Combination Therapy with Cyclophosphamide and Zymosan on a Spontaneous Mammary Cancer in Mice*

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SUMMARY

The results of experiments in which surgery, cyclophosphamide, and zymosan have been combined as therapy on a spontaneous mammary adenocarcinoma in CD8Fi female mice are evaluated. Zymosan, combined with cyclophosphamide and enucleative surgery, significantly enhanced the therapeutic effect. The mechanism of zymosan action as an immune phenomenon mediated by the host is considered. The data furnished additional evidence that immune phenomena may be strengthened to afford more effective treatment in cancer and are considered of particular significance inasmuch as they were obtained with spontaneous neoplasms.

The cure or control of spontaneous mammary cancer in mice has been the subject of intensive research by our group (5) and others (4, 11). We have found that maximal effects of chemotherapy are obtained when the bulk of tumor tissue is removed surgically, prior to the administration of chemotherapy (6).

This enhanced effect may be due to reducing the number of tumor cells into a range that can be controlled by host defenses, such an antibody. Support for the concept that host defense mechanisms were operative in influencing chemotherapeutic response came from experiments with transplanted tumors, in which it was shown that a chemotherapeutic effect could be reversed by the simultaneous administration of cortisone, a known depressant of the reticuloendothelial system (RES). In contrast, the concomitant administration of zymosan, which is recognized for its capacity to increase the ability of the host to form antibodies (9), augmented the chemotherapeutic effect (7).

Cortisone reversal of chemotherapeutic response following surgery was also obtained with spontaneous neoplasms (7). The present report documents data indicating that zymosan can augment the effect of chemotherapy following surgery upon spontaneous mammary cancer in mice.

MATERIALS AND METHODS

General—CD8Fi female mice (25-30 gm.), each bearing a single, well established, spontaneously occurring mammary adenocarcinoma, were used. All animals were housed in plastic cages in an air-conditioned room at a constant temperature of 76°F. They received Purina Laboratory Chow and water ad libitum.

The alkylating agent, cyclophosphamide, was the chemotherapeutic agent chosen for this study. It was prepared in 0.85 per cent saline at 3 mg/cc and given to the animals daily at a dosage of 30 mg/kg of body weight. Three experiments in which slightly different dosage schedules were followed are reported (Table 1). In all cases, the injection of cyclophosphamide was initiated on the day following surgical removal of the tumors.

Zymosan, an insoluble polysaccharide at high molecular weight, was obtained from the Nutritional Biochemical Corporation. It was prepared at a concentration of 2 mg/cc in 0.85 per cent saline, and the suspension was sterilized by being boiled for 1 hour in a water bath, was allowed to cool, and was administered at a dose of 20 mg/kg of body weight. In the three experiments zymosan was given at slightly different time intervals, but it always was administered a number of days (9-13 days) before the surgical removal of the tumors. A second injection was given 1 or more days after the surgery. In Experiments 2 and 3 a third injection was given 13 days after surgery.

Both drugs were prepared immediately prior to injection and were administered intraperitoneally. The schedule of injections for the three experiments is summarized in Table 1. In each experiment, the tumors of all mice were surgically enucleated. The mice then were divided into three groups of seventeen to thirty-five each. Following surgery all groups were treated as follows:

Group A, no further treatment; Group B, cyclophosphamide alone; Group C, cyclophosphamide and zymosan.

Tumor recurrence was determined weekly by manual palpation of the animals and was considered recurrent if present either at the original site of surgery or anywhere else in the animal. Animals that died before the termination of the experiment were: (a) eliminated from the total number of animals in the experiment if they did not bear a tumor at the time of death or (b) included with those ani-
TABLE 1

INJECTION SCHEDULE OF CYTOXAN AND ZYMOSAN RELATIVE TO SURGERY

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Cytoxan</th>
<th>Zymosan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. injections*</td>
<td>No. injections</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>3</td>
</tr>
</tbody>
</table>

* Cytoxan was injected daily beginning 1 day after surgery.

