Moloney Sarcoma Virus-Induced Tumors in Mice: Inhibition or Stimulation by (Poly rI)·(Poly rC)[1] (35627)

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The synthetic double-stranded RNA (poly rI)·(poly rC) (homopolymer pair of polyriboinosinic acid and polyribocytidylic acid) has been reported to suppress the growth of murine sarcoma virus (MSV) (Moloney strain) *in vitro* (mouse embryo fibroblasts) (1, 2) and *in vivo* (newborn mice) (3). Because of the interferon stimulating capacity of (poly rI)·(poly rC) *in vitro* (4) and *in vivo* (5) and the inhibitory effect of interferon on MSV replication *in vitro* (6–8), the antitumor activity of (poly rI)·(poly rC) in MSV-infected mice has been attributed to the production of endogenous interferon (3). However, recent studies by Larson *et al.* (9) have shown that (poly rI)·(poly rC) is not always effective in the treatment of Friend leukemia in mice and that adverse as well as beneficial effects may be obtained depending on the dosage of drug and time of administration. We now report that the antitumor effect of (poly rI)·(poly rC) also depends on the age of the animals and that (poly rI)·(poly rC) inhibits MSV tumor formation in 4–6-day-old mice but stimulates MSV tumor formation in older (20-day-old) mice.

Materials and Methods. Randomly bred Swiss-Webster albino mice (either 20-day-old females or 4- to 6-day-old males and females) were obtained from Simonsen Laboratory, Gilroy, California. Litters of 4–6-day-old mice were pooled and then randomly divided into experimental and control groups (10 mice and 1 mother/cage). The Moloney strain of murine sarcoma virus (Moloney leukemia virus pseudotype) [M-MSV (MLV)] was generously provided by Dr. I. L. Weissman, Stanford University, Stanford. It has been maintained through serial passages in (C57BL/6 × BALB/c) F_1 hybrids. A virus stock was obtained by inoculating newborn BALB/c mice subcutaneously in the back region, harvesting the tumors 12 days later and preparing a cell-free virus extract from the pooled tumor tissues according to Moloney (procedure A) (10). The final virus pellet was suspended with a Teflon pestle homogenizer in a solution containing 0.25 *M* sucrose and 0.00075 *M* Tris–HCl, pH 7.2 (11). The virus stock was stored in aliquots at −70°. (Poly rI)·(poly rC) was prepared by mixing equal amounts of poly rI and poly rC (both purchased from P-L Biochemicals, Inc., Milwaukee) at 1 mg/ml of saline for 2 hr at 45°. Complex formation was evidenced by (a) hypochromicity (at 260 nm); (b) sharp thermal transition (62°); and (c) capacity to inhibit vesicular stomatitis virus plaque formation in human skin fibroblasts at 0.001 μg/ml. (Poly rI)·(poly rC) was stored in aliquots at −20°.

Groups of 30 mice (4–6 days old) were inoculated intramuscularly (right thigh) with 0.05 ml of a 1:100 virus stock dilution. (Poly rI)·(poly rC)-treated mice were injected intraperitoneally with 0.1 ml of either 100 or 20 μg of (poly rI)·(poly rC) on alternate days, from 1 day before until 17 days after virus challenge.

Results. Figure 1 indicates that (poly rI)·(poly rC) partially reduced tumor development in 4- to 6-day-old mice: tumors appeared later in the (poly rI)·(poly rC)-

