Murine Sarcoma and Leukemia Viral Infection of Mice: Effect of Long-term Treatment with Poly I·Poly C (35602)

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Synthetic polyinosinic polycytidylic RNA (poly I·poly C) has been found to inhibit cytolytic (1–8) as well as oncogenic (8–10) viruses. In addition, poly I·poly C suppresses the development of transplanted murine (11–13) and guinea pig (14) tumors of known or unknown viral etiology. Poly I·poly C is a potent inducer of interferon (1–10). Synthetic polyribonucleotides enhance humoral and cellular immunological response of mice (15–17). The mechanism of suppression of virus-induced murine sarcoma by poly I·poly C is at present unclear.

Studies reported herein suggested that although poly I·poly C suppressed the development of murine sarcoma and substantially reduced the mortality in infected mice, the treatment was ineffective against the murine leukemia virus (MuLV) present in large excess in virus stocks of murine sarcoma virus. *In vivo* studies with Rauscher and Friend leukemia viruses suggested that poly I·poly C treatment was only effective in suppressing the replication of low challenge doses (20 to 25 mouse infectious units) of these viruses.

Methods and results. Poly I·poly C. Poly I·poly C was prepared at a concentration of 1 mg/ml in phosphate buffered saline (PBS) with 5×10^{-3} *M* $MgCl_2$ as previously described (1, 9). PBS with $MgCl_2$ was used for inoculation of control mice.

Murine sarcoma virus. Virus stocks of Moloney strain of murine sarcoma virus (M-MSV) were prepared as 10%-clarified mouse tumor extracts. A pseudotype of this virus with the coat of Friend leukemia virus (18), designated herein as M-MSV(FLV), was used. The virus stocks were stored at —70°. The infectivity titers were determined by tumor induction assay in NIH Swiss mice and by focus assay in NIH Swiss mouse embryo fibroblast cultures (19). Newborn mice were inoculated with 5 tumor-inducing units of the virus containing approximately 10^2 focus-forming units (FFU) of MSV and 10^4 infectious units of Friend leukemia virus (COMUL test) (19).

Murine leukemia viruses. Tissue culture adapted strains of Friend and Rauscher leukemia viruses (19) were propagated *in vivo* in NIH Swiss mice for two passages. Virus stocks were prepared as clarified 10% extracts of infected spleens.

Mice. NIH Swiss mice used in these experiments were obtained from the Animal Production Section of the National Institutes of Health. Ten newborn mice were housed with 1 mother/cage. The murine sarcoma virus was inoculated subcutaneously into the back region of mice on the day following birth. Treatment with poly I·poly C and control PBS was given by subcutaneous injection in the same area as virus inoculation.

Complement fixation (CF) test for murine leukemia virus (COMUL test.) The presence of murine leukemia group-specific (gs) CF antigens in the spleens of mice was examined by direct test of 10% extracts of spleens in CF test against sera containing gs antibodies to murine leukemia virus (19). The presence of infectious leukemia virus was tested by the CF induction test in NIH Swiss mouse embryo fibroblast cultures (COMUL test) (19).

Exp. 1. Long-term treatment on infection with murine sarcoma virus and associated leukemia virus. One hundred mice were inoculated subcutaneously on the day of birth with 0.1 ml of poly I·poly C (100 μg/mouse). One hundred control mice were similarly inoculated with 0.1 ml of PBS. On the following day, the mice were inoculated subcutaneously with 5 tumor-inducing units of M-MSV(FLV). Treatment was continued

TABLE I. Effect of Long-Term Treatment of Mice with Poly I · Poly C on Infection with Murine Sarcoma and Associated Leukemia Virus.

Treatment	Mortality[a]	Maximum tumor incidence (%)	Incidence of lymphoma (%)	Presence of murine leukemia virus			
				CF antigen[b]		Infectious virus[c]	
			Day:	80	160	80	160
None (control)	0/40	0	0	0/20	1/20	0/16	0/20
PBS	51/99	63	11.5	29/29	8/8	8/8	8/8
Poly I · Poly C	27/99	<1	12.3	30/37	24/27	8/8	24/27

[a] The following number of surviving mice were sacrificed on day 80: control, 20; PBS, 30; Poly I · Poly C, 45.

[b] Number of spleen extracts positive for antigen (CF titer ≧ 1:8)/total number tested at this dilution.

[c] Number of spleen extracts positive for infectious murine leukemia virus/total number tested.

on alternate days for 80 days. On day 80 after virus inoculation, half the surviving mice in each group were sacrificed and autopsied. Tissues were collected for histopathological studies and virus detection. The remaining mice were maintained and observed for an additional 80 days. The surviving mice were then sacrificed and similarly examined. Mice that were moribund or found dead during the experimental period were autopsied and examined.

Mice infected with M-MSV(FLV), which were given placebo treatment with PBS, developed sarcoma. Most of these animals succumbed to the disease (Table I). Animals that died or were sacrificed invariably showed gross and histopathological signs of sarcoma and/or lymphoma. On the other hand, virus inoculated mice which were given poly I·poly C treatment, failed to develop sarcoma and the majority of these mice survived. However, the poly I·poly C treated animals which died and a proportion of those animals which were sacrificed had gross and histopathological evidence of lymphoma. Spleens of mice of both groups, whether enlarged or not, contained CF viral antigens, as well as infectious murine leukemia virus, as determined by direct CF test of spleen extracts and by CF induction test in NIH Swiss mouse embryo cultures (Table I). Morphological transformation of inoculated mouse embryo cultures was not observed. Parallel groups of 20 control mice, which were not inoculated with virus but given PBS or poly I·poly C treatment, appeared normal. Their spleens did not contain viral antigen or infectious murine leukemia virus.

