Serum Properdin in Tumor-bearing Mice. II. The Influence of Tumors of Different Origin*

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SUMMARY

The growth of three tumors, of different cell type and origin, was associated with low levels of serum properdin in mice. This relationship was not observed in mice bearing a myeloid leukemia. Shigella antibody titers also remained relatively unaltered in the latter animals. Depression in properdin occurred toward the end of survival, at a time when the tumor hosts had reduced their food consumption and were losing weight. Similar serological changes could be produced by food deprivation; this finding suggests that the relationship is probably nonspecific.

It is known that both circulating antibodies and cellular responses may be evoked by the presence of tumors in mice (17). On the other hand, a depression rather than increase in serum properdin, a so-called "primordial antibody," and of bacterial antibodies has recently been observed in mice with transplanted, isogenic mammary tumors (16). Since genetic deviation can occur in mouse tumors transplanted through many generations, even in strains of origin (11), the possibility exists that these results are related to the development of antigenic differences between the tumor and normal host tissues.

The authors, therefore, undertook to study the effect of tumors of different origin, genotype, and transplant generation on serum properdin under conditions similar to those previously described (16). The additional neoplasms studied included two induced in the host strain, a leukemia which arose some time ago in a mouse of the same strain but different subline, and a sarcomatous tumor previously carried over many years in unrelated, random-bred stock.

MATERIALS AND METHODS

Eight- to 10-week C57BL/6J male mice bred in the authors' laboratory were used. They were caged in groups of four to six and were allowed Purina Laboratory Chow and water ad libitum. Sequential bleedings of individual male tumor recipients were made at 7-day intervals. Approximately 0.4 cc. of blood was aspirated from the orbital venous plexus on each occasion, and the sera obtained were stored at -12° C. to -20° C. until all samples in a particular experiment had been collected. Properdin assays on separate samples from each mouse were then carried out, using a modification of Pillemer's technic as previously described (16). The range of serum properdin in normal mice by this procedure ranged from 15 to 30 units. As before, titers were interpreted as being significantly depressed only when they were persistently less than one-half of that present prior to tumor transplantation. A standard slide agglutination technic was used in determining anti-Shigella paradysenteriae B titers before and following the intraperitoneal injection of 10⁹ alcohol-killed organisms.

The C1498 myeloid leukemia and the induced tumors were minced following excision, and fragments were inoculated subcutaneously into the right thigh with a 15-gauge trocar. Control mice received fragments of kidney from normal isologous donors. In two experiments the myeloid leukemia was inoculated, instead, as a cell suspension in cold isotonic saline after being forced through a tissue press. Approximately 25 X 10⁴ nucleated cells in 0.03 cc. were injected subcutaneously into each recipient by this means.

In the case of Sarcoma 37, ascites tumor cells were mixed with an equal volume of cold isotonic saline, and 0.2 cc. of the final suspension, containing approximately 12 X 10⁶ cells, was injected intramuscularly. Aseptic precautions were observed in all instances. Sites of inoculation were measured in two dimensions with calipers to determine tumor growth, and mice were weighed 3 times weekly. All mice were autopsied and microscopic sections studied as indicated by the gross findings.

With the exception of Sarcoma 37, no spontaneous tumor regressions occurred.

DESCRIPTION OF TUMORS

Sarcoma 37 (S-37).—This tumor arose spontaneously as a mammary adenocarcinoma in a female mouse of the

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† Transplants derived from another individual of the same inbred strain and, presumably, of the same genotype.

