

# Depression of Host *versus* Graft Immunity and Stimulation of Tumor Growth following Partial Hepatectomy<sup>1</sup>

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## SUMMARY

Tumor growth was measured in inbred (C3H and C3Hf) and hybrid (C3H × C57BL/6 F<sub>1</sub> and BALB/c × C3H F<sub>1</sub>) mice after partial hepatectomy, sham hepatectomy, and hind limb amputation. Epithelial tumors (mammary carcinoma) and mesenchymal tumors (methylcholanthrene-induced and spontaneous tissue culture) were used in order to determine the tissue specificity of the tumor growth stimulation. Immunological parameters were defined by: (a) utilization of tumors that were either antigenic or nonantigenic, and (b) measurement of the host *versus* graft response in partially hepatectomized mice. Partial hepatectomy and the control operations were done on the same day as the tumor cell inoculation. All tumors, regardless of their tissue type or antigenicity, grew significantly better in partially hepatectomized mice as compared with the control mice. There was a significant positive correlation between the magnitude of tumor growth stimulation and the degree of antigenicity of the tumor. Last, skin allograft survival was significantly prolonged in the hepatectomized mice.

The results suggest that: (a) the stimulation of tumor growth in hepatectomized mice is not tissue specific as previously reported; and (b) the antigenicity of a tumor is not necessary for growth stimulation after partial hepatectomy.

## INTRODUCTION

Following the removal of a portion of the liver, the remaining lobes undergo a rapid and intense proliferation (5). Paschkis *et al.* (9) studied the effect of partial hepatectomy on transplantable tumors and found that there was a stimulation of the growth of the Fisher hepatoma and the Flexner-Jobling carcinoma. However, there was no apparent stimulation of mesenchymal tumors (*i.e.*, Murphy lymphosarcoma and Jensen sarcoma). They suggested that regenerating liver releases growth-promoting agents that stimulate both the liver and certain types of epithelial tumors. Trotter (16) subsequently investigated the effect of partial hepatectomy on the time of appearance of s.c. hepatoma transplants. Early transplant generation hepatomas appeared sooner in partially hepatectomized mice, but late generation hepatomas were refractory to the effects of partial

hepatectomy. She postulated that early transplant generation tumors were still responsive to the hormonal milieu, while tumors of later generations had lost this responsiveness in the course of tumor progression. Trotter suggested that this stimulus may be related to the growth-promoting agent that stimulated cell multiplication in the normal liver after partial hepatectomy. Recently, Lee (7) has shown that the weights of s.c. hepatomas in partially hepatectomized mice were higher than in control rats. In addition, hepatoma DNA synthesis was increased between 18 and 36 hr after partial hepatectomy. He postulated that the increased growth of the hepatomas may have been due to the regulatory mechanisms governing the proliferation of hepatocytes.

The present experiments were therefore designed to answer the following questions. (a) Does partial hepatectomy stimulate the growth of mesenchymal tumors? (b) Does partial hepatectomy depress cell-mediated immunity?

## MATERIALS AND METHODS

Tissue specificity was determined by the use of epithelial tumors (mammary carcinoma) and mesenchymal tumors (MCA<sup>2</sup>-induced sarcoma). The immunological parameters were defined by using antigenic or nonantigenic tumors, or hosts in which the tumor antigens would be weakly immunogenic (*e.g.*, C3H mammary tumor given to C3H mice). Skin allografts were done to assay the competency of the host-*versus*-graft response in partially hepatectomized mice.

Male mice of the inbred strains C3H/HeN1cr (C3H), C3Hf/HeN1cr (C3Hf), and A/He (A), and hybrid strains C3H/HeN1cr × C57BL/6J1cr F<sub>1</sub> (C3H × BL F<sub>1</sub>) and BALB/cAnN1cr × C3H/HeN1cr F<sub>1</sub> (C × C3H F<sub>1</sub>) were used.

The epithelial tumor used was a mammary adenocarcinoma (MT2) that had arisen spontaneously in a C3H female and was in its 3rd transplant generation at the time of the experiment. The mesenchymal tumors were MCA-induced sarcomas or tumors from tissue culture-transformed cells. MCA 2075 was induced in a C female with a 5% MCA pellet (12) and was in its 3rd transplant generation. MCA 2045 arose in a C3H male that had been treated with a 5% MCA pellet and was in its 10th transplant generation. The tissue culture tumor (TC72) arose by spontaneous transformation *in vitro* from C3H × BL F<sub>1</sub> fetal cells. Mammary tumor cell suspensions were prepared by treating the tumor mince

<sup>1</sup> The work was supported by USPHS Grants CA-08856, CA-05255, CA-06927, and RR-05539 from the NIH and by an appropriation from the Commonwealth of Pennsylvania.

