Influence of Synthetic Double-Stranded Ribonucleic Acid (Poly I:C) on SV_{40} Viral Oncogenesis and Transplant Tumor in Hamsters[1] (34397)

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Studies conducted in these laboratories showed that double-stranded ribonucleic acids derived from several sources were active inducers of interferon and of enhanced host resistance to viral infection both *in vitro* and *in vivo* (1–6). One of these inducers, synthetic polyriboinosinic:polyribocytidylic acid complex (poly I:C, $rI_n{\cdot}rC_n$, or rI:rC), was selected for use in studies concerned with the effect of such complexes on virus-induced neoplasia. The influence of poly I:C on the oncogenesis of adenovirus type 12 in newborn hamsters (7) and on Friend leukemia in mice (8) has been reported. The present paper summarizes the findings in studies of the effect of poly I:C on SV_{40} viral oncogenesis and on development of SV_{40} transplant tumor in hamsters.

Materials and Methods. Hamsters. Six-week-old male and newborn golden Syrian hamsters of both sexes were obtained from the random-bred closed colony of Lakeview Hamster Colony, Newfield, New Jersey. For practical purpose, this colony of animals may be regarded as essentially syngeneic.

Tumor cells. The F5-1 line (9, 10) of SV_{40} hamster tumor cells used for these studies was shown to be free of infectious virus and to contain specific tumor T antigen as measured by the complement-fixation test (11). This tumor line originated in a hamster that had received SV_{40} virus when newborn and was from the hamster colony described above. The F5-1 cells were grown as monolayers in stationary or roller bottles and were used at passages 30 to 66. The cells were harvested in Hanks' balanced salt solution (HBSS) after 5-min exposure to 0.25% trypsin (Difco), washed once, and resuspended to the desired concentration of viable cells. Cell counts were done using a model B Coulter counter and the percentage of viable cells was determined by the trypan blue exclusion method.

Virus. The SV_{40} virus (VA 45-54 strain) had been passed 5 times in grivet monkey kidney cultures (GMK) and had a titer of $10^{-7.6}$ $TCID_{50}$/0.1 ml in GMK.

Poly I:C. The individual homopolymers were purchased from Miles Laboratories, Elkhart, Indiana or were made in these laboratories. Solution of the homopolymers and the poly I:C complex were prepared in phosphate buffered saline solution (PBS) (0.006 *M* sodium phosphate, 0.15 *M* NaCl) as described previously (2) and were furnished by Drs. A. K. Field and A. A. Tytell of these laboratories.

Experimental design. SV_{40} transplant tumors. One-ml volumes of F5-1 tumor cells at the desired concentration were transplanted subcutaneously at "0" time into the scapular region of adult male hamsters. Poly I:C or PBS (placebo control) was given intraperitoneally in 0.1 or 1.0-ml volume in single or multiple doses at the times and concentrations indicated in the text.

SV_{40} virus-induced tumors. Litters of newborn (14–18 hr old) hamsters were pooled and the pups were distributed at random into the experimental groups. At "0" time, 0.2 ml of undiluted SV_{40} virus was inoculated subcutaneously into the scapular region when the animals were still $\leq$ 24 hr old. Poly I:C or PBS (placebo control) was given intraperitoneally in 0.1-ml volume in single or multiple doses at the times and concentrations indicated in the text.