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* One unit of properdin is that quantity which, in the presence of an optimal amount of zymosan, completely inhibits 120 units of C'3 in 1 ml. of properdin-free serum during 1 hour's incubation at 37° C.
Imperial Cancer Research Fund Stock in 1906. It later underwent transformation to an anaplastic tumor, which has been described by Stewart et al. (20). The tumor was obtained from Dr. I. Diller of the Institute for Cancer Research, Philadelphia, who had been carrying it for many years in the ascites form in random-bred ICR mice. Since October, 1961, it has been maintained in the ascites form in C57BL/6J female carriers in the authors' laboratory. After intramuscular injection the tumor invades regional lymph nodes and, on occasion, the peritoneal cavity by direct extension. Distant metastases are generally not seen. Growth of this neoplasm as a solid tumor in the C57BL male causes host death in 3-6 weeks. Spontaneous regressions occur in about 2 per cent.

Methylcholanthrene-induced (MC-I) tumor.—This fibro-

### TABLE 1

**Serum Properdin Levels in Mice with Different Tumors**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day Relative to Tumor Transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-6</td>
</tr>
<tr>
<td>S-A 37</td>
<td>24</td>
</tr>
<tr>
<td>MC-I tumor*</td>
<td>24</td>
</tr>
<tr>
<td>R-I† Lymphoma</td>
<td>30</td>
</tr>
<tr>
<td>C1498 leukemia</td>
<td>20</td>
</tr>
</tbody>
</table>

* Ninth transplant generation.
† Hematocrits in these mice were 25 per cent or less at times shown.
‡ Second transplant generation.
Italicized numbers indicate significantly depressed values.
Sarcoma was induced in male mice of the local subline by applying 6 per cent 3-methylcholanthrene in a 95:5 benzene-mineral oil suspension on the dorsum 3 times weekly from May through August, 1961, for a total of 30 applications. Mice receiving the first transplant generation survived 2-3 months. However, subsequent transplant generations, in both carriers and experimental male C57BL/6J mice, have grown more rapidly, killing the recipients as early as the 4th or 5th week. Mice carrying the first, fifth, eighth, and ninth transplant generations were assayed. Although metastases are limited to adjacent lymph nodes, this tumor also tends to infiltrate adjacent tissues such as skin and peritoneum. Microscopically, it consists of sheets of fusiform cells.

**Radiation-induced (R-I) lymphoma.**—This tumor originated in the thymus of a C57BL/6J male mouse 6 months following a series of four weekly whole-body exposures of 150 roentgens each administered in this laboratory. It was first transplanted in December, 1962, and thereafter maintained in female carriers of the same subline. Mice carrying the first through the fourth transplant generations were assayed in the present study. The lymphoma spreads widely, eventually involving the spleen, kidneys, lungs, bone marrow, as well as peripheral, retroperitoneal, and mediastinal lymph nodes. Death invariably occurs about 1½ months after transplantation.

**Myeloid leukemia (C1498).**—This is an undifferentiated leukemia which arose spontaneously in a C57BL/6J mouse in 1941. It was obtained from the Jackson Memorial Laboratory in May, 1962, and has since been carried in C57BL/6J females bred in the authors' laboratory through more than 55 transplant generations. The tumor shows evidence of local infiltration at the site of trochar inoculation. It quickly becomes widely disseminated, usually killing the host within 10-16 days after being transplanted in this manner. Death is postponed until the fourth week if a saline suspension of 25 × 10⁴ cells rather than tumor fragments are transplanted.

**RESULTS**

Significant depressions in serum properdin occurred in mice carrying the Sarcoma 37 and both induced tumors (R-I and MC-I). As previously reported in the case of a mammary adenocarcinoma (16), this occurred shortly before death. Results in typical experiments with each of the four tumors studied are shown in Table 1. The total incidence of low properdin titers in recipients of the S-37, MC-I, and R-I neoplasms was 27.3, 33.3 and 66.7 per cent, respectively (Chart 1). These changes could not be correlated with tumor growth rates in individual animals. In contrast, mice given inoculations of the C1498 myeloid leukemia rarely showed depressions (incidence = 6.1 per cent), and, with a single exception, 42 kidney-recipient controls likewise demonstrated no effect.