Received August 28, 1975; accepted February 5, 1976.

<sup>2</sup> The abbreviations used are: MCA, methylcholanthrene; HBSS, Hanks' balanced salt solution.

with collagenase (2.5 mg/ml) for 1 hr, washing the cells 3 times in HBSS and resuspending them in HBSS without calcium and magnesium. The MCA- and tissue culture-induced tumors were treated with a mixture of DNase (0.005%) and Pronase (0.25%) for 0.5 hr, washed 3 times in HBSS, and resuspended in HBSS (12). Tumor cell viability was determined by the trypan blue exclusion technique. A viable cell dose of  $2 \times 10^6$  mammary tumor cells or  $5 \times 10^5$  sarcoma cells was injected s.c. into the middorsal region of each mouse. The size of the tumor was determined by measuring the smallest diameter of each tumor at various times after tumor cell inoculation. The identity of the mouse (whether experimental or control) was unknown at the time of tumor measurement.

Partial hepatectomy was done in the morning of the same day as the tumor inoculation. The hepatectomy was performed according to the techniques of Higgins and Anderson (6). Skin grafting was carried out by transplanting full-thickness allogeneic grafts (A/He donors) on the day of hepatectomy (2, 15). Graft survival was assayed by determining the amount of graft rejected at a given time. Leg amputations were done by removing the left hind limb just below the head of the femur. Data were analyzed by the Mann-Whitney U test (14).

Antigenicity was determined by immunizing 12 mice with trocar pieces of the test tumor and sham immunizing a control group of 12 mice. After the tumors had grown for 10 days, they were excised. At the time of tumor excision, sham operations were done on the controls. Ten days later, each group was irradiated (whole-body) with 500 R and, on the following day, each received a cell suspension of  $5 \times 10^5$  sarcoma cells or  $2 \times 10^6$  mammary tumor cells. The data were analyzed when the mean tumor size of the controls was approximately 5 mm. The antigenic ratio was the mean size of the controls divided by the mean size of the immunized group. A value of 1 or less was taken to mean that the tumor was nonantigenic. Conversely, a value greater than 1 meant that the tumor was antigenic (1, 12). All antigenic ratios were tested in the syngeneic strain.

**RESULTS**

**Growth Stimulation of Mammary and MCA-induced Sarcomas.** In order to condense the data of the pilot experiments into a practical form, a series of charts are presented showing median values. The confirmatory experiments are presented in a tabular form with the data ranked in order. Chart 1 represents the median difference in size of the mammary tumor as a function of time. It can be seen that the mammary tumor in the partially hepatectomized mice was significantly larger than those in the controls. The differences between the pairs became significant at Day 14 and remained so until the experiment was terminated on Day 25. These results were compatible with those in the literature (16). The growth of MCA 2075 is depicted in Chart 2. A significant difference in the median tumor sizes occurred at 10 days and at 21 days. These results were confirmed (Table 1) by repeating the experiment and evaluating the data on Day 10 (i.e., the 1st point of the curve in Chart 2).

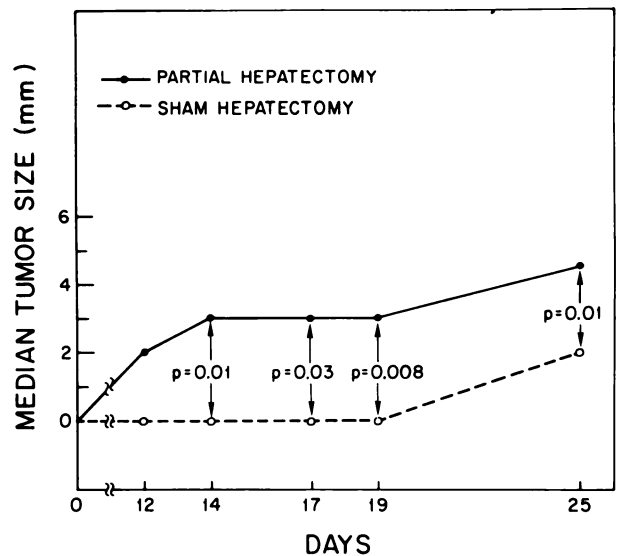


Chart 1. A s.c. dose of  $2 \times 10^6$  MT2 tumor cells was injected on the same day (Day 0) as partial hepatectomy and sham hepatectomy. Tumor growth was measured at the intervals shown on the chart. Probability values computed by Mann-Whitney U test.

