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Early Growth of Experimental Tumors and the Onset of Concomitant Immunity*

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Abstract—*On repeated experiments, the rate of take of a given threshold inoculum of T241, B-16, SV40, L1210 or rat Wilms tumor in their syngeneic strains was not increased by additional tumor implanted concomitantly in other site(s), suggesting that early tumor growth is not dependent on an overwhelming by the tumor load of systemic host factors. There is no evidence* in vivo *of immune response in allogeneic or syngeneic tumors initially, following inoculation. Such evidence, in the form of tumor rejection or decreased rate of challenge take, does not appear at any definite interval from the time of inoculation, but its onset varies with the size of initial inoculum and coincides approximately with the time of early palpability of the tumor nodule. The take of a threshold inoculum apparently depends on its interaction with local host factors.*

INTRODUCTION

FOR MOST experimental systems, there is a minimum (threshold) number of tumor cells required for survival and growth of the transplanted tumor inoculum. Increasing the number of implanted cells will raise the frequency of successful tumor implantation. Ultimately, a number of cells is reached which will result uniformly in successful take of the tumor transplant. The question remains as to the relative role of local and/or systemic host factors in determining the outcome of transplants of threshold numbers of tumor cells.

The following experiments were designed to evaluate, in different tumor systems, the effect of varying the total tumor dose to the host on the fate of individual threshold inocula. The immunogenicity of the tumors under study was indicated by demonstration of concomitant immunity. Finally, *in vitro* studies of growth in cell culture were performed.

MATERIALS AND METHODS

Animals

Three strains of 6 to 9-week-old male inbred mice (of the same age for each experiment), C57BL/6J, Balb/cCR and DBA/2Ha-D, were obtained from the West Seneca Breeding Colony, Roswell Park Memorial Institute, and maintained under standard conditions on Teklad 4% fat rat and mouse chow and tap water *ad libitum*. Wistar Furth male rats, 12 weeks old, were purchased from Microbiological Laboratories and maintained on stock pellets (Teklad) and water *ad libitum*.

Tumors

T241, a Lewis sarcoma syngeneic to C57BL/6J mice, was originally obtained from Warren Cole in 1965 and has been serially maintained *in vivo* using the trocar method. A culture line of this tumor was initiated in 1974 and aliquots of 10^6 cells were frozen (−70°C) in a 10% glycol solution of RPMI 1640 media with 10% fetal calf serum and 2% penicillin and streptomycin. B-16, a spontaneous murine melanoma also syngeneic to C57BL/6J mice [1], was obtained from Jackson Laboratories (Bar Harbor, Maine) in 1974 and has been serially passaged in C57BL/6J mice via trocar. *In vitro* cultures were also established for B-16 and sterile ampules frozen, as with T241. TU-5, a transplantable mouse tumor line of SV40-transformed Balb/cCR kidney cells, was obtained from Dr. D. R. Dubbs [2]. L1210, a lymphoid leukemia which originated in 1948 after skin painting with methylcholanthrene in DBA/2 mice [3], was obtained in the 50th passage from Dr. Gerald Grindey. The ascitic tumor was maintained by serial passage of 10^6 cells intraperitoneally, twice a week. Wilms

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tumor, a rat sarcoma [4], was obtained in its 50th generation from Dr. Philip Tomashefsky. At the time of this experiment, the tumor was in its 84th generation at this Institute in adult male Wistar Furth rats. It was called Wilms tumor because of its origin in renal parenchyma.

Preparation of cells

T241, B-16 and TU-5 cells were harvested similarly. Confluent flasks were trypsinized using 0.25% trypsin with 0.2% EDTA. RPMI 1640 media with 10% calf serum was used for dilution. Final counting solutions were prepared using 0.2% Trypan blue in saline and cell counts were made using a hemocytometer. All cell suspensions for injection were kept on ice and simultaneously mixed with a magnetic stirrer. One-milliliter aliquots were drawn up and each animal received 0.1 ml subcutaneously. Subcutaneous injections were given carefully and confirmed by the visual appearance of the characteristic elevations. In the process of injection, control animals were alternated individually with non-controls in each experiment.

