Serum Properdin in Tumor-bearing Mice. I. Comparison with Natural and Immune Antibodies*

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SUMMARY

Growth of a transplanted, isologous mammary carcinoma resulted in depressed titers of serum properdin and Shigella paradyenteriae antibody in mice. This tumor had no apparent deleterious influence on natural anti-sheep hemagglutinins. Tumor antigen preparations did not produce these effects. The likelihood that these alterations in immune response are secondary rather than direct effects is suggested by their constant association in time with the preterminal state and by the observation that properdin levels could be preserved by zymosan injections without influencing tumor growth.

In the decade since the existence of a naturally occurring, circulating euglobulin termed “properdin” was first announced (11) many questions have arisen concerning the true nature of this substance. Properdin was originally said to be multipotential, being bactericidal, hemolytic, and virus-neutralizing in the presence of complement and the cation magnesium. Pillemer believed that properdin differed from antibody but stated that properdin might be a primordial type of antibody. Other investigators (9, 10) have subsequently challenged this interpretation.

Nelson (9), for one, has collected evidence suggesting that the phenomena ascribed to the properdin system can be explained in terms of classical antibody in combination with complement. Lepow (8) has ventured the opinion that the properdin system may be a group of nonspecific factors which are conceptually analogous to complement and which participate in certain kinds of antigen-antibody reactions to produce inactivation of C’3 or cellular damage.

An interesting characteristic of properdin has been described in its relationship to the growth of transplantable tumors. A depression in serum levels of properdin has been observed in hosts of different tumors (2, 4–6); however, the results reported have varied.

An effort has been made in the present study to eliminate some variables by sequentially sampling the same mice. In consideration of the possible relationship between properdin and classical antibody, determinations of natural anti-sheep hemagglutinins and immune antibodies to Shigella organisms have also been performed. To further evaluate the interdependence of properdin and tumor growth, attempts have been made to influence one or the other by local x-radiation or zymosan administration.

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MATERIALS AND METHODS

C57BL/6J mice of both sexes, closely inbred in the authors’ laboratory, weighing 18–25 gm. and 9–11 weeks of age, were used in these studies. They were allowed Purina Laboratory Chow and water ad libitum and were caged in groups of four to six.

The isologous mammary adenocarcinoma BW10232 was transplanted subcutaneously to the right thigh of these mice. These tumors were minced immediately following excision, and fragments 2 mm. in diameter weighing approximately 20 mg. (wet weight) were introduced by means of a 15-gauge trocar. Controls were given inoculations subcutaneously, at the same time, of fragments of kidney from normal donors of the same subline and sex. Aseptic precautions were observed throughout. Sites of inoculation were measured with calipers to determine tumor growth, and recipients were weighed 3 times weekly. All mice were autopsied on death, and tissue sections were examined microscopically when deemed necessary.

This mammary tumor (MT) arose spontaneously in a C57BL/6J female. Since being obtained from the Jackson Memorial Laboratory, it has been carried in C57BL males of the authors’ subline through more than 50 transplant generations in over 1500 recipients without appreciable alteration in growth pattern or a single instance of spontaneous regression. The tumor characteristically remains confined to the subcutaneous tissues of the thigh without infiltrating muscle or skin. Grossly, it is white, soft in consistency, and moderately vascular. Pleural effusions and, occasionally, ascites may be present, but no enlarged lymph nodes or other metastases have been seen in the untreated animal. Microscopically, it consists of small cells in a mixed, undifferentiated, and glandular pattern with interspersed foci of ischemic necrosis. Numerous blood-filled sinusoids or thin-walled vessels are present, but little
or no hemorrhage. Severe anemia and death invariably occur 4–4½ weeks after transplantation.

**Serological procedures.**—All mice were serially bled from the ophthalmic venous plexus with sterile pipettes. Approximately 0.4-cc. samples were aspirated for the properdin and the Shigella assays from the same mice at weekly intervals, before and after tumor transplantation; 0.15 cc. of blood was aspirated twice weekly for the hemagglutinin assay. Sera obtained on centrifugation after clotting were immediately frozen and stored at −12° to −20°C. until assayed.

Properdin assays were performed by a modification of Pillemer's procedure (12). Each batch of RP and R3 was prepared from pooled normal sera obtained from at least ten human donors. These reagents were kept at −12° C. and used within 2 weeks of preparation. Anti-sheep hemolysin was obtained from Cappel Laboratories and from the Baltimore Biological Laboratory. Freshly prepared batches of sheep red cells in Alsever's solution received weekly were used. Zymosan, Fleischmann Standard Brands lot #98-551, Type A, was ground with a glass homogenizer and suspended in isotonic saline immediately before use in the assay. (When injected into experimental mice the zymosan suspensions, 1 mg/cc., were, in addition, placed in a boiling water bath for 30 minutes and used within 24 hours.)

One unit of properdin in human serum has been defined as the quantity which, in the presence of an optimal amount of zymosan, completely inhibits 120 units of C'3 in 1 ml. of RP during 1 hour's incubation at 37°C, thereby preventing hemolysis of sensitized sheep red cells (11). In most runs 10–20 per cent hemolysis could be used as the end-point. Occasionally, the poor hemolysis obtained with normal mouse sera indicated a deficiency of C'3 activity in the RP and necessitated using the first trace of hemolysis (5–10 per cent) as the end-point instead. To circumvent problems encountered in preparing standardized reagents, pooled normal mouse serum was always run simultaneously with the experimental samples as an additional control. Since, in the first step of the assay, the reaction of zymosan with mouse properdin may be inhibited by the presence of properdin-free mouse complement, the euglobulins in all samples were first precipitated and then reconstituted by properdin-free mouse complement.