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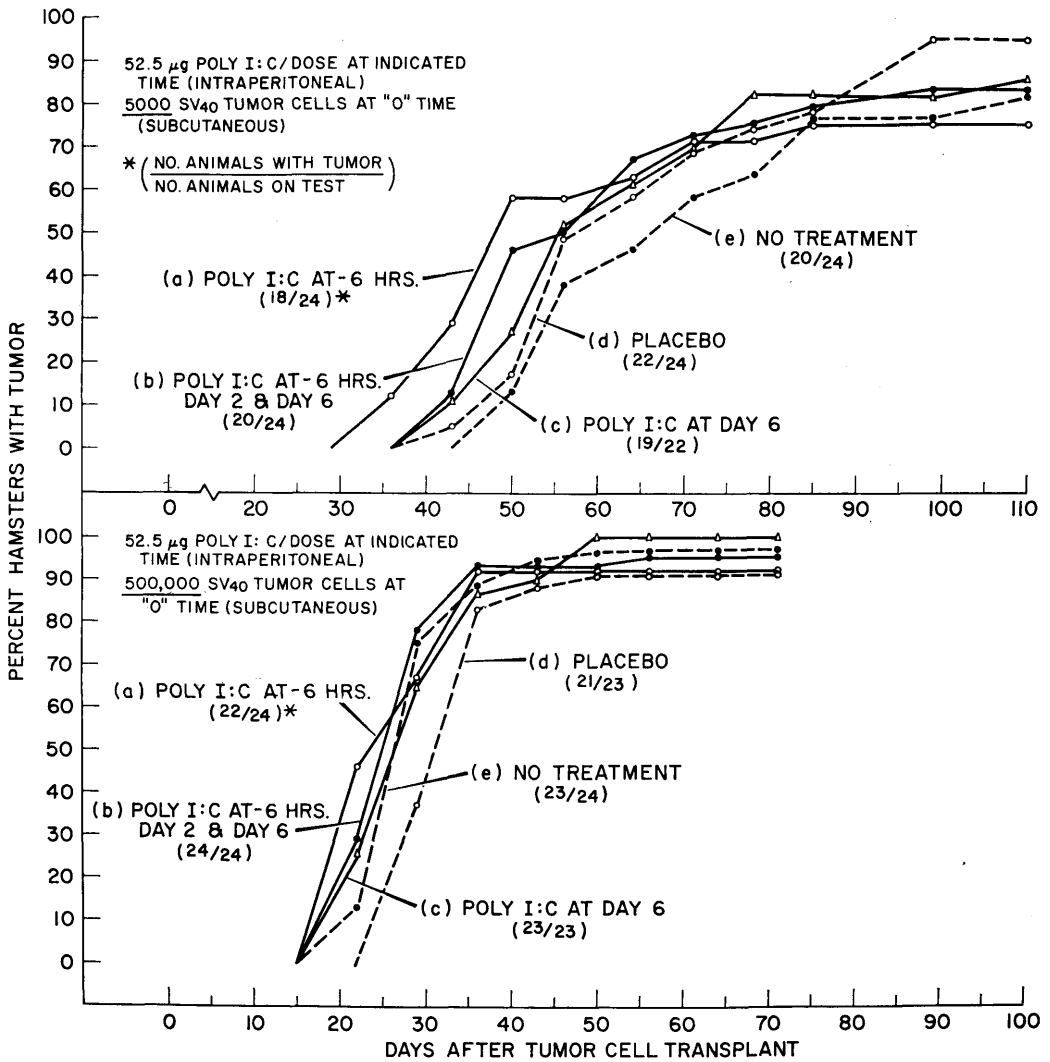

FIG. 1. Effect of time of inoculation and number of doses of Poly I:C on SV_{40} transplant tumor development in hamsters.

Expression of results. All animals were examined weekly for tumors. Animals which died in the course of the experiments, as well as those which were sacrificed at termination of the experiments, were autopsied and the presence or absence of tumors was observed. The findings were expressed as "percentage of hamsters with tumors" representing 100 times the number of animals which developed tumor divided by the total number of animals in the group with or without tumor, minus those which died from nonspecific causes. In the SV_{40} virus study, the percentages were corrected for nonspecific deaths according to standard life table procedures.

Results. Effect of time of inoculation and number of doses of poly I:C on SV_{40} transplant tumor incidence in hamsters. Groups of 24 adult hamsters were treated with single or multiple doses of 52.5 μg (0.525 mg/kg) of poly I:C before and/or after subcutaneous inoculation of 5000 or 500,000 SV_{40} tumor cells. The treatment regimens used were as follows: (a) one dose of poly I:C at —6 hr; (b) 3 doses of poly I:C at —6 hr, day 2, and day 6; (c) 1 dose of poly I:C on day 6;

(d) 3 doses of placebo at −6 hr, day 2, and day 6; and (e) no treatment. Figure 1 shows that poly I:C administered according to these schedules had little or no lasting effect on the incidence of palpable tumors as compared with the placebo and untreated control groups. There was some indication that administration of poly I:C, especially when given 6 hr before inoculation of 5000 tumor cells, might lead to slightly earlier appearance of palpable tumors in a portion of the animals. Essentially, the same results were obtained in a second experiment which is not presented here.