L1210 was obtained from tumor-bearing DBA/2 mice which were killed on day 4 after injection of 10^6 L1210 cells. Ascitic fluid was aspirated with a 25-gauge needle through the exposed peritoneum and dispersed into a sterile beaker with 20 ml Hanks balanced salt solution (HBSS).

Design of experiments

1. In each of the initial experiments, there were three groups of 10 mice each (a total of 213 mice in experiments of Table 1). Group I received a single subcutaneous (sc) injection of a given number of T241 or B-16 cells (D) in the right upper flank (RUF) or right medial thigh (RMT). Group II received 10 injections of D cells, each of the same tumor, in 10 distinct sites (i.e., a $10 \times D$ total tumor dose). Sites injected were the upper and lower flanks, the lateral and medial aspects of the thigh bilaterally, and upper and lower abdomen. Group III received a $10 \times D$ tumor dose in the same site as Group I (Table 1). Each horizontal line on this table, comprising three groups of mice, represents one experiment. The three groups of mice within each experiment were injected from the same batch of cells on the same day.

2. In repeat experiments for the B-16, the effect of injecting three or nine more sites with 10^3 cells in each additional site was studied on the take of a 10^3 cells inoculum in the RMT.

3. Then, several groups of mice were injected with 10^3 cells of B-16 in the RUF (15 mice), right axilla (10 mice), RMT (15 mice), and lateral aspect of right forelimb over the deltoid area (10 mice).

4. Subsequent experiments utilized two groups of 21 C57BL/6J mice each receiving the same tumor dose at one and the same site (10^3 T241 cells in RMT), Group II animals also receiving an additional ten-fold dose in the contralateral side simultaneously. The same was repeated with two groups of 20 mice each for the B-16 tumor (10^3 cells as threshold inoculum).

5. To establish that these results were not peculiar to C57BL/6J, experiments were repeated in Balb/cCR mice with their syngeneic tumor SV40 (10^4 cells as threshold inoculum). Further repetitions used the syngeneic system of L1210 (10^3 cells) in DBA/2Ha-D mice using a 5-times higher dose in the contralateral side of Group II mice and the syngeneic system of Wilms tumor in Wistar Furth rats (threshold dose 1.5×10^4 cells).

6. Concomitant immunity was sought against T241 and B-16 tumors. T241 4.45×10^4 was injected s.c. in the left flank in three groups of five mice each. One week, 10 days and 14 days later they were injected respectively with T241 cells s.c. in the right flank as well as their respective controls. The same experiment was

Table 1. Effect of tumor dose on frequency of growth of implants in C57 BL/6J mice injected at a single site or in multiple sites

				Group I, D		Group II ($D \times 10$ sites)		Group III, ($D \times 10$ sites)		
Tumor	Tumor dose	Mice per group	Site	No. of takes	Diameter (mm)	Number of takes at site	Diameter (mm)	No. of takes	Diameter (mm)	Day of tumor measurement (T.M.)
241	5×10^2	10	RUF	0		0		2	15.0	38
	10^3	10	RUF	2	3.0	1	4.0	10	7.0	31
	5×10^3	10	RUF	10	4.0	3	5.0	10	6.5	25
	10^4	10	RUF	6	3.5	3	3.0	10	5.0	20
B-16	10^3	10	RUF	1	5.0	2	4.0	4	8.5	28
	10^3	10	RMT	8	3.0	8	2.5	10	4.0	12
	5×10^3	10	RMT	10	4.0	10	4.0	10	8.0	12

repeated for B-16 using 3×10^4 cells as primary inoculum.

7. The time of onset of concomitant immunity was then sought in allogeneic combinations. Nine DBA/2Ha-D mice were injected with 5×10^5 B-16 cells in the right flank.