Table 1  
Ranked data showing increased growth of MCA 2075 in hepatectomized C x C3H F<sub>1</sub> mice 10 days after partial hepatectomy

Tumor size (mm)	
Sham hepatectomy	Partial hepatectomy <sup>a</sup>
0	0
0	1
0	1
0	2
1	3
1	3
1	3
2	5
2	5

<sup>a</sup> Tumor growth greater in hepatectomized group;  $p = 0.01$ .

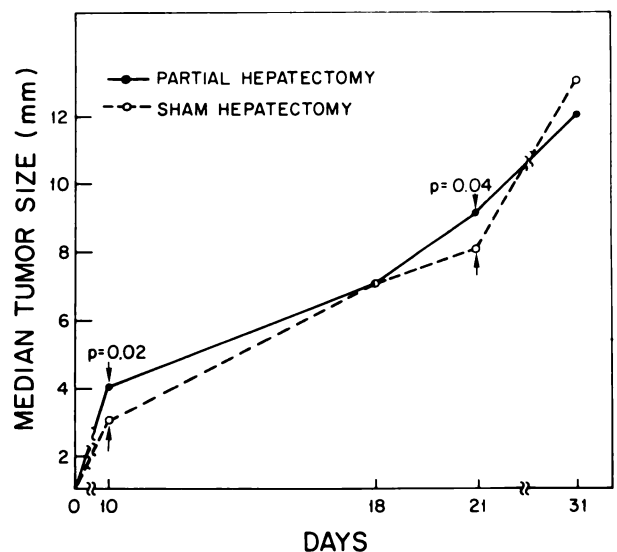


Chart 2. Effects of partial hepatectomy on the growth of MCA 2075.

In order to expand this observation, the experiment was repeated using a 2nd MCA tumor (MCA 2045). A significant difference in median tumor sizes became evident on Day 5 and persisted until the end of the experiment on Day 14 (Chart 3). Results of the confirmatory experiment are presented in Table 2. In this experiment, a hind limb amputation was added to test the effects of nonspecific trauma on tumor growth. Tumor size was again significantly greater in the hepatectomized group and leg amputation did not appear to affect tumor size.

**Prolongation of Allograft Survival by Partial Hepatectomy.** C x C3H mice were grafted with full-thickness A/He skin in order to determine whether partial hepatectomy interfered with delayed type hypersensitivity. Analysis of the data revealed that graft rejection proceeded significantly more slowly in the hepatectomized group from Day 9 through Day 16 (Chart 4).

**Growth of Nonantigenic Tumors.** Since allograft immunity was found to be depressed, it was postulated that immunity to tumor antigens might also have been impaired.

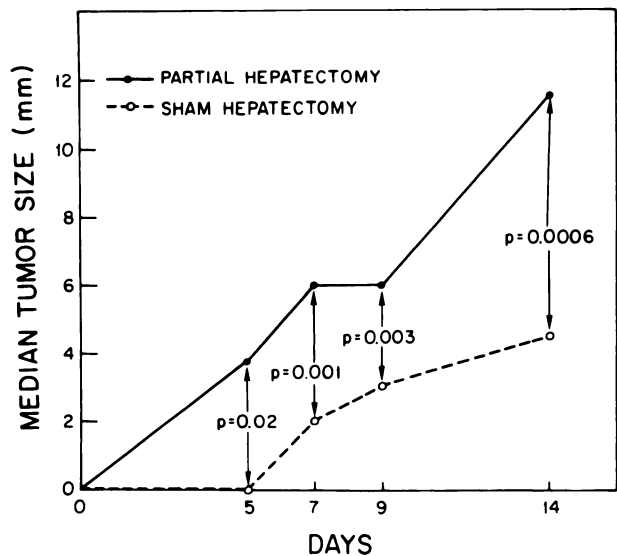


Chart 3. Effects of partial hepatectomy on the growth of MCA 2045.

Table 2

Ranked data showing increased growth of MCA 2045 in hepatectomized C x C3H F<sub>1</sub> mice 7 days after hepatectomy. Sham hepatectomy versus leg amputation, p = 0.2; partial hepatectomy versus leg amputation, p = 0.002; partial hepatectomy versus sham hepatectomy, p = 0.02.