Inasmuch as mammary tumor hosts also develop low titers of Shigella antibody (16), the influence of the C1498 leukemia on immune Shigella antibodies was determined. Only two of ten mice bearing the leukemia developed depressions in these titers, as compared with twelve of fifteen mammary tumor-recipients, but, as in the properdin studies, only one or two blood samples could be obtained after leukemia inoculation before death supervened. Even when survival time and, hence, the number of samples were doubled by inoculating fewer tumor cells in suspension serum, properdin levels rarely fell in these leukemia recipients (one out of seventeen subjects).

Body weights of the tumor-bearers tended to parallel tumor growth, rising until several days before death, at which time both declined. This sequence of events as observed in a group of mice with the radiation-induced lymphoma is shown in Chart 2. It is apparent that properdin levels decline sharply about the time that body weight gain ceases. It is noteworthy that the recipients of the C1498 leukemia in which serum properdin was relatively little affected continued to gain weight until death.

Tumor-bearing mice were observed to gradually curtail their food intake during the course of tumor growth from the usual 3-4 gm. daily to as little as 0.5 gm. shortly before death. Additional studies were, therefore, performed to determine the effect of diminished solid food

![](chart1.png)

**CHART 1.**—Frequency of properdin depression in C57BL tumor-bearers. Each column designates the incidence of properdin depression observed in four to five experiments for each of the tumors shown. Results in mice with the mammary tumor shown for comparison are taken from Reference 16.

![](chart2.png)

**CHART 2.**—The tumor sizes and body weights shown are the mean values of ten mice that received the third transplant generation of the radiation-induced (R-I) lymphoma. Properdin levels in individual recipients are shown as separate points. None of ten normal controls (not shown) whose sera were assayed at similar intervals had equivalent depressions in serum properdin.
been found in carriers of three of the four tumors studied.

The variations in serum properdin observed in mice with different tumors in the present investigation are difficult to explain. Similar variations have been described in rodents with homologous and heterologous tumors (4, 8, 9). No correlation between tumor transplant generation and properdin levels is seen in the present data.

DISCUSSION

A significant decline in serum properdin has repeatedly been found in carriers of three of the four tumors studied. Previous work in this laboratory has shown a similar response in mice with mammary tumors (16). The fact that it first appears shortly before death suggests that it may be secondary to the deteriorated condition of the host. This hypothesis, invoking an indirect rather than direct mechanism, is supported by the lack of correlation between tumor growth rates and properdin levels in individual mice and the fact that a similar effect can be elicited by semi-starvation or other forms of stress such as hemorrhagic shock (3, 5), whole-body irradiation (12, 15), bacteremia (1), and dust inhalation (13). The possibility that anemia causes this effect has been tested by a duplicate study.

A less prominent side-effect of tumor growth was the production of anemia. However, this occurred with some frequency only following the transplantation of the MC-I tumor (Table 1).

Whereas the disseminated, radiation-induced lymphoma had a markedly deleterious influence upon this end-point, the also widely disseminated, transplantable myeloid leukemia had relatively little effect. The considerable involvement of the reticuloendothelial tissues in the R-I mice may explain their proclivity for properdin depression. The fact that C1498 leukemia recipients continued to gain weight and to appear outwardly healthy suggests that their usual state of nutrition and metabolism may have been relatively intact up to the time of death.

It is of interest that tumor-bearing animals have been reported to show increased rates of destruction of circulating antibody (21), inasmuch as properdin has been interpreted by some workers as being a mixture of naturally occurring antibodies. Various manifestations of impaired immune response have also been recorded in humans with cancer (6, 7, 14, 18, 19). The parallelism between anti-Shigella and properdin titers in the leukemia and MT recipients (16) speaks in favor of a common origin of classical antibody and properdin as suggested by Benacerraf and Sebestyen (2). It can be assumed that any impairment of immune response does contribute to the eventual demise of the tumor host, but further deductions regarding the role of properdin per se cannot be made at this time, owing to the paucity of information regarding its nature and role in immunity.

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