8. Subsequently, L1210 5×10^6 cells were injected s.c. in the right medial thigh of Balb/cCR, C57BL/6J and DBA/2Ha, 7 mice per group; this was repeated using L1210 5×10^5 cells and L1210 $\times 10^4$ cells. In similar experiments, B-16 5×10^5, 5×10^4 or 5×10^3 cells were injected s.c. in the right medial thigh of C57BL/6J, DBA/2Ha and Balb/cCR, using seven mice per each size of inoculum and strain. In other experiments, 5×10^3 L1210 (10 mice per strain) or 10^3 L1210 cells (8 mice per strain) were injected s.c. in the right flank of C57BL/6J, DBA/2Ha or Balb/cCR.

9. Ten C57BL/6J mice, injected previously with 5×10^3 or 10^3 cells L1210 without take, were injected five weeks later s.c. in the left flank with 10^4 cells L1210 each, as were 10 control mice. Ten C57BL/6J mice, having received previously L1210 cells with appearance of tumor nodule followed by complete regression, were also injected with 10^4 cells in the left flank on the same day.

10. Concomitant immunity was then sought in syngeneic hosts. C57BL/6J mice were inoculated with 5×10^5 T241 or B-16 in the right flank, and at the time of early palpability of the developing nodule or 1 week later the challenge, consisting of 5×10^3 cells of the same tumor, was given in the left flank. This size of primary inoculum gives rise to palpable tumor within one week to nearly all mice. It was at this point that the challenge inoculum was given (Table 2). Concomitant immunity was also sought in L1210 system, using 10^4 cells as the primary inoculum and 10^3 cells as the challenge inoculum. Similar experiments were performed using 5×10^5 or 10^5 cells of SV40 in Balb/cCR mice as the primary inoculum and 5×10^4 or 10^4 cells as challenge inoculum, given when the primary inoculum had become a palpable tumor (Table 2).

11. A group of 10 control C57BL/6J mice were injected with 5×10^3 T241 cells in RMT, while another group of 10 mice received 5×10^3 cells in RMT. The last group also received treatment with hydrocortisone 1 mg i.p. on the

Table 2. Concomitant immunity

Mice	Tumor and dose	Challenge takes	Control takes	χ^2 and significance	
C57BL/6J					
	T241 5×10^3 on palp.	0/10	6/10	$\chi^2 = 8.57$ $P < 0.005$	
					$\chi^2 = 16.94$ $P < 0.00005$
	T241 5×10^3 1 week after palp.	3/10	10/10	$\chi^2 = 10.77$ $P < 0.002$	
	B-16 5×10^3 on palp.	3/9	10/10	$\chi^2 = 9.74$ $P < 0.002$	
					$\chi^2 = 15.08$ $P < 0.0001$
	B-16 5×10^5 1 week after palp.	5/9	10/10	$\chi^2 = 5.63$ $P < 0.02$	
	T241 5×10^3 on palp.	6/29	9/11	$\chi^2 = 12.71$ $P < 0.005$	
DBA/2Ha	*L*1210 10^3 on palp.	1/5	7/8	$\chi^2 = 5.92$ $P < 0.02$	
Balb/cCR	SV40 5×10^4 on palp.	1/8	9/10	$\chi^2 = 10.81$ $P < 0.002$	
					$\chi^2 = 25.02$ $P < 0.0001$
	SV40 10^4 on palp.	0/8	9/10	$\chi^2 = 14.40$ $P < 0.0002$	

same day and thrice weekly for four weeks thereafter.

In vitro *studies*

The *in vitro* ability to develop a growing cell culture was studied for both B-16 and T241 tumors by inoculating 5×10^3 or 5×10^2 cells in Petri dishes, using five dishes per each dose and tumor. Then the studies were repeated by inoculating 5×10^2, 4×10^2, 2×10^2, 10^2, 5×10 or 10 cells in five Petri dishes for each dose-tumor.

