 4.

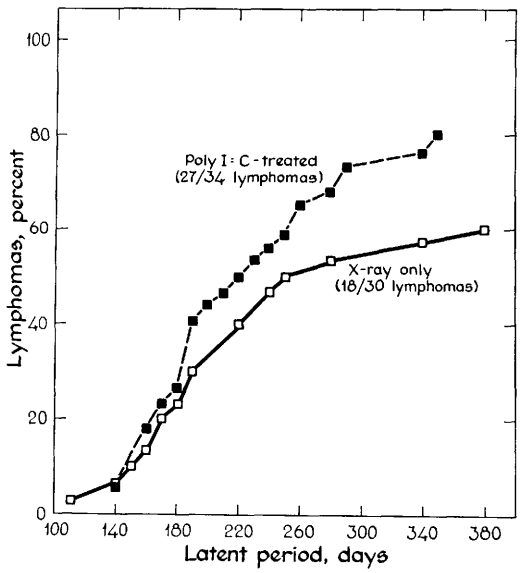

FIG. 3. Effect of poly I:C on X-ray-induced lymphoma development.

A slightly lower X-ray dose, 125 R, was used in an experiment designed to assess the effect of poly I:C administration; polymer treatment was continued for a period of 3 months. The results (Fig. 3) were unexpected: there was no evidence of protection in the polymer-treated group, which had even more lymphomas (80%) than the control group (60%); the difference, however, is not significant ($p > .1$).

Leukemic cell growth. It has been reported (9, 10) that interferon and interferon inducers may inhibit the growth of tumors, even those for which no viral etiology is known. In order to determine whether such inhibition might be an additional factor in our present studies, the following experiment was carried out. Leukemic lymphocytes were injected intravenously into adult C57BL mice which, several hours earlier, had received an intraperitoneal injection of either interferon (approx 1000 units/0.3 ml dose), poly I:C, or control fluid. The latter injections were repeated the following day, and then three times weekly for 2 weeks. Leukemia deaths clustered between 12 and 19 days after inoculation of tumor cells. There was no appreciable difference between the control and the interferon-treated groups. In the animals receiving poly I:C, there was a delay in mortality of about 3 days (Fig. 4).

Discussion. From the evidence obtained, it appears that interferon can inhibit the development of lymphosarcomas in C57BL mice, whether induced by RadLV or by exposure to ionizing radiation. The effect is one of delayed onset of the disease, sometimes accompanied by a reduction in its frequency. It is manifested only when the lymphomagenic stimulus is submaximal, but this may be due merely to the insufficient potency or total dosage of the interferon preparations used. Unpublished work by De Maeyer and Duplan and Gresser (cited by De Maeyer, 10th Int. Cancer Congr., Houston, 1970) also indicated a lack of effect of interferon on the incidence of radiogenic lymphomas, under conditions of maximal leukemogenic stimulus (175 R/exposure, for 4 weekly exposures).

The fact that radiogenic lymphoma is inhibited at all by interferon is nevertheless of interest, since it provides additional evidence for the theory that X-rays elicit lymphoma through a viral intermediary. The reported inhibitory effect of interferon on tumor cell growth, however, clouds the interpretation of its mode of action in the lymphoma induction system. The experiment reported above (Fig. 4) does not indicate inhibition of the growth of transplanted lymphoma cells, but in view of the sensitivity of the system to such factors as intensity of disease-producing stimulus and amount of interferon administered, one cannot conclude that interferon may not exert a suppressive effect on the growth of autochthonous cells after they undergo neoplastic transformation in the course of virus or radiation leukomogenesis.

The results with poly I:C are difficult to understand: it does not alter the course of radiation leukemogenesis, although it may have a very slight delaying effect on death following transplantation of lymphoma cells. One explanation for the former effect may be that the animals, having recently been irradiated, could not respond optimally to its action. Further, the antitumor activity of this polymer may be mediated by some aspect of host resistance, *e.g.*, cellular immunity (10),

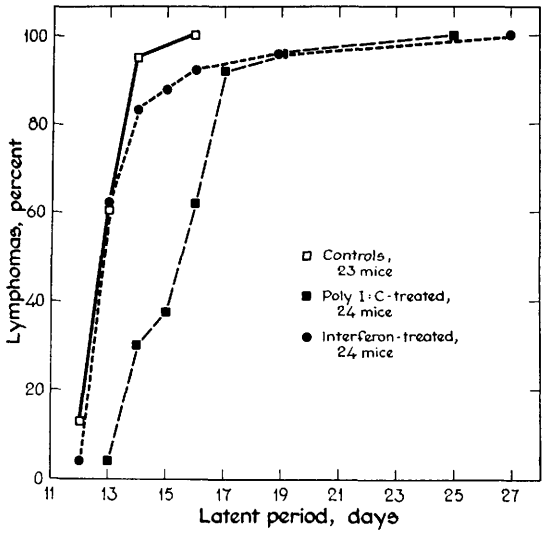

FIG. 4. Effect of interferon and of poly I:C on survival time of mice following intravenous injection of tumor cells.

which may be less important in the induction of neoplasia by irradiation. Interestingly, long-term treatment with interferon inhibits both spontaneous and superinfection leukemogenesis in AKR strain mice (5), whereas treatment with poly I:C (which, however, was begun late in life, when the leukemogenic process was well under way) was reportedly ineffective (11).

Summary. Long-term administration of interferon resulted in the partial suppression of radiation-induced lymphosarcoma development in strain C57BL mice. The inducer of interferon, polyinosinic acid–polycytidylic acid, was without effect under the same conditions. Interferon also protected C57BL mice against the induction of these tumors by a virus, RadLV, but did not modify the death rate following injection of tumor cells.

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