A Transient Cytotoxic Host Response to the Rous Sarcoma Virus-Induced Transplantation Antigen (34851)

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Tumor-specific transplantation antigens (TSTA) were demonstrated in chemically induced neoplasms by Foley (1) and by Prehn and Main (2). Subsequently, TSTA were reported in other experimental tumor systems, *i.e.*, in "spontaneous" tumors, and in tumors induced by oncogenic viruses, chemical carcinogens, and by implanted inert materials. Current data indicated that the immunity engendered by the host against the TSTA involves mechanisms similar to those which are responsible for homograft rejection. Many hypotheses have been postulated to explain the growth of tumors as regards host responses to homografts. Some involve the antibody enhancement phenomena (3) immunodepression in the case of chemically induced tumors (4), and tolerance or paralysis (5). Mikulska *et al.* (6), have demonstrated a suppressed immunological status in the host to its tumor, which recovers after removal of the tumor. This suggests that the host is capable of initiating an immune response, but in the process of tumor growth the immune mechanism is "turned off" or "paralyzed." The information reported here attempts to examine the time-relationship of this phenomenon after implantation of tumor cells. It involves *in vitro* cell culture techniques by which the cell-associated immunological status of the host can be assessed.

Materials and Methods. A transplantable fibrosarcoma was induced in newborn female inbred Fischer rats by subcutaneous inoculation of 10^4 PFU of Schmidt-Ruppin strain Rous sarcoma virus. The virus was obtained from Dr. Ray Bryan, National Cancer Institute, Bethesda, Maryland. A selected primary solid tumor, which appeared approximately 4½ months later, was excised and minced under asceptic conditions. The small fragments, in minimal essential media (MEM), were then aspirated vigorously in a 1-ml syringe and 0.1 ml of this cell suspension was then inoculated subcutaneously into the interscapular region of newborn Fischer rats. The tumor was transplanted twice through newborn rats and thereafter into rats of weanling age. Tumors of the fourth to the sixth cell passages in inbred weanling female Fischer rats were used in the experiments which are described below. Four to ten animals were used to assess immunity for each interval studied. However, at times lymphoid cells from two different animals were pooled.

In order to assess the cell-mediated immune status of the host, the passaged tumor was excised aseptically into Eagle's MEM at pH 7. The tumor tissue was then minced with scissors to very fine fragments, and washed once in MEM; and the tissue fragments were then resuspended in prewarmed 0.25% solution of trypsin (Difco 1:250) in phosphate-buffered saline (PBS) for 30 min at 37° in a combination water bath–shaker. The dispersed cells were then decanted through sterile gauze, centrifuged, and washed twice in MEM at 4°. The cells were counted by hemocytometer and 10^6 morphologically intact cells (88% viable as determined by trypan blue exclusion test) were inoculated subcutaneously in the dorsal region of the rat.

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TABLE I. Response of Lymphoid Cells from RSV Tumor-Bearing Rats Inoculated with 10^6 Tumor Cells.

Days after inoculation of RSV tumor cells	RSV tumor cells (% survival ± SE)		
	Lymphoid cells from:		
	Normal rats		Tumor-bearing rats
10	100 ± 16.9	(4)[a]	90.5 ± 8.26
15–21	100 ± 4.9	(9)	47.3 ± 6.8
22–29	100 ± 8.9	(11)	102 ± 5.56
32–38	100 ± 3.8	(10)	126 ±16

[a] Number of rats in each group.

Assays for host sensitization to the inoculated tumor cells were performed by *in vitro* cytotoxicity tests (7). RSV tumor cells, propagated in tissue culture, were suspended in Waymouth's 752/1 medium (Gibco) plus 10% heat-inactivated calf serum; and 3×10^5 and 1×10^6 cells were seeded in Leighton tubes and in 25-mm petri dishes, respectively. Twenty-four hours later the inoculated and uninoculated control animals were anesthetized by ether, exsanguinated from the heart, and the brachial, inguinal, and mesenteric lymph nodes were removed aseptically, pooled, and washed in MEM. The lymph nodes were teased through a sterile 60-mesh stainless steel screen, the cells were washed twice and adjusted to a final concentration of 1 and 1.5–2.0 $\times$ 10^6 cells per ml in Waymouth's medium containing 5% heat-inactivated fetal calf serum. 2×10^6 of the lymph node cells were added to the Leighton tubes and 4.5–6.0 million cells were added to each petri dish of "target" tumor cells. The mixed cultures were incubated for 48 hr at 37° in a mixture of 5% CO_2 and 95% air and then examined for surviving target tumor cells. The nutrient medium was replaced by PBS, and then by 2 ml of 0.25% trypsin. After incubation at 37° for 20–25 min, chilled PBS was added to the cell–trypsin mixture. The detached cells were then counted by hemocytometer and from this the percentages of surviving tumor cells were calculated. Tumor cells and lymphocytes were differentiated on the basis of size and morphology.