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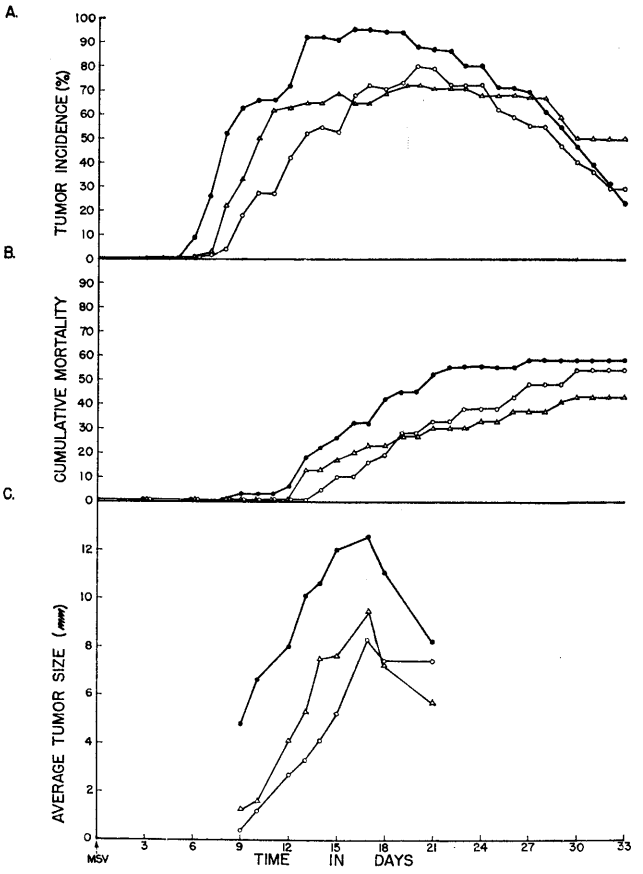

FIG. 1. Effect of (poly rI)·(poly rC) on tumor incidence, mortality, and tumor size in mice infected with MSV when 4 to 6 days old (30 mice/group): (●) control; (○) (poly rI)·(poly rC): repeated doses of 100 μg intraperitoneally at 1 day before and 1, 3, 5, 7, 9, 11, 13, 15, and 17 days after MSV challenge; (△) (poly rI)·(poly rC): repreated doses of 20 μg intraperitoneally at 1 day before and 1, 3, 5, 7, 9, 11, 13, 15, and 17 days after MSV challenge.

treated than in the control groups, more control mice developed tumors and died with tumors than treated mice, and, when tumors developed, they were smaller in size in the treated than in the untreated groups. Similar but more dramatic antitumor effects were observed by Sarma *et al.* (3) in mice *neonatally* infected with MSV and injected with (poly rI)·(poly rC) in the same area as the virus.

To further explore the effect of (poly rI)·(poly rC) on MSV tumor growth in older animals, groups of 30 mice were inoculated intramuscularly (right thigh) with 0.1 ml of a 1:10 virus stock dilution at the age of 20 days. (Poly rI)·(poly rC) treatment was given as a single intravenous dose of 50 μg 2 hr before virus challenge or as repeated intramuscular injections of 40 μg at 1 day before and 1, 3, 6, 9, and 13 days after virus challenge. Both treatment regimens (Fig. 2), as well as repeated intraperitoneal injections of 40 μg of (poly rI)·(poly rC), markedly enhanced tumor development: tumors appeared earlier and regressed later in the (poly rI)·(poly rC) treated than in the control groups; tumor incidence and death rate with tumor were higher in the treated than in the untreated groups. Finally, tumors of control mice were smaller in size than tumors of (poly rI)·(poly rC)-treated animals.

Discussion. Although the antiviral, antitumor, antibacterial, and antiprotozoal effects of (poly rI)·(poly rC) are well documented (12), (poly rI)·(poly rC) has recently been