Effect of single graded doses of poly I:C on SV_{40} transplant tumor development in hamsters. Previous studies (8) showed that poly I:C could either increase or decrease spleen weight in mice inoculated with Friend leukemia virus depending on the dose of drug which was given. There was a suggestion, in the present studies, of earlier tumor appearance in treated animals and a dose–response experiment was therefore carried out. Groups of 24 adult hamsters were treated with 1 dose of poly I:C 6 hr before subcutaneous inoculation of 1000 or 5000 SV_{40} tumor cells. The test groups were as follows: (a) 0.8 μg (0.008 mg/kg) poly I:C; (b) 3.3 μg (0.033 mg/kg); (c) 13.1 μg (0.131 mg/kg); (d) 52.5 μg (0.525 mg/kg); (e) 210 μg (2.1 mg/kg); (f) placebo; and (g) no treatment. As shown in Fig. 2, the rates for development of palpable tumor and the final tumor incidences were essentially the same in all the treated and the control groups. Similar findings were obtained in a second experiment not recorded here in which the same concentrations of poly I:C were tested in the same way using 5000 SV_{40} tumor cells/animal for transplant.

Effect of prolonged treatment with poly I:C on SV_{40} transplant tumor development in hamsters. Groups of 24 adult hamsters (60–107 g) were treated daily with graded doses of poly I:C for 28 days. The first dose was given 6 hr before subcutaneous inoculation of 5000 or 500,000 SV_{40} tumor cells. The test groups were as follows: (a) 0.6 μg (0.006 mg/kg) poly I:C; (b) 2.5 μg (0.025 mg/kg); (c) 10 μg (0.1 mg/kg); (d) 40 μg

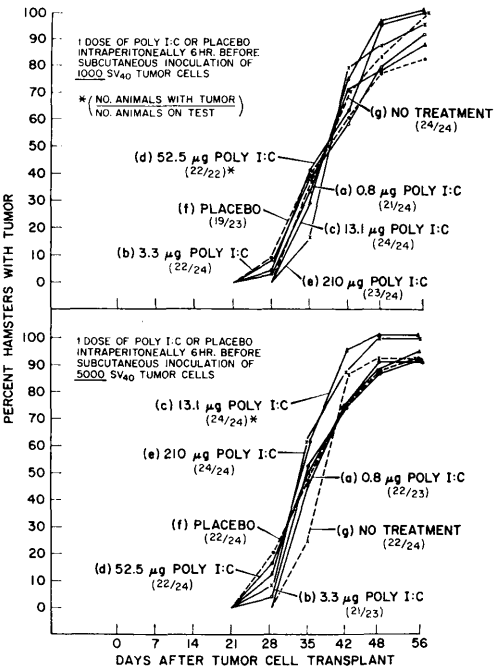

FIG. 2. Effect of amount of Poly I:C given in a single dose on SV_{40} transplant tumor development in hamsters.

(0.4 mg/kg); (e) 160 μg (1.6 mg/kg); (f) placebo; and (g) no treatment. Some tumors appeared before treatment was completed, especially in the 500,000 tumor cell transplant group. Whether this happened or not, treatment was carried out for the full 28 days. Figure 3 shows that the incidence of palpable tumor was not significantly altered by the poly I:C treatment at any of the dose levels during the 28-day time period it was given. No regressions were noted in tumors which appeared before completion of the treatment. The minor differences which were seen were within the range of experimental variation.

Effect of prophylactic and/or therapeutic treatment with poly I:C on SV_{40} virus oncogenesis in newborn hamsters. Groups of newborn hamsters were given poly I:C (52.5 μg/dose) prior to and/or after subcutaneous inoculation with SV_{40} virus in the regimens shown in Fig. 4. This included (a) a single dose of poly I:C given before virus inoculation; (b) one dose given before and one dose given after virus; (c) one dose given before and eight doses after virus; (d) 7 doses given

