The Ineffectiveness of Zymosan in Altering the Heterotransplantation of Tumors to Rats

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The successful transplantation and serial propagation of heterologous tumors through successive generations of laboratory animals are sought for experimental purposes. Total-body irradiation of hosts, alone or in combination with cortisone injections, is now being used to alter the natural immunity of hosts to foreign tissues (1, 10). The discovery by Pillemer that a normal serum euglobulin, properdin, has nonspecific bactericidal, virucidal, and hemolytic activities (4, 6) has suggested that this globulin might play a role in host resistance to tumor transplantation.

Ross and his associates demonstrated that the properdin titer falls to low levels following total-body irradiation (8). Toolan has demonstrated that total-body irradiation reduces resistance to heterologous tumor transplantation (10). It seemed possible, therefore, that a reduction in properdin titer might aid heterologous tumor growth.

Zymosan, the insoluble residue from yeast cells that have been digested with trypsin and extracted with water and alcohol, has been demonstrated to cause marked reduction in serum properdin levels of laboratory animals (7).

It was the purpose of this study to evaluate the effect of zymosan administration on transplantation and serial propagation of heterologous tumors in rats.

MATERIALS AND METHODS

White, female Sprague-Dawley rats weighing 40–100 gm. were used as the experimental animals. Various mouse tumors were selected for the heterotransplantation because of differences in their growth characteristics. Sarcoma 37 (S-37) and Krebs ascites (K-2) grow in several strains of mice. These tumors were maintained in N.I.H. white mice in this laboratory. Ascitic fluid obtained from the latter was diluted 1:20 with normal saline, and an inoculum of 0.15 ml. was given intramuscularly into a hind leg of the rat to be studied. In two series, 0.3 ml. of the same dilution was given intraperitoneally. C3HBA and S-91 tumors are strain-specific in C3H and DBA strain mice, respectively. Suspensions of cells of these tumors which had been grown intramuscularly in the specific strains were made by passing solid tumor through a cytosieve (9). An inoculum of 0.15 ml. of a 1:10 normal saline dilution was given intramuscularly into the rat’s hind leg. In a single series, 0.3 ml. of the same dilution of S-91 melanoma was given intraperitoneally.

HeLa was derived originally from an epidermoid carcinoma of a patient with carcinoma of the cervix and has been maintained in tissue culture. One ml. of a cell suspension (in a concentration of 7–20 million cells/ml) was given intraperitoneally to each rat in the series.

Zymosan1 was prepared for injection by a method described by Pillemer et al. (5). Cortisone acetate was used for comparison as a conditioning agent. A preliminary median lethal dose study was made with zymosan, and with zymosan in combination with cortisone. Doses of zymosan of over 175 mg/kg in combination with cortisone were toxic, but doses of 150 mg/kg of zymosan were well tolerated.

Tumor-inoculated animals were divided into four separately treated groups with ten animals in each. One group received 150 mg/kg of zymosan intravenously 72 and 24 hours before and 48 hours after tumor transplantation. A second group received 150 mg/kg of zymosan 72 and 24 hours prior to tumor inoculation and, in addition, 3 mg. of cortisone acetate subcutaneously at the time of tumor inoculation and 48, 96, and 144 hours later. Animals comprising a third group received 6 mg. of cortisone acetate at the time of tumor inoculation and 48, 96, and 144 hours later. The fourth group received only subcutaneous penicillin, which

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1 Lot No. 5B171, Standard Brands, Incorporated; tested for activity by Pillemer.
was also given to animals in all groups in doses of 20,000 units on the day of tumor transplantation and 48 and 96 hours later.

Rats that received S-37 and K-2 ascites tumor were sacrificed 10–13 days following transplantation. Rats that received S-91, C3HBA, and HeLa tumors were sacrificed 21 days following transplantation. Tumor takes were distinguished by the presence of a progressively enlarging mass. Representative sections were fixed for histological examination. In addition, some of these tumors were passed back to the donor mouse strains in which they were maintained prior to hetero-transplantation.

Following sacrifice, solid tumors were excised, "cytosedwed," and transplanted to groups of animals prepared similarly with zymosan and cortisone. This tumor was diluted 1:5 with normal saline (0.9 per cent NaCl), and 0.15 ml. was injected intramuscularly. The number of animals used in successive passages depended on the amount of tumor available for transfer from the previous generation. Serial passage was carried out as long as the tumors continued to grow.