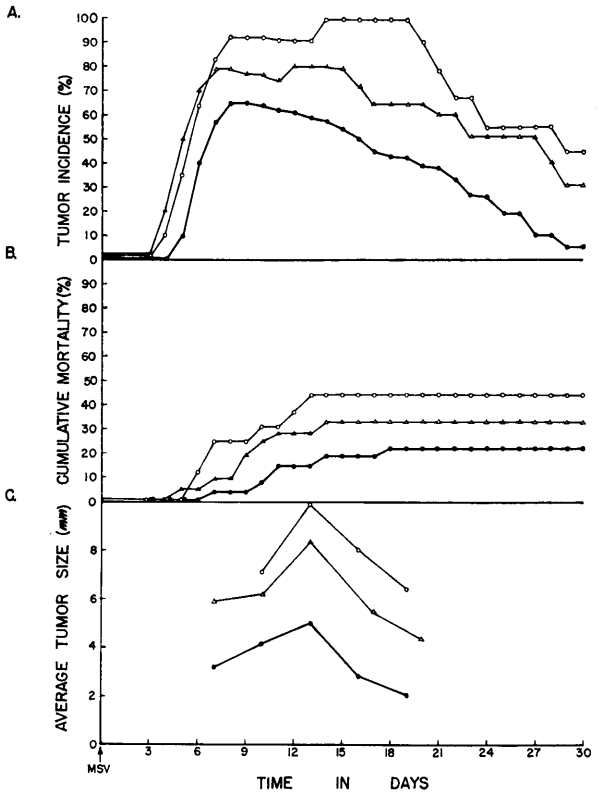

FIG. 2. Effect of (poly rI)·(poly rC) on tumor incidence, mortality, and tumor size in mice infected with MSV when 20 days old (30 mice/group): (●) control; (○) (poly rI)·(poly rC): single dose of 50 μg intravenously at 2 hr before MSV challenge; (△) (poly rI)·(poly rC): repeated doses of 40 μg intramuscularly at 1 day before and 1, 3, 6, 9, and 13 days after MSV challenge.

found ineffective against some bacterial infections [such as *Mycobacterium leprae* (13)] and tumors [such as spontaneous leukemia in AKR/J mice (14) and transplanted tumors induced by SV 40 (15), polyoma virus (16), and methylcholanthrene (17)]. Friend leukemia (9) and radiation leukemia (18) and some protozoal infections such as *Leishmania donovani* (19) and *Trypanosoma cruzi* (20, 21) could be stimulated with (poly rI)·(poly rC) under some conditions. Paradoxically, many of these processes which were not effected or rather stimulated by (poly rI)·(poly rC), are effectively inhibited by interferon itself [Friend leukemia (22), spontaneous leukemia in AKR mice (23), and radiation leukemia (18)].

The differing effects of (poly rI)·(poly rC) and interferon on several malignant processes suggest that (poly rI)·(poly rC) does not act through interferon production alone. It might also exert a direct and selective chemotherapeutic effect on tumors or act through the stimulation of cellular immunity (24). Accordingly, the differential effects of (poly rI)·(poly rC) on MSV tumor growth in 4–6-day-old and 20-day-old mice might be due to differences in interferon production, direct antitumor action or immune responsiveness between younger and older mice. However, newborn mice respond equally well with interferon production to (poly rI)·(poly rC) as older mice (3), and there is no experimental evidence that suggests that (poly rI)·(poly rC) might have a different direct action on tumors in older than in younger animals. The differential effects of (poly rI)·(poly rC) in

younger and older mice seem to be related to differences in their immune responsiveness as described below.

Both virus-neutralizing (25) and anti-TSTA (tumor specific transplantation antigen) antibodies (26), and even circulating virus–antibody complexes (27) have been demonstrated in mice infected neonatally with MSV. Although immunosuppressive agents such as cortisone (28), antilymphocytic serum (29), X-irradiation (30), and thymectomy (31) increased the incidence of progressively growing MSV tumors and death, a consistent relationship between antibody production and tumor regression could not always be established (30, 31). Immune sera of mice infected with MSV have been shown to contain both virus-neutralizing and anti-TSTA antibodies (25, 26). If this immune serum was transferred, it inhibited tumor growth in mice neonatally infected with MSV (25) but enhanced the growth of grafts of MSV induced tumors in adult syngeneic recipients (26); the tumor-inhibiting effect was ascribed to virus-neutralizing antibodies and the tumor-enhancing effect to anti-TSTA antibodies (25, 26). It is tempting to speculate that (poly rI)·(poly rC) which stimulates both humoral and cellular immune response (32–35), enhances preferentially virus-neutralizing antibody production in younger and anti-TSTA antibody production in older animals.

Summary. These studies demonstrate a major effect of age of animals on the effect of (poly rI)·(poly rC) on Moloney sarcoma virus-induced tumors. In 4–6-day-old mice, tumors appeared later in the treated than in the control group, whereas in 20-day-old mice, treatment enhanced tumor formation.

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