Tumor size (mm)		
Sham hepatectomy	Leg amputation	Partial hepatectomy
0	0	3
0	0	3
0	0	4
0	0	4
1	0	5
2	0	5
2	0	5
3	2	6
3	3	7
5	4	8
7	4	10
8	5	Died
8	11	Died

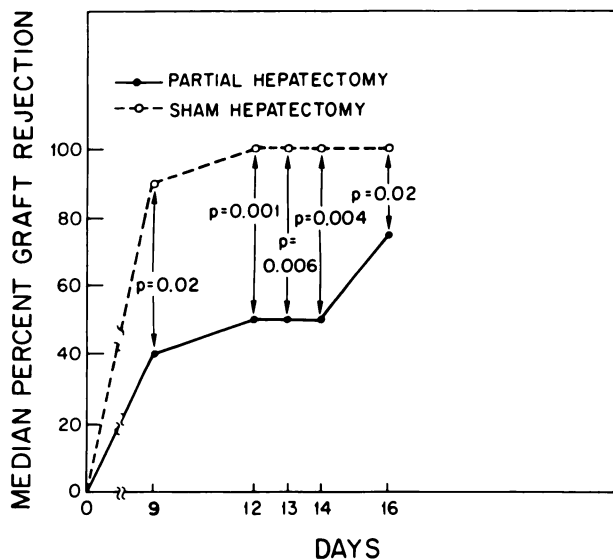


Chart 4. Delayed rejection of allografts in partially hepatectomized C x C3H F<sub>1</sub> mice.

Table 3

Separate experiments showing the increased growth of the nonantigenic tissue culture sarcoma in partially hepatectomized C3H x BL F<sub>1</sub> mice 5 days after tumor inoculation and hepatectomy

Tumor size (mm)			
Sham hepatectomy	Partial hepatectomy	Sham hepatectomy	Partial hepatectomy
0	0	0	0
0	3	0	2
0	3	0	2
2	4	0	2
2	4	0	2
3	4	0	2
3	4	0	3
3	4	0	3
3	5	0	3
4	5	0	3
4	5	0	3
4	5	0	4
4	5	0	4
4	8	0	4
		1	4
		2	4
		3	4
		3	4
		4	4
		4	5
		4	5
		4	5
		4	5
		4	5
		4	5
		4	5
		5	5
		6	6
		7	7
		7	7
		7	

p = 0.003

p = 0.003

To determine whether impairment of antitumor immunity could account for larger tumors in the hepatectomized mice, the growth of a nonantigenic tissue culture sarcoma (TC72) was determined in partially hepatectomized mice. The experiment was performed 3 times. Two of the experiments were pooled for statistical evaluation (Table 3). It can

be seen that there was significant growth stimulation of the tissue culture sarcoma in the hepatectomized mice as compared with the sham-operated controls.

In addition, separate experiments were performed in which a spontaneous C3H mammary tumor (MT2) was inoculated into partially hepatectomized C3H mice. This tumor was not immunogenic in C3H mice (antigenic ratio, 0.8). It can be seen from Table 4 that the growth of MT2 was significantly greater in the hepatectomized mice. These results demonstrated that immunogenicity of the tumors was not a prerequisite for growth stimulation after partial hepatectomy.

**Correlation between Tumor Antigenicity and Stimulation of Tumor Growth.** The next step was to determine whether there was any correlation between tumor antigenicity and the magnitude of tumor growth stimulation. In order to test this, a stimulation ratio for each tumor was calculated on the present data by dividing the mean tumor size of the hepatectomized group by the mean tumor size of the con-

Table 4

Ranked data showing increased tumor size (MT2) in the partially hepatectomized C3H mice 3 days after tumor inoculation and partial hepatectomy

$p = 0.02.$

Tumor size (mm)	
Sham hepatectomy	Partial hepatectomy
0	3
2	3
2	3
2	3
2	3
2	3
3	3
3	3
3	4
3	4
4	5
4	5
4	5
4	5
4	5
5	6

Table 5

Antigenic ratios and stimulation ratios of MCA, tissue culture, and mammary tumors

The Spearman coefficient was calculated from the paired antigenic ratio and stimulation ratio for each tumor.

Tumor	Antigenic ratio	Stimulation ratio	Spearman rank correlation coefficient and $p$ value
MCA 2045	2.9	2.7	
MCA 2075	1.9	2.5	
MT2(C3Hf) <sup>a, b</sup>	1.8	1.9 <sup>c</sup>	+1, $p = 0.01$
TC72	0.9	1.7	
MT2(C3H) <sup>a</sup>	0.8	1.3 <sup>c</sup>	

<sup>a</sup> Test mice were C3H or C3Hf.

