Enhanced Growth of a Polyoma Transformed Tumor Cell in *Spirometra mansonoides*-Infected Hamsters[1] (37412)

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(Introduced by R. G. Douglas, Jr.)

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The sparganum larva of the tapeworm *Spirometra mansonoides* elaborates a growth stimulating factor, termed sparganum growth factor (SGF), which produces a significant increase in size and weight of experimental hosts of different species (1). This effect is not a result of increased food consumption alone and is dependent on the number of larvae inoculated (2). For a complete review of the biology of *S. mansonoides* see Ref. (3).

The growth factor displays many properties of growth hormone (4). Rats deprived of growth hormone by hypophysectomy gain weight, resume growth of long bones and exhibit increased protein synthesis and liver glycogen content after infection with spargana (5).

Knowing the general growth stimulating effects of infection with *S. mansonoides,* we decided to investigate its influence on the growth of a transplantable polyoma transformed tumor cell line in golden hamsters. The tumor cell chosen for study was the HTC-3049-91 TC line (6) which produces a nonmetastasizing, encapsulated tumor mass that can be easily removed *in toto* and weighed. Since tumor cells are independent of the normal restraints of cell growth and maintenance, we wanted to know how such a tumor graft would respond to SGF *in vivo.*

Materials and Methods. Weanling male golden hamsters were infected subcutaneously with spargana larvae suspended in a small volume of normal saline. Larvae were recovered at the end of each experiment in the fascia about the neck. Each animal was weighed three times a week for the duration of the experiment.

At 10 days postinfection with the spargana, appropriate cell suspensions of the tumor cell were injected subcutaneously into each of four abdominal quadrants (7). The sites received 10, 100, 1000 and 10,000 cells, respectively, in a volume of 0.1 ml. The progress of tumor development was determined by palpation at every third weighing. Experiments were terminated at the first sign of tumor necrosis, at which time each tumor was removed and weighed. The number of cells required to produce a palpable tumor in 50% of the inoculated animals within 30 days (TD_{50}) was determined by the method of Reed and Muench (8) from the incidence of tumors on the final day of the experiment.

Results. Two experiments were performed. In the first, 18 hamsters infected with a mean dose of nine spargana (9.4 ± 0.7) had a significantly greater mean body weight (142.3 ± 11.9) than 12 uninfected controls (128.2 ± 9.0) ($p < 0.01$). The mean tumor weights in the infected animals were 1.5–2 times greater at the 10, 100 and 1000 cell levels than those of the controls, but the weights were too variable for the differences to be significant. Since this experiment suggested that the implanted tumor had responded to a dose of spargana that was sufficient to produce only a small stimulating effect on the host, a second experiment was carried out using a higher dose of spargana.

In the second experiment, 13 hamsters were inoculated with 25–30 (mean 27.4 ± 2.2) spargana/animal with 12 animals kept as controls. As shown in Fig. 1, sparganainfected animals began to display growth

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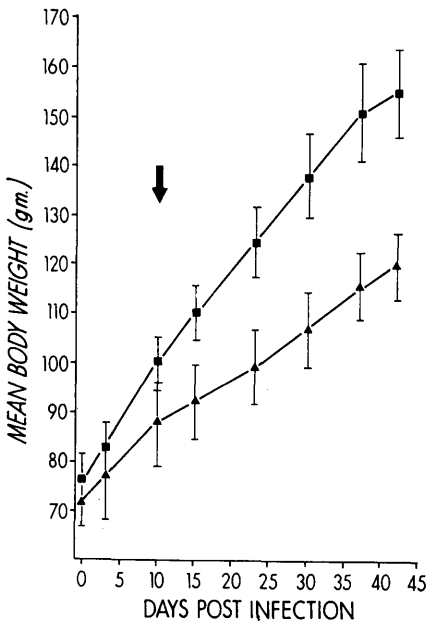

FIG. 1. Accelerated growth of male golden hamsters infected with the sparganum larvae of *S. mansonoides*. Thirteen animals, infected on Day 0 with 25–30 spargana, and 12 controls were inoculated subcutaneously on Day 10 with 10, 100, 1000 and 10,000 tumor cells in each of the four abdominal quadrants. Arrow indicates implantation of tumor cells; (▲—) control mean body weight in grams ± standard error of the mean; (■—) infected mean body weight in grams ± standard error of the mean.

stimulation within the first 4 days postinfection. They continued this trend until termination of the experiment. The rate of appearance of palpable tumors arising from tumor cells implanted on Day 10 is shown in Fig. 2. Tumor cell growth is so rapid at the 1000 and 10,000 cell levels that all of the animals in both control and infected groups were positive by 12 days following implantation. However, at the two lower doses of 10 and 100 cells, *Spirometra*-infected animals showed enhanced tumor development. Using the data from the 10 cell site, the TD_{50} was found to be approximately 5 cells for the spirometra group and 28 cells for the control group.