In vivo, 5×10^2 B-16 cells where injected s.c. in the right flank of each of 15 mice, and 5×10^2 T241 cells were injected s.c. in the right flank of each of 15 mice.

RESULTS

1. The multiply injected mice (Group II), having received a 10-times higher total tumor dose, did not have an increased rate of takes for the same site over the Group I singly injected animals. Among 40 Group II mice injected with T241 there were seven takes in RUF, compared to 18 takes at the same site in 40 Group I mice ($P < 0.05$). There were 20 takes in 30 multiply injected mice with B-16 versus 19 takes at the same site in 30 singly injected mice (Table 1).

2. Repeat experiments with B-16 showed no difference in takes of 10^3 inoculum given alone (3/5) or with an additional three-fold (3/5) or nine-fold tumor dose (3/5) in an equal number of additional sites.

3. Tumors grew well after implantation into medial and lateral aspects of the thighs. They did less well after implantation into the s.c. tissue of the abdomen and the flank. Within the multiply injected animals (Group II) of Table 1, there were 54 takes of 70 injected sites in RMT versus 20 takes of 70 sites in RUF, ($P < 0.001$). This difference of anatomical sites was further confirmed in the B-16 tumor. There were 8/15 takes in RMT versus 1/15 takes in RUF ($P < 0.01$), while there were 2/10 in the right axilla and 0/15 in the deltoid area.

4. The effect of the presence of a single contralateral ten-fold larger inoculum (10*D*) did not result in a higher rate of successful implantation and growth of the lower inoculum (*D*). In the controls, 12 of 21 T241 implants grew, whereas four of 21 implants grew in the bilaterally injected mice ($P < 0.05$). In the B-16 series, there was no difference between Group I and Group II, there being 15 takes of 20 implants in the singly injected group and 16 of 20 implants in the group with contralateral ten-fold larger inoculum.

5. In Balb/cCR mice with syngeneic SV40 tumor implanted, there were fewer takes (1 of 20 implants) in the mice given simultaneously a contralateral 10*D* injection, as compared to controls (14 of 20 implants) ($P < 0.0001$). In the DBA/2Ha mice with L1210 tumor, there was no difference in the rate of takes (8/8 in both groups); however, the rate of initial growth of a 10^3 inoculum, as reflected in average tumor diameter, in mice given a five-fold inoculum in the contralateral side was not increased compared to controls. In Wistar rats with syngeneic Wilms tumor, there were 2/8 takes in the singly injected group versus 1/8 in the group with additional ten-fold contralateral inoculum.

6. In the initial experiments seeking concomitant immunity, no definite evidence could be obtained for T241 at 7 (challenge 3/5 takes, control 4/5), 10 (challenge 2/5 takes, control 3/5) or 14 (challenge 2/5, control 5/5) days. For the B-16 tumor at 7, 10 and 14 days there were, respectively, 5/5, 5/5 and 5/5 takes in the challenge groups, and 5/5, 3/4 and 5/5 takes in the control groups. It should be noted that the primary inoculum which had been used to incite immunity was a small one, producing palpable nodules at $2\frac{1}{2}$–3 weeks.

7. Inoculating B-16 melanoma in DBA/2Ha mice, it was found that some takes (4/9) did appear but became palpable 21 days after inoculation, grew for one week and then regressed completely in one more week in all but one of the four mice.

8. With different-sized inocula of L1210, it was found that larger inocula became palpable in their allogeneic hosts (C57BL/6J and Balb/cCR) faster (in 5 days) and disappeared faster (in 18–22 days), while smaller inocula were slower to become palpable (in 8 days) and disappear (in 28–38 days). There were takes in all mice with these inocula. Using smaller inocula of L1210 (10^3 or 5×10^3), takes appeared (in 20–30% of C57BL/6J and 80–90% of Balb/cCR mice) as late as day 17 after inoculation in mice which were able to reject their tumor.