<sup>b</sup> Tumor growth stimulation data not shown ( $p = 0.01$ ).

<sup>c</sup> The stimulation ratio of MT2(C3Hf) was significantly larger than the stimulation ratio of MT2(C3H),  $p = 0.04$ .

trol group (Table 5). A Spearman rank correlation coefficient was computed by comparing the paired stimulation ratios and the corresponding antigenic ratios for each tumor. A statistically significant positive correlation was found ( $p = 0.01$ ) which suggested that the greater the antigenicity of the tumor, the greater the growth stimulation of the tumor after partial hepatectomy. Additional evidence supporting this correlation was demonstrated by comparing the stimulation ratios of MT2 in C3H and C3Hf mice. The tumor was nonimmunogenic in the C3H strain and its stimulation ratio was significantly less than in the C3Hf strain in which the tumor was immunogenic (Table 5).

DISCUSSION

The growth stimulation of certain epithelial tumors following hepatectomy has been previously documented (7, 9, 16). In contrast, stimulation of s.c.-implanted mesenchymal tumors has not, until now, been found (7, 9). As a consequence of the work on humoral factors in liver regeneration, it was proposed that the growth stimulation of tumors was due, at least in part, to these humoral agents. In support of this hypothesis, Lee (7) has found that the incorporation of tritiated thymidine into hepatoma cells after partial hepatectomy closely followed the time course of thymidine incorporation into the regenerating liver. Paschkis (9) concluded that the agent was selective for epithelial tumors because the Jensen sarcoma and the Murphy lymphosarcoma were not stimulated by partial hepatectomy. Subsequently, Paschkis (10) and others (3, 4) demonstrated that a variety of normal tissues (epithelial and mesenchymal) showed growth stimulation as a consequence of partial hepatectomy. This could be explained by the increased growth hormone after hepatectomy (8). In addition, the present experimental results showed stimulation of mesenchymal as well as epithelial tumors. Because of these results, it seems doubtful that the agent(s) were organ or tissue specific.

In order to rule out nonspecific stress as the cause of tumor growth stimulation (17), a hind limb amputation control was included in one experiment. No tumor stimulation was evident in this group. However, this control may not be totally adequate when compared to partial hepatectomy and, as a consequence, stress cannot be totally excluded as a factor.

Whatever the real and/or hypothetical mechanisms that are involved in liver regeneration, an impairment of the cell-mediated immune response could alone explain the increased growth of epithelial and mesenchymal tumors after partial hepatectomy. This hypothesis was supported by the data that showed significant decreases in the rate of rejection of allografts in partially hepatectomized mice. However, a nonantigenic mesenchymal and a nonantigenic epithelial tumor showed significant growth stimulation after hepatectomy, suggesting that other factors may have played a role in the stimulation of tumor growth. Conceivably, after partial hepatectomy, all the tumors were stimulated by the nonimmunological humoral factors associated with liver regeneration. Furthermore, the greater magnitude

of growth of the antigenic tumors resulted from the depression of cell-mediated immunity. An alternative hypothesis for the observed significant positive correlation between antigenicity and tumor growth stimulation may be found by examining the immunostimulation hypothesis of tumor growth advanced by Prehn and Lappé (13) and Prehn (11). The hypothesis, stated briefly, was that immune reactivity to a tumor may occasionally be adequate to control it but that lesser degrees of immunity may actually promote the growth of the tumor. Therefore, as a consequence of partial hepatectomy, the reactivity of the immune response was lowered to a less inhibitory and perhaps even to a stimulatory level. This conferred a selective growth advantage to the antigenic tumors while it had little effect on nonantigenic tumors.

In conclusion, the growth stimulation of tumors in partially hepatectomized mice represents a complex biological series of events. The initial impetus for stimulation may occur as a result of the nonimmunological growth-promoting agents related to liver regeneration. The subsequent enhancement of the growth of the antigenic tumors seems likely to be a consequence of impairment of the cell-mediated immunological response.

#### ACKNOWLEDGMENTS

It is a pleasure to acknowledge and to thank Dr. R. T. Prehn and Dr. S. Sorof for their valuable aid in the review of this manuscript. I also wish to thank Lorraine Collins and Charlotte Rivell for their expert technical assistance.

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