The Failure of Freund’s Adjuvant To Affect the Survival of Mice with Transplanted Sarcoma 180*

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SUMMARY

The treatment of CFW Swiss mice with complete Freund’s adjuvant before and after the transplantation of Sarcoma 180 intraperitoneally or subcutaneously did not affect the survival of the mice or rejection of the tumor transplants.

Attempts to prevent or augment the growth of transplants of tumors have been numerous (8). Many of these have employed the use of exogenous agents directly affecting the tumor cells. Others have attempted to stimulate host defenses against tumor growth, as for example by the induction of an inflammatory process. In recent years attention has been attracted to the role of the reticulo-endothelial system in the host defenses against transplanted tumors. Alterations in the cell population of the spleen and lymph nodes associated with spontaneous and transplanted tumors have been described. Efforts to increase host resistance to transplantable tumors of mice by stimulation of the reticulo-endothelial system have been reported (1, 2, 5, 6, 9).

Old, Clark, and Benacerraf (5) have indicated that certain strains of mice infected with Bacillus Calmette-Guérin will reject the solid form of Sarcoma 180, and that mice inoculated with Ehrlich ascites tumor, when infected by this organism, survive twice the expected length of time. These authors have suggested that the increased host resistance to the tumor is the result of proliferation and increased phagocytic activity of the reticulo-endothelial cells.

Working on a similar hypothesis, that the host defense against transplanted tumors might be directly correlated with a hyperactive and hyperplastic reticulo-endothelial system, the effect of complete Freund’s adjuvant containing Mycobacterium butyricum on the development of the ascitic and solid (subcutaneous) forms of Sarcoma 180 was studied. Administration of complete Freund’s adjuvant leads to the proliferation of reticulo-endothelial cells (4, 7) and frequently to an increase in antibody production.

MATERIALS AND METHODS

Young male and female CFW Swiss mice, which weighed 16–20 gm., were used in these experiments. The animals were divided into six groups: Freund’s adjuvant1 administered intraperitoneally before transplantation of ascites Sarcoma 180; Freund’s adjuvant administered intraperitoneally after transplantation of ascites Sarcoma 180; Freund’s adjuvant administered subcutaneously before transplantation of ascites Sarcoma 180; Freund’s adjuvant administered subcutaneously before transplantation of ascites Sarcoma 180; Freund’s adjuvant administered intraperitoneally after transplantation of Sarcoma 180 subcutaneously; and, Freund’s adjuvant administered subcutaneously before transplantation of Sarcoma 180 subcutaneously (Table 1).

Sarcoma 180 was always transplanted with the use of 0.25 cc. of undiluted ascitic fluid. Freund’s adjuvant was given in doses ranging from 0.01 to 0.5 ml.

RESULTS

The inoculation of adjuvant had no effect on prolonging the survival of the animals, or on their capacity to reject tumor implants (Table 1). However, the animals that received large doses of adjuvant (0.5 ml.) 1 day before or 1 day after transplantation of ascites Sarcoma 180 died more...
rapidly. Except for these instances no statistically significant variations in the survival of the experimental versus the control tumor animals were found either as a function of dose, time of injection, or route of administration of adjuvant.

When the adjuvant was given after the ascites form of Sarcoma 180, those mice with ascites had ruffled, wet fur. These changes commenced within 1 hour after injection and persisted for several days. A similar but less severe and more transitory type of response occurred in those animals which received only Freund’s adjuvant or adjuvant prior to the development of recognizable ascites. There was a large amount of solid tumor in the peritoneal cavity.

Whether the decrease in survival was due to a depression of the animals’ resistance to the growth of the tumor or the summation of two severe injuries, the tumor and the inflammatory reaction produced by the adjuvant, is not apparent. The adjuvant is an irritant, and the degree of the inflammatory response and the reaction of the reticulo-endothelial system parallel the size of the dose given by any one portal. This may be similar to other types of injury, which have been reported to enhance tumor growth (3, 10).

**TABLE 1**

**Effect of Administration of Freund’s Adjuvant on the Survival of CFW Mice with Transplanted S-180**

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Route of injection of adjuvant</th>
<th>Injection schedule of adjuvant</th>
<th>Conc. of adjuvant injected/mL/animal</th>
<th>Per cent of animals dead in days after S-180</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Transplantation of S-180 Intraperitoneally</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 controls (I.P.)</td>
<td>Intraperitoneal</td>
<td>1, 3, 7, 17, 30 and 40 days before S-180</td>
<td>0.05</td>
<td>9 13 17</td>
</tr>
<tr>
<td>(see note)</td>
<td></td>
<td></td>
<td>0.50</td>
<td>No statistically significant change over controls</td>
</tr>
<tr>
<td>310 (see note)</td>
<td>Intraperitoneal</td>
<td>1, 3, 5, 7, 9 days after S-180</td>
<td>0.25</td>
<td>“ “ “</td>
</tr>
<tr>
<td>80</td>
<td>Subcutaneous</td>
<td>1, 4, 8, 12 days before S-180</td>
<td>0.25</td>
<td>“ “ “</td>
</tr>
<tr>
<td><strong>B. Transplantation of S-180 Subcutaneously</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>126 controls (SQ)</td>
<td>Intraperitoneal</td>
<td>1, 4, 8, 11 days before S-180</td>
<td>0.25</td>
<td>22 35 70</td>
</tr>
<tr>
<td>87</td>
<td></td>
<td></td>
<td>“ “ “</td>
<td>No statistically significant change over controls</td>
</tr>
<tr>
<td>75</td>
<td>Intraperitoneal</td>
<td>1, 4, 8, 11, 14 days after S-180</td>
<td>0.25</td>
<td>“ “ “</td>
</tr>
<tr>
<td>93</td>
<td>Subcutaneous</td>
<td>1, 4, 8, 13 days before S-180</td>
<td>0.25</td>
<td>“ “ “</td>
</tr>
</tbody>
</table>

* Note: The animals receiving 0.5 ml. of adjuvant 1 day before or after S-180 all died in less than 10 days.

There were no deaths as a consequence of injection of Freund’s adjuvant intraperitoneally in 145 control animals or subcutaneously in 80 control animals.

**DISCUSSION**

Alteration of the reticulo-endothelial system with complete Freund’s adjuvant failed to prolong the survival of CFW Swiss mice given injections of ascites Sarcoma 180, or alter the course of the growth of Sarcoma 180 implanted subcutaneously. There was evidence that large doses of Freund’s adjuvant given intraperitoneally resulted in a more rapid death of the animals inoculated with the ascites form of Sarcoma 180. The amount of ascitic fluid in these animals was far less than that in the corresponding control animals at the same time. Gross examination of the animals showed a

On the basis of these experiments it would appear that the host defenses against Sarcoma 180 are not improved simply as a result of an increase in the reticulo-endothelial population. The increased resistance of mice toward transplanted S-180 after inoculation with Bacillus Calmette-Guérin shown by Old, Clark, and Benacerraf (5) would appear to be more complicated than a mechanism associated solely with the proliferation and phagocytic ability of the reticulo-endothelial system.

**REFERENCES**

2. BRADNER, W. T.; CLARKE, D. A.; and STOCK, C. C. Stimu-
Schoenberg and Moore—Freund’s Adjuvant and Tumor-bearing Mice

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