Different-sized inocula of B-16 (5×10^5, 5×10^4 or 5×10^3) were inoculated in the medial thigh of C57BL/6J, Balb/cCR and DBA/2Ha. There were six takes of seven mice in the Balb/cCR (5×10^5 B-16 cells) which had regressed in all mice by day 31. There were five takes of seven mice given 5×10^4 cells in Balb/cCR mice, of which four regressed by day 38, but one mouse presented tumor progression. There were seven takes of eight Balb/cCR mice given 5×10^3 B-16 cells which continued to appear up to day 28, grew for one more week and then regressed in all but two which showed tumor progression.

9. There were 5/10 takes in C57BL/6J mice injected with 10^4 cells L1210 in left flank (having had prior contact with L1210 without take) which slowly regressed. There were seven takes of 10 C57BL/6J control mice injected with 10^4 cells L1210 which also regressed, while there was no take in the 10 C57BL/6J injected with 10^4 cells L1210, which had prior temporary take and regression of L1210 tumor.

10. Concomitant immunity was shown on challenging C57BL/6J mice with T241 or B-16 tumors at the time of early palpability, or 1 week later, of a previously injected inoculum of the same tumor (Table 2). Evidence of concomitant immunity was also elicited for L1210 in DBA/2Ha mice and for SV40 in Balb/cCR (Table 2).

11. In the experiment dealing with immunosuppression with hydrocortisone, by day nine after inoculation there were two takes in the control group, with average diameter 3.5 mm, and one take in the treated group with diameter 1 mm, while by day 27 there were 10/10 takes in the control group, with average diameter 8.7 mm, and 9/9 takes in the treated group, with average diameter 10.9 mm.

In vitro *studies*

Four days after inoculation of 5×10^3, 10^3 or 5×10^2 cells, there was confluent growth in all plates for both B-16 and T241. In the second series of experiments, one week after inoculation of 5×10^2 there was growth in all plates, but this slower with the smaller inocula, particularly 5×10 or 10 cells. However, one week later there was confluent growth in all plates.

There were no takes in 15 mice injected with 5×10^2 cells of B-16 and no takes in 15 mice injected with 5×10^2 cells of T241.

DISCUSSION

In the above experiments there was a significant difference in the rate of tumor growth when the same-sized inoculum of tumor was implanted at various anatomical sites. The rate of takes in the subcutaneous tissue of the thigh was significantly higher than that of the flank. The difference in takes of tumors in different tissues has been previously described [5, 6], but this variation in the same tissue according to the anatomical site is less well known; it may relate to the vascularity of the area or other factors, and points to the importance of local host factors on the takes of threshold inocula.

There is abundant clinical and experimental evidence that weak and generally unsuccessful immune resistance develops in the host during at least a portion of the evolution of a tumor [7–10].

The immunosurveillance theory, stating the capacity of the immune system to destroy aberrant cells with malignant potential [11], would suggest that this capacity is overcome or overwhelmed when a palpable tumor appears.

The participation of the immune response, if any, remains, however, enigmatic in the control of early tumor inocula. In repeated experiments with the five tumor systems of this report the take of a threshold inoculum was the same (B-16, L1210, Wilms) or suppressed (T241, SV40) compared to controls when additional tumor load was given simultaneously at other distinct site(s); the take of a threshold inoculum is not facilitated by the simultaneous implantation of a separate ten-fold larger inoculum which takes regularly, and thus the earlier take of the latter does not signify an overwhelming of a potential host response.

In a previous study it was also concluded that the chance of take at a site is not influenced by the simultaneous inoculation of other sites [12].

Previous studies have shown the immunogenicity of L1210 [13], B-16 [1] and SV40 [2] tumors. It has been previously suggested that the cellular response to a tumor inoculum rises to a maximum at one week after implantation and then decreases precipitously, while the humoral response attains its maximum at two weeks [14].