RESULTS

The results are summarized in Table 1. Animals given tumor intraperitoneally are grouped with those that received intramuscularly. The number of animals used in successive passages depended on the amount of tumor available for transfer from the previous generation. Serial passage was carried out as long as the tumors continued to grow.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>ZYMOBAN, 150 MG/KG</th>
<th>CORTISONE, 6 MG/KG</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Generation</td>
<td>Generation</td>
<td>Generation</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>S-37</td>
<td>25/39*</td>
<td>1/56</td>
<td>0/12</td>
</tr>
<tr>
<td>Per cent takes</td>
<td>72</td>
<td>2.8</td>
<td>0</td>
</tr>
<tr>
<td>K-2</td>
<td>4/45</td>
<td>0/28</td>
<td>-†</td>
</tr>
<tr>
<td>Per cent takes</td>
<td>9</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>S-91</td>
<td>2/45</td>
<td>2/56</td>
<td>0/6</td>
</tr>
<tr>
<td>Per cent takes</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CSHBA</td>
<td>0/11</td>
<td>-</td>
<td>0/11</td>
</tr>
<tr>
<td>Per cent takes</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HeLa</td>
<td>3/32</td>
<td>-</td>
<td>9/34</td>
</tr>
<tr>
<td>Per cent takes</td>
<td>15</td>
<td>-</td>
<td>37</td>
</tr>
</tbody>
</table>

* Number of tumor takes/number of transplantations.
† Indicates that the amount of tumor available from the previous generation was insufficient for transfer.

There was a significant difference, however, between rats treated only with zymosan and those treated only with cortisone. Only 9 per cent of those treated with zymosan alone grew tumors, while 45 per cent of the animals treated with cortisone alone had tumor takes. On attempted serial passage only a small percentage of K-2 tumors could be propagated; these were in groups treated with zymosan and cortisone in combination and cortisone alone. The more strain-specific S-91 tumor took poorly, but equally, in all groups. There was no growth with attempted serial passage. There was no growth in any group of rats into which C3HBA was transplanted. Tumor takes occurred only in the initial transplantation with HeLa tumor. They took significantly better.
which tumors did take and those in which tumors did not take, indicating that lack of weight gain was the result of treatment rather than of tumor growth. There were no deaths of rats that received cortisone, but those animals that received repeated doses of 6 mg. appeared ill during the week following cortisone administration.

Initial weights of rats in each of the groups were comparable. There was no correlation between weights at the time of tumor inoculations and incidence of tumor takes in this host weight range of 40–100 gm.

**DISCUSSION**

Herbut and Kraemer (2) and Palm (3) found that zymosan had a positive effect in conditioning rats for a single tumor heterotransplantation. Many tumors have survived heterologous transplantation for a single generation in hosts prepared by irradiation and cortisone. However, serial propagation of these tumors has failed in most instances. To our knowledge, there have been no reports of attempted serial propagation of heterologous tumors in animals prepared with zymosan.

This study demonstrates that the intravenous administration of zymosan is ineffective in decreasing resistance to heterologous tumor growth. Heterotransplantation of the nonstrain-specific S-37 ascites tumor resulted in initial growth in almost equal numbers in all prepared groups. S-37 was passed in four successive generations of rats prepared with cortisone and with cortisone and zymosan. However, this tumor could not be passed to control or zymosan-treated rats after the second generation. K-2 ascites tumor also is not strain-specific, but there were a smaller number of initial takes than occurred with S-37. The lack of positive effect of zymosan on passage of K-2, while evident with initial transplantation, became even more definite with the first serial passage.

It is interesting that there were few takes of S-91 melanoma in any of the groups, and there were no takes of C3HBA. This suggests that there may be an inverse correlation between strain specificity of a tumor and propensity for heterotransplantation.

The facts that zymosan alone is ineffective and a combination of zymosan and cortisone is no more effective than cortisone alone in enhancing heterotransplantation indicate that cortisone is the agent responsible for alteration of host resistance. As can be seen from Table 2, cortisone alone or in combination with zymosan affected the weight gain of rats adversely. The difference in the average per cent increase in weight of cortisone-treated rats and controls sacrificed at 21 days following treatment was not as marked as the difference in weights in these groups of rats sacrificed at 13 days. This is owing to the facts that cortisone administration was discontinued 6 days following tumor transplantation and that animals showed signs of recovery from the effects of cortisone administration within a week. Individual growth curves give evidence to this.

**SUMMARY**

The value of zymosan as a conditioning agent of Sprague-Dawley rats for reception and serial propagation of heterologous tumors was studied utilizing mouse tumors (Sarcoma 37, Krebs ascites, C3HBA, and S-91) and a human epidermoid tumor (HeLa) maintained in tissue culture.

Zymosan was found to be ineffective in enhancing heterologous tumor growth.

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**REFERENCES**


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