The weights of individual tumors are shown in Fig. 3. The spargana-infected animals had a significantly larger tumor burden ($p < 0.02$) at the lower cell doses. These data corroborate the difference observed between the rates of appearance of palpable tumors in the two groups.

Discussion. The tumor cell line studied here grows as a semiautonomous cell when as few as 5–10 cells are inoculated subcutaneously into golden hamsters. The ability of the cell to survive as a graft in a new host is dependent upon its intrinsic ability to grow *in vivo*, as well as the nature of the environment into which it is introduced. This latter point is illustrated by the fact that animals previously immunized with polyoma virus display a marked resistance to the transplantation of polyoma tumor cells (9) and animals subjected to thymectomy show enhanced tumor growth (10). The results reported here demonstrate that the *Spirometra*-infected animals provide a more suitable environment for tumor cell growth than the control animals. Furthermore, that this may be related to the dose of SGF is

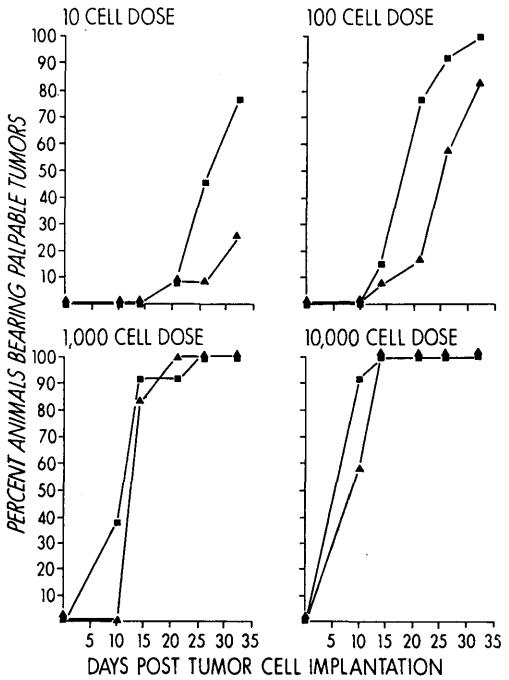

FIG. 2. Rate of appearance of palpable tumors in control and *Spirometra*-infected animals described in Fig. 1. (▲—) percentage of control animals with tumors; (■—) percentage of infected animals with tumors.

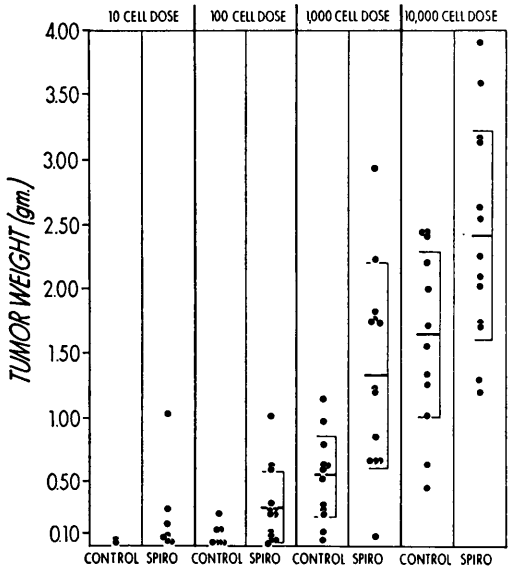

FIG. 3. Histogram of tumor weights at each cell dose in control and *Spirometra*-infected animals described in Fig. 1. Thirty days after implantation of tumor cells the animals were sacrificed and the tumors were removed *in toto* and weighed. Solid bar, mean value (g/animal); bracket, standard error of the mean.

suggested by the results of the first experiment in which tumor cell growth was enhanced by a dose of spargana (9 per animal) which produced only a moderate growth stimulation in infected animals.

We have demonstrated enhanced tumor growth in spargana-infected hamsters. This effect may be due to a direct response of the tumor cell to the growth factor or arise from an induced immunosuppressive state. Similarly, enhanced growth may be an indirect consequence of overall host stimulation by the parasite or a combination of these effects. However, it is more likely that the tumor cell is responding in an indirect manner. SGF has been shown to cause both the synthesis of somatomedin (sulfation factor) and linear growth in hypophysectomized rats in the absence of detectable rat growth hormone (11). Since somatomedin is released in response to growth hormone and is directly responsible for stimulation of growth of the animal, the observed enhanced tumor growth probably reflects the response of the tumor cell to somatomedin induced by SGF. *In vitro* experiments are presently underway to test directly the cellular response to SGF.

Summary. The growth of a polyoma virus transformed tumor cell implanted in golden hamsters infected with *Spirometra mansonoides* spargana larvae was studied to determine the effect of the sparganum growth factor (SGF) on tumor growth. The rate of appearance of palpable tumors and relative tumor size were increased in infected animals while the tumor dose 50% was decreased fivefold.

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