Demonstration of concomitant immunity was attempted initially in our study by inoculating C57BL/6J mice with what retrospectively were shown to be small inocula of T241 or B-16 cells giving rise to palpable nodules $2\frac{1}{2}$–3 weeks later. Then at 7, 10 or 14 days, respective groups of mice were challenged with a threshold inoculum of the same tumor. However, there was no evidence of concomitant immunity.

The timing of the onset of immune response was then sought using allogeneic tumors. In their case there is a strong immune response against tissue histocompatibility antigens [15], and one could deduce the onset of an effective immune response by simply observing the growth and regression of these tumors. In the first experiment there were 4/9 takes of B-16 melanoma in four DBA/2Ha mice. For three of the mice, which were able to reject their tumor, it became evident that although they potentially had this capability, they allowed the tumor nodule to appear three weeks after inoculation and to grow for one more week, and then caused its regression.

In further experiments with L1210 using

larger inocula (5×10^6 cells), the tumor became palpable, grew for a week or so and then regressed completely by day 22 in all the allogeneic mice. With 5×10^5 cells the tumor nodules regressed completely by day 28, while with 5×10^4 cells the tumor nodules regressed completely by day 38. Using lower inocula of L1210 (i.e., 5×10^3 or 10^3 cells) in C57BL/6J or Balb/cCR mice, nodules continued to appear by day 12 and day 17 after inoculation, indicating that there was no effective immune response up to this time, even though these allogeneic mice were able to reject their tumor later.

Using B-16 tumor, with 5×10^5 cells all the Balb/cCR mice had rejected completely their tumor by day 31 (there was continuous shrinkage from day 16, more actively after day 21), while with 5×10^3 cells nodules continued to appear by day 28 and were actively growing by day 31.

With larger tumor inocula it was found that there was nearly 100% take in the allogeneic hosts. With smaller inocula the incidence of take was less, but generally it appeared that more mice in this group progressed to death. Also, there was no appreciable difference in the various experiments in the timing of the appearance of these tumor nodules between the syngeneic and allogeneic hosts, suggesting again that there was no effective immune response in the allogeneic mice prior to the appearance of tumor nodules. Furthermore, prior inoculation without take in an allogeneic host did not confer immunity from further challenge with the same tumor, while prior take and regression conferred complete immunity.

With the above knowledge, concomitant immunity was sought again in syngeneic tumors. A larger primary inoculum was used, giving rise to a palpable nodule in one week or so. Challenge now with a threshold inoculum at the time of early palpability of the primary inoculum produced evidence of concomitant immunity in B-16, T241, L1210 and SV40 tumors (Table 2).

Treatment of C57BL/6J mice with hydrocortisone did not increase the rate of initial takes of T241, but the tumor nodules grew subsequently faster compared to those of control mice.

The onset of immunity was also followed in mice inoculated with tumor by the serial application of an *in vitro* assay. In a study already published, *in vitro* evidence of immunity was obtained for colon carcinoma (MCA-38) and B-16 in the C57BL/6J mice [16]. In these experiments specific leukocyte adherence inhibition assay correlated with concomitant tumor immunity to these tumors, which was present from the time of early palpability of the rising tumor nodule [16].

The above data are not compatible with the immunosurveillance theory, which postulates that a competent immune system can eradicate microscopic amounts of autochthonous tumor. It is very hard to see how this could happen when even an allogeneic host does not mount an effective immune response except after the appearance of a tumor nodule.

The notion that a certain number of tumor cells is required for a successful take because such a number of tumor cells is required to find among them one or two biologically strong cells capable of initiating tumor growth *in vivo* is apparently not correct. Under conditions of cell culture, nearly all cells are capable of initiating growth. In the performed *in vitro* studies, as low a number as 10 cells of T241 or B-16 are capable of further multiplication and formation of large colonies, while *in vivo* inocula smaller than 10^3 cells do not take.