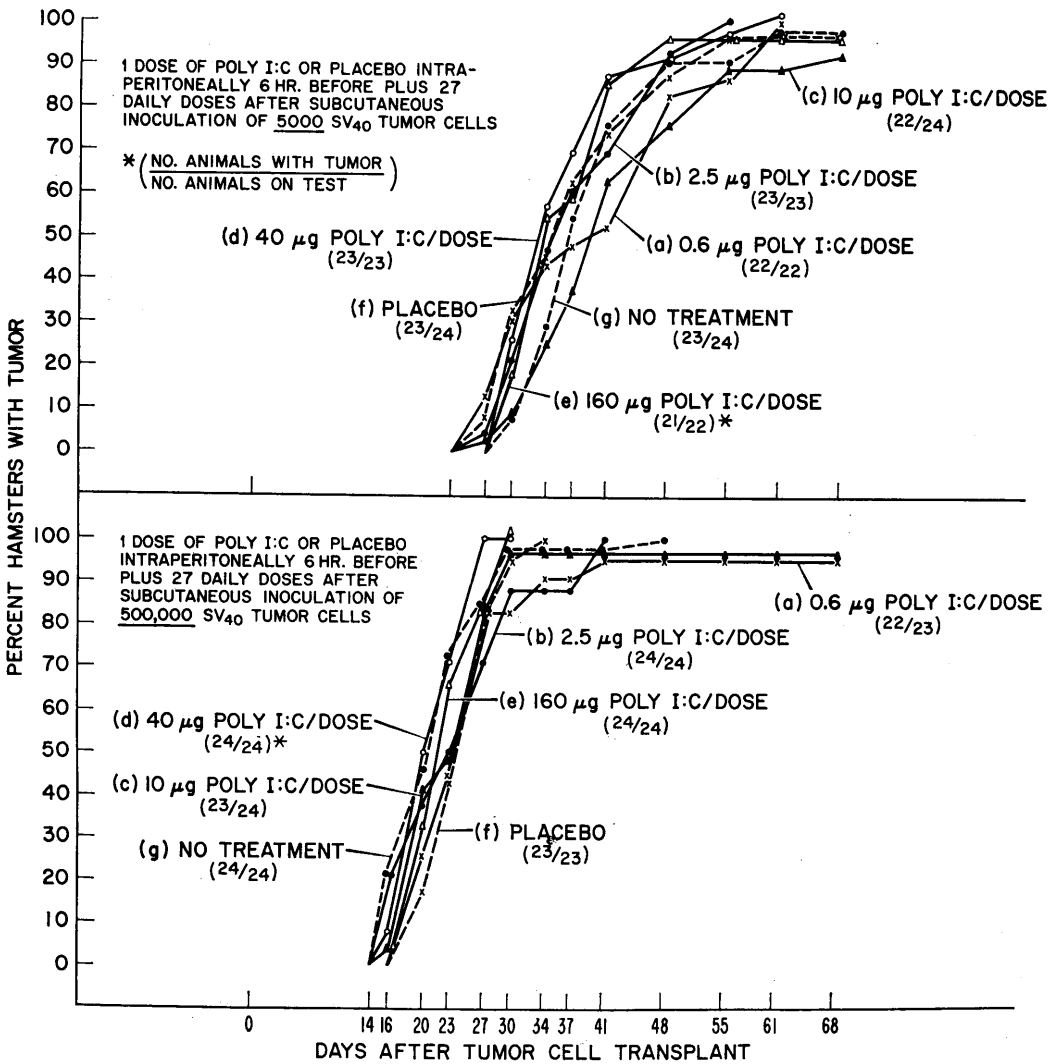

FIG. 3. Effect of daily treatment with Poly I:C on SV_{40} transplant tumor development in hamsters.

only after virus; (e) placebo given in the same regimen as for group 3; and (f) no treatment. The findings presented in Fig. 4 show that poly I:C administered at the indicated time intervals had little or no effect on SV_{40} virus tumorigenesis as tested in newborn hamsters which received a large dose ($10^{7.9}$ $TCID_{50}$) of SV_{40} virus when less than 24 hr of age. The percentage of treated animals with tumors was generally within the range of experimental variation between the 2 control groups. The only indication for a protective effect was a slight delay in detec-

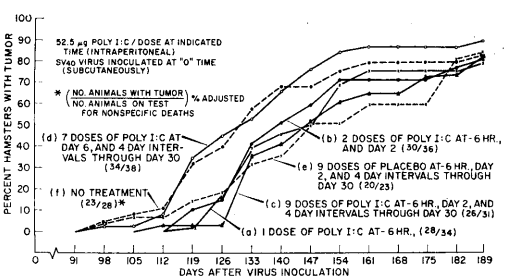

FIG. 4. Effect of treatment with Poly I:C on SV_{40} virus oncogenesis in newborn hamsters.

tion of early tumors among those groups of animals which were given poly I:C before virus. Thus, the first tumors were detected at 98 days (14 weeks) among the 2 control groups and the poly I:C post-treatment group whereas the first tumors were detected at 112 or 119 days (16 or 17 weeks) among the 3 groups which received the dose of poly I:C before virus was given.