Exp. 2. Effect of poly I·poly C treatment on murine leukemia virus infection. Newborn mice were divided into groups of 5 mice/cage with one mother. Half the total number of mice each received 100 μg of poly I·poly C subcutaneously and the remaining half received PBS. The following day, decimal dilutions of Rauscher and Friend leukemia viruses were inoculated in 0.1-ml amounts/mouse using 5 treated and 5 control mice/virus dilution. The treatment with poly I·poly C and PBS was continued on alternate days for 21 days. The mice were sacrificed and 10% crude extracts of each spleen was prepared. The spleen samples were tested in CF test vs MSV rat sera to determine the presence of murine leukemia gs antigen. Results shown in Table II indicate that poly I·poly C treatment suppressed the development of CF viral antigen in the spleens of mice inoculated with 25 or lower input mouse infectious units of the Friend or Rauscher leukemia viruses.

Discussion. Mouse interferon has been found to inhibit both murine leukemia and sarcoma viruses (21–24). In *in vitro* studies we found poly I·poly C to be effective in suppressing the morphological transformation of cells by murine sarcoma virus. Continuous treatment of cells with poly I·poly C was

TABLE II. Effect of Poly I • Poly C Treatment on the Murine Leukemia Group-Specific Antigens in the Spleens of NIH Swiss Mice Infected with Decimal Dilutions of Murine Leukemia Viruses.

Leukemia virus	Treatment	CF viral antigens in spleen[a]; Log_{10} virus dilution inoculated						Titer[d]
		—1	—2	—3	—4	—5	—6	
Friend	PBS		2/2[c]	5/5[b]	4/5	0/5	0/5	$10^{4.4}$
	Poly I • poly C	5/5	5/5	1/2[c]	0/5	0/5	0/5	10^{3}
Rauscher	PBS	5/5	5/5	5/5	4/5	1/5	0/5	$10^{4.5}$
	Poly I • poly C	4/5	4/5	3/5	2/5	0/5	0/4	$10^{3.2}$

[a] Spleens of 10 control animals did not have CF antigen at 1:8 dilution.

[b] Number of spleen extracts positive for antigen (CF titer $\geq$ 1:8)/total number tested at this dilution.

[c] Reduction in number due to early nonspecific deaths.

[d] Virus titer computed by the method of Reed and Muench (26).

also effective in suppressing the replication of murine sarcoma and leukemia viruses. However, treatment of infected cells after the establishment of viral infection was without effect in reducing CF viral antigen titer of infected cells.

Treatment of murine sarcoma virus-infected mice with poly I·poly C resulted in a suppression of the development of sarcoma and consequent mortality even when treatment was commenced after the establishment of virus infection (9). However, under the conditions of our experiments, poly I·poly C treatment did not have an inhibitory effect in preventing the replication of 10^4 infectious units of the murine leukemia virus present in the murine sarcoma viral inoculum. Thus, the spleens of treated mice were found to contain CF viral antigen and infectious murine leukemia virus capable of replicating in mouse embryo cultures. Our poly I·poly C *in vivo* studies reported herein similarly indicate that treatment of mice infected with up to 25 infectious units of Rauscher and Friend leukemia viruses inhibited the capacity of the virus to replicate and induce CF viral antigens in the spleens of the inoculated mice. However, at higher challenge doses, the treatment did not have this inhibitory effect. Our previous (9) and present experiments suggest that prolonged treatment with poly I·poly C at the dosage used is effective against virus-induced murine sarcoma, even when commenced after inoculation of virus (9). However, such treatment with poly I·poly C is without significant effect in altering the course of infection by the murine leukemia virus present in excess of 25 infectious units in the murine sarcoma viral inoculum. While the development of lymphoma in the treated mice appears to be due to a lack of inhibitory effect of poly I·poly C on the replication of the large challenge dose of infectious murine leukemia virus, the suppression of the development of sarcoma in these treated mice may be due to the inhibition of the replication of the low dose of sarcoma virus present in the viral inoculum, or due to other unknown modes of action of poly I·poly C on sarcoma cells. Other investigators have made similar observations on the occurrence of lymphoma and recurrence of sarcoma in mice inoculated with the Moloney strain MSV and treated for a limited period of time with poly I·poly C (25).

Summary. Prolonged treatment with poly I·poly C was effective in suppressing the development of virus-induced murine sarcoma and the resultant mortality in infected mice. However, treatment at the dosage used was ineffective in preventing the infection of these mice by the murine leukemia virus present in excess in stocks of murine sarcoma virus. The poly I·poly C treated virus-infected mice eventually developed lymphoma, and their spleens contained both murine leukemia, group-specific, complement-fixing viral antigens and infectious murine leukemia

virus. These experiments suggest that poly I·poly C treatment may not be effective against Friend and Rauscher leukemia viruses at high challenge doses.

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