Animals showing tumor recurrence if they had a tumor at the time of death. The total number of animals that died before the termination of the three experiments was: 44 out of 78 for Group A, 34 out of 72 for Group B, and 25 out of 75 for Group C. These deaths were caused by needle trauma, excessive tumor growth, or drug toxicity. Only nine of the 25 deaths in Group C could be attributed to drug toxicity. Changes in the average weight of the animals in each group were recorded daily. In the three experiments reported, the average maximum weight loss was 11 per cent, a degree of weight loss that in our experience has never per se influenced tumor growth.

The three experiments were terminated at different times—namely, 47, 62, and 83 days after surgery in Experiments 1–3, respectively. The palpation data are reported for a 56-day time period (Chart 1), which serves to note the differences in recurrence rates between the groups; following this period the differences gradually merged into a recurrence rate that was the same in all groups.

**Surgical.**—The tumor-bearing animals used in these experiments were 9–12 months of age. They were anesthetized with sodium pentobarbital (Nembutal) at a dose of 85 mg/kg intraperitoneally. Under aseptic conditions the skin was incised, and all tumor was stripped out grossly. The wound was closed with Michel clips, which were removed 1 week later.

**RESULTS**

In accordance with a previous report (5), treatment of this spontaneous tumor with cyclophosphamide following surgery (Group B) resulted in lower recurrence rates than treatment with surgery alone (Group A). However, the lowest recurrence rates are shown to be in Group C, the group in which a combination of surgery, cyclophosphamide, and zymosan was employed.

The results of the three experiments are recorded in Table 2 and Chart 1. This composite graph (Chart 1) closely resembles each graph that was made of the three individual experiments. The composite graph is a plot of per cent nonrecurrence versus the time in days after surgery. Nonrecurrence indicates the absence of a tumor, not only at the original site of surgery, but anywhere in the animal.

The graph demonstrates that the triple combination of surgery, cyclophosphamide, and zymosan consistently resulted in the lowest recurrence rate. Fifty-six days after surgery the recurrence rate of those groups receiving surgery, cyclophosphamide, and zymosan was 25 per cent lower than that of those groups treated with only surgery and cyclophosphamide (Table 2).

**DISCUSSION**

Previous experiments have shown that zymosan alone does not influence the recurrence rate of surgically enucleated spontaneous tumors in mice.1 When administered in combination with a cancer chemotherapeutic agent,

1 D. S. Martin, unpublished data.
However, a synergistic effect has been shown to occur and has resulted in a decrease in the recurrence rate of surgically enucleated spontaneous tumors.

The mechanism whereby zymosan has produced these results is not clear. The possibility that the results reflect an immune reaction mediated by the host, however, is consistent with data obtained by others. Blattberg (1, 2) has reported that sera from rabbits and guinea pigs given injections of zymosan showed increased bactericidal activity against Escherichia coli B, which could be removed by absorption of the sera with these organisms. Old et al. (9) found that zymosan induced reticuloendothelial hyperplasia, decreased host susceptibility to bacterial challenge, and protected Swiss mice against challenge with Sarcoma 180. Bradner et al. (3) have demonstrated a significant degree of Sarcoma 180 tumor loss as a result of zymosan administration. In addition, alteration in serum properdin levels following administration of zymosan has been established (10), and there appears to be a correlation between the properdin level and the resistance of a host to tumor tissue (3, 12). In view of the demonstration by Nelson (8) that the characteristics of properdin are consistent with its being a natural antibody to zymosan, all of the above reports indicate that zymosan is antigenic and that antibodies produced in response to its administration can cross-react with other biologic materials, presumably because they share common antigenic determinant groups with this polysaccharide.

It seems reasonable to suppose that a similar mechanism may be operative in the reported results with spontaneous mammary tumors. Antibodies to zymosan may cross-react with antigenic groups on the tumor cells, alter the integrity of the cells, and render them more susceptible to cyclophosphamide.

Various schedules of zymosan administration have been investigated in 35 separate experiments in which zymosan has been given in combination with cyclophosphamide as well as with other chemotherapeutic agents. Doses have ranged from 0.25 mg/kg to 150 mg/kg of body weight, the number of injections has been varied, and the administration, in relationship to the time of surgery and chemotherapy, has been altered. These data reveal a critical relationship between the dose of zymosan and the time of its administration. The experiments reported here represent the most effective regimen that was obtained. The relationships between the dosage schedule, the titer of antibody to zymosan, and anti-tumor effect are being investigated.

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REFERENCES


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