Discussion. Previous reports (2, 5, 6, 12) from these laboratories recorded the discovery that poly I:C is a highly effective inducer of interferon and of resistance to lytic viral infections *in vivo* and *in vitro*. Additionally, it was shown (7) that the substance gave marked protection in newborn hamsters against the development of internal but not of subcutaneous tumors at the injection site following subcutaneous inoculation of adenovirus 12. Minor beneficial or adverse effects on Friend leukemia in mice were noted (8), depending on the time of administration of the drug. In the present study, single or multiple doses of poly I:C given intraperitoneally before and/or after subcutaneous inoculation of SV_{40} virus in newborn hamsters showed only minor delay in appearance of tumors at the injection site and no apparent effect upon the ultimate development of tumor. These findings are similar to the ones reported (7) earlier for poly I:C in relation to the lack of suppression of development of adenovirus 12 tumors at the subcutaneous injection site. Unlike adenovirus 12, SV_{40} virus does not produce internal tumors following subcutaneous inoculation and hence, this parameter could not be measured in the SV_{40} virus system.

The present studies with SV_{40} transplant tumor were carried out with tumor cells and host animals derived from the same random-bred closed hamster colony and representing an essentially syngeneic system. The single or multiple doses of poly I:C given intraperitoneally in varying amount before and/or after subcutaneous transplant of 1000 to 500,000 SV_{40} tumor cells in adult male hamsters had little or no effect upon the percentage of animals which subsequently developed tumor. Levy *et al.* (13) have reported findings in tests of poly I:C of different origin than ours on several transplant tumors in mice. Their results were expressed in terms of length of survival rather than percentage of animals which developed tumor. Though direct comparisons are not possible, the lack of beneficial effect on SV_{40} transplant tumor in the present study resembled the weak or near negative results reported by Levy in tests of poly I:C given in different dosage and regimen (100 μg/mouse, 3 times/week) against L1210 and subcutaneous B1237 lymphoma in mice. The findings in the present work were in contrast to the definitely positive results obtained by Levy against J96132 reticulum cell sarcoma and MT1 tumor and the lower level activity against a BALB/c mouse fibrosarcoma and ascitic B1237 lymphoma in mice.

It is not unexpected that different results be found in tests of the effect of poly I:C against virus-induced primary tumor in contrast to transplant tumor; in tests of poly I:C against tumors of different kind in different animal species and sex; in tests employing different dosage and time regimens for drug and for tumor; and in tests using poly I:C of diverse source and composition.

Poly I:C is known to have at least 3 activities: (a) induction of interferon, (b) stimulation, in the presence of antigen, of ordinary immune mechanisms, and (c) marked toxicity for several animal species at relatively low dosage (H. Peck *et al.*, unpublished). Interferon induced by poly I:C might be expected to be active against tumorigenesis by at least some viruses if given in advance of virus and it might be expected to influence virus-dependent neoplasia such as certain of the leukemias caused by RNA viruses; by contrast, it would not be expected to be active against virus-free transplant tumors unless mechanisms which are presently unknown be involved. Hamsters do not develop detectable circulating interferon following poly I:C (A. K. Field *et al.*, unpublished) but may do so in the course of viral infections (14). Stimulation by poly I:C of ordinary immune mechanisms (15, 16) might bring about an adverse effect on certain tumors if antibody against tumor be augmented (immunologic enhancement), or might have

a beneficial result if cell-mediated immunity be increased. Female animals are generally more responsive immunologically than are male animals (17, 18). This was also indicated previously (7) in the greater protection afforded female than male animals by poly I:C against adenovirus 12 internal tumor. It may be worth noting that male animals only were employed in the present transplant tumor studies. Poly I:C of present composition is highly active biologically and carries a relatively high level of toxicity which may vary according to source, preparation, and lot. Toxicity may include an effect on the vascular system and alteration of blood coagulation (H. Peck *et al.*, unpublished) which may not be observable grossly. Toxicity alone might account for tumor suppression or destruction by poly I:C, especially when given in multiple doses after the appearance of tumor. It is evident that the interrelationships between host, tumor, and poly I:C may be quite complex and the outcome may be difficult to predict in any particular circumstance.

Summary. Studies were carried out to measure the effect of a synthetic double-stranded RNA, poly I:C, on SV_{40} transplant tumor growth and SV_{40} viral oncogenesis in hamsters. Single or multiple doses of poly I:C given intraperitoneally before and/or after subcutaneous inoculation of SV_{40} virus into newborn hamsters had little or no effect on the percentage of animals developing subcutaneous tumors later in life. Likewise, single or multiple doses of varying amounts of poly I:C administered intraperitoneally before and /or after subcutaneous inoculation of 1000 to 500,000 virus-free SV_{40} tumor cells had little or no effect on the rate of tumor appearance in adult male hamsters.

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