Compared to *in vitro* culture, with *in vivo* growth the early growth of small inocula is impaired either due to the existence of local non-specific host factors destroying tumor cells or due to relative lack of favorable conditions for their growth. The number of cells present in a threshold (or an above-threshold) inoculum may be providing a protective effect or increased reserve against local adverse host factors and may help elicit favorable host factors (e.g., through sufficient elaboration of angiogenesis factor).

On the basis of the above experiments, it appears that the host immune system does not manufacture an effective immune response at any definite interval after tumor inoculation. Rather, such intervals seem to vary with the size of the initial inoculum, being longer for small inocula; a certain amount of antigenic stimulation is apparently required for the initiation of the immune response which corresponds roughly with the early palpability of the tumor nodule. It appears that the take of experimental threshold inocula is not dependent upon the result of an interaction with systemic host factors which, when overcome, allow the clinical appearance of a nodule; it is, rather, an interaction between the injected cells and local adverse host conditions.

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REFERENCES

1. BYSTYRN JC, BART RS, LIVINGSTON P, KOPF AW. Growth and immunogenicity of murine B-16 melanoma. *J Invest Dermatol* 1974; **63**: 369–373.
2. KIT S, KIRIMURA T, DUBBS DR. Transplantable mouse tumor line induced by injection of SV40-transformed mouse kidney cells. *Int J Cancer* 1969; **4**: 384–392.
3. LAW LW, DUNN TB, BOYLE PJ, MILER JH. Observation on the effect of a folic acid antagonist on transplantable lymphoid leukemias in mice. *J Natl Cancer Inst* 1949; **10**: 179–192.
4. TOMASHEFSKY P, FURTH J, LATTIMER JK, TANNENBAUM M, PRIESTLY J. The Furth–Columbia rat Wilms tumor. *Trans Am Assoc Genitourin Surg* 1971; **63**: 28–36.
5. KRONMAN BS, WEPSIC HT, CHURCHILL WH, ZBAR B, BORSOS T, RAPP GH. Immunotherapy of cancer: an experimental model in syngeneic guinea pigs. *Science* 1970; **168**: 257–259.
6. APFFEL CA, PETERS JH. Rejection of lethal ascites tumors following subcutaneous inoculation. A phenomenon of antigenic expression. *J Natl Cancer Inst* 1967; **39**: 1129–1139.
7. SOPHOCLES AM, JR, NAKLER SH. Immunologic aspects of cancer. *Surg Gynecol Obstet* 1971; **133**: 321–331.
8. DUTCHER J. Immunity and neoplasms. *Ann Intern Med* 1972; **129**: 143–144.
9. HAUSCHKA TS. Tumor immunity. In: ROSE NR, MILOGROM F, VAN OSS CJ, eds. *Principles of Immunology*. New York, Macmillan, 1973.
10. COGGIN JH, JR, AMBROSE KR, DIERLAM PJ, ANDERSON NG. Proposed mechanisms by which autochthonous neoplasms escape immune rejection. *Cancer Res* 1974; **34**: 2092–2101.
11. BURNET MF. Immunological surveillance in neoplasia. *Transplant Rev* 1971; **7**: 3–25.
12. WOODRUFF MFA, DUNBAR N. The effect of cell dose and distribution on the development of a transplanted tumour. *Eur J Cancer* 1974; **10**: 533–537.
13. BONMASSAR E, BONMASSAR A, VADLAMUDI S, GOLDIN A. Immunologic response of mice bearing leukemia L1210. *Cancer* 1971; **27**: 1356–1362.
14. KALISS N, BRYANT BF. Factors determining homograft destruction and immunological enhancement in mice receiving successive tumor inocula. *J Natl Cancer Inst* 1958; **20**: 691–703.
15. SNELL GD. Histocompatibility genes of the mouse. II. Production and analysis of isogenic resistant lines. *J Natl Cancer Inst* 1958; **21**: 843–877.
16. LEVESON SH, TAN MH, KARAKOUSIS C. The leukocyte adherence inhibition (LAI) assay correlates with transplantation immunity. *Proc Am Assoc Cancer Res* 1977; **18**: 150.