Results. The data in Table I indicate that lymphocytes from the rats which had been inoculated 10 days previously with 10^6 viable tumor cells showed a low-level cytoxic response. The maximum response which was reflected in the survival of only 51.1% of the tumor cells was observed on Days 15–21 after the inoculation of tumor cells. From Days 22 on the lymphoid cells from the tumor-bearing rats did not destroy the target cells. Tumors appeared in the inoculated rats at 8–10 days after inoculation of tumor cells. No exact correlation was found between tumor size and the immune status of the host: however, average tumor size of approximately 2-cm diameter coincided with maximum cytotoxic response. Average tumor diameter in the nonresponsive rats was approximately 5 cm.

The data in Table II portray a similar pattern of transient response, but the time sequence of the cytotoxic effect appeared earlier. These rats were inoculated with the same line of tumor cells, but after it had been passaged four times in tissue culture. Inoculation of 10^6 tumor cells (96% viable by trypan blue exclusion test) induced palpable tumors 7 days after inoculation as compared with 8–10 days in the previous experiments. The accelerated appearance of tumors may be a result of a higher percentage of viable cells in the inoculum and/or of some selective enhancement of the tumor cells through their propagation in tissue culture.

Specificity of the cell-associated immune reaction is demonstrated in Table II. Lymphoid cells which destroyed RSV tumor cells were inactive on sarcoma cells which had been induced in Fischer rats by polyoma virus.

Discussion. The experimental data recorded here support the recent report of Barski and Youn (8) on a transient host-immune response to inoculated Rauscher virus-transformed T5 tumor cells, with a subsequent state of "paralysis." Our data suggest that an actively growing solid tumor in some way "paralyzes" or reverses the initial cell-mediated immune responses of the host, as determined by *in vitro* cytotoxicity tests (6, 8). This is supported by experimentally derived results involving X-ray-inactivated tu-

TABLE II. Response of Lymphoid Cells from RSV Tumor-Bearing Rats to RSV and Polyoma-Induced Tumor Cells.

	RSV tumor cells (% survival ± SE)		Polyoma tumor cells (% survival ± SE)	
	Lymphoid cells from:		Lymphoid cells from:	
Days after tumor inoculation	Normal rats	Tumor-bearing rats	Normal rats	Tumor-bearing rats
9	100 ± 3.0	51.6 ± 6.2	100 ± 16.7	117 ± 20.9
16	100 ± 13.4	114.9 ± 7.14	—	—

mor cells, and by surgical extirpation of tumor after which the lymphoid tissue of the host then regained the capacity for a cytotoxic response through appropiate *in vitro* tests (6, 8). While our results do not clarify the mechanism(s) involved in this state of "paralysis," antibody enhancement, tolerance, or some humoral immune-depressive factor (9) associated with a population of growing tumor cells have been offered as possible explanations (10). Possibly, one or more of these factors are operating to cause the unresponsive state of the lymphoid cells on the target tissue. This observed phenomenon has been explained by the fact that the majority of sensitized lymphoid cells are assembled at tumor site. However, from histological examinations of germ-free tumors significant numbers of lymphoid cells have not been observed in or around the tumors.

Our data suggested that there was an "enhancing" effect of the "paralyzed" lymphoid cells on the target tissue. Although this may not be significant statistically, there is nevertheless a suggestive trend toward a stimulatory effect in excess of the normal "feeder" effect associated with exposure to unsensitized lymphoid cells.

If tumor cells continue to grow in the host as a result of immunological disability, then control will depend on some means of immunological enhancement. Possibly, tumor cells are more efficient as immunological depressants than "normal" tissues, and it could be important to determine its nature. It is significant that tumor-bearing rats whose lymphoid cells had reached the "paralyzed," or nonresponsive stage, *in vitro*, were physically indistinguishable from tumor-free controls when judged by size, by physical condition, and by general appearance. Thus, the lack of cytoxic cell response in the *in vitro* tests could not be attributed to a general state of debilitation in the tumor-bearing hosts.

Recently some evidence has accumulated in our laboratory that the RSV TSTA may have been altered, decreased quantitatively, or lost entirely. Preliminary data have shown that when the same RSV tumor that was used throughout all of the above experiments was used after 16 passages in Fischer rats no reactions were found when assayed during the usual time intervals. This has also been the case with another RSV primary now in the 5th animal passage. There are reports in the literature where TSTA in solid tumors, induced by so-called defective oncogenic viruses, appeared to have been lost (11). Further work on this problem is currently being conducted in our laboratory. It may be important, in this regard, to limit such experiments to "primary" tumors or to close derivatives thereof.

Summary. This report is concerned with the time-relationship of cell-mediated immune reactions by hosts with expanding transplanted solid tumors. Inoculation of 10^6 serially passaged tumor cells elicited peak lymphocyte cytotoxic responses between 16 and 21 days followed by a state of immunological "paralysis." Rats which were inoculated with 10^6 tumor cells which had been passaged four additional times in tissue culture gave lymphocytes with peak cytotoxic effects at 9 days after implantation and "paralysis" by 16 days. A stimulatory effect over and above the normal "feeder" effect by unsensi-

tized lymphoid cells was noted when "paralyzed" cells were overlayed on the target cells. This "paralysis" could not be attributed to a general state of debilitation in the tumor-bearing hosts.

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