Assay of induced and natural antibodies.—0.1-ML suspensions of 10⁶ alcohol-killed Shigella paradysenteriae B organisms were injected intraperitoneally 8 days before tumor transplantation. Induced anti-Shigella titers were determined by a standard slide agglutinin technic. A decrease in tube dilution to less than one-half of the maximum observed in response to the antigen was considered significant.

For the hemagglutinin assay, 0.05 ml of 1 per cent suspensions of washed sheep erythrocytes was added to serial dilutions of experimental mouse sera. Hemagglutination was observed after 2 hours' incubation at room temperature and compared with saponin standards.

**Irradiation procedure.**—The mice were immobilized on a rotating platform without anesthesia. Except for the lower extremity into which the tumor had been introduced 2 weeks previously, the bodies of these mice were carefully shielded from the x-ray beam with ½ inch thickness lead. X-ray exposures were administered with a G.E. Maxitron apparatus under the following physical conditions: 250 kvP, 30 ma., 72 cm. FSD, ½ mm. Cu, 1 mm. Al added filtration, HVL = 0.95 mm. Cu. Roentgen output, including backscatter, was 77 r/min. Dose rates were monitored with a calibrated Victoreen ionization chamber before and after each exposure. The radiation exposure of the shielded parts of the experimental subjects was found to be 1–2 per cent of the dose received by the unshielded extremity.

**TABLE 1**

<table>
<thead>
<tr>
<th>EXPERTMENT</th>
<th>DAY RELATIVE TO TUMOR TRANSPLANTATION</th>
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<tbody>
<tr>
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<td>-6</td>
</tr>
<tr>
<td>I</td>
<td>15</td>
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<td>26</td>
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<td>24</td>
<td>30</td>
</tr>
<tr>
<td>30</td>
<td>26</td>
</tr>
</tbody>
</table>

Italicized numerals indicate significant changes.

* Hematocrits were 25% or less at times shown in Experiment I. Hematocrits were not performed in Experiment II.
RESULTS

Tumor recipients first showed a decrease in properdin toward the end of the 2d week after transplantation (Table 1). In five replicate experiments, a total of 23 out of 52, or 44.3 per cent of such mice, demonstrated this effect, whereas no decline occurred in any of the 31 controls bled concomitantly at similar intervals. As shown in two typical experiments (Table 1), changes in titer became apparent as death approached. The over-all deterioration of the host at this time was reflected by weight loss and anemia. On occasions when tumor hosts survived longer than usual, properdin levels were not influenced until later. In a limited study on C57BL/6J females, six out of seven mammary tumor recipients likewise developed low properdin titers.

TABLE 2
SHIG. PARADYSENTERIAE B ANTIBODY TITERS IN C57BL MICE

<table>
<thead>
<tr>
<th>Group</th>
<th>Day relative to tumor transplantation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>-9</td>
</tr>
<tr>
<td>MT recipients*</td>
<td>80 160</td>
</tr>
<tr>
<td></td>
<td>80 160</td>
</tr>
<tr>
<td>Controls</td>
<td>160 960</td>
</tr>
</tbody>
</table>

*10⁶ alcohol-killed organisms were injected intraperitoneally into male mice 8 days before transplantation.

Titters are given as the reciprocals of the serum dilutions. In a duplicate experiment seven of eight MT recipients and only one of eight controls demonstrated a fall in titer.

TABLE 3
NATURAL ANTI-SHEEP HEMAGGLUTININS IN MAMMARY TUMOR-BEARING C57BL MICE

<table>
<thead>
<tr>
<th>No. mice assayed</th>
<th>Titters after transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measurable titers present before transplantation</td>
</tr>
<tr>
<td></td>
<td>No. increased</td>
</tr>
<tr>
<td></td>
<td>9 18</td>
</tr>
<tr>
<td></td>
<td>No titers originally present</td>
</tr>
<tr>
<td></td>
<td>10 16</td>
</tr>
<tr>
<td>Totals</td>
<td>19 34</td>
</tr>
</tbody>
</table>

Induced Shigella antibodies.—Response to the Shigella antigen was less pronounced and less sustained in tumor-bearers than in normal controls. Titers fell to less than half the maximum within 2–3 weeks after tumor transplantation in eleven of fourteen MT-recipient as compared with one of fifteen normal controls (Table 2). As in the case of serum properdin, the depression in titer once present persisted until death.

As an additional control Shigella antigen was injected into normal mice, and serum properdin levels were then determined at weekly intervals. No increase in properdin titers took place in these animals.

Hemagglutination studies.—Approximately half of the tumor-bearing mice prior to tumor inoculation had naturally occurring anti-sheep hemagglutinins. Although findings after tumor transplantation varied somewhat, certain general patterns could be discerned (Table 3). Zero baseline titers tended to remain as such. In the females there was either no significant change—i.e., less than one tube dilution difference—or a tendency to develop higher titers as the tumor grew progressively. In the males that were evaluated on this basis there was usually no change.

Exogenous influences on tumor growth and serum properdin.—

Local tumor irradiation: Mammary tumor growth was temporarily inhibited by a single exposure to 3,000 roentgens, thereby enabling tumor recipients to survive approximately (1½–2) times as long as usual. Serum properdin levels were depressed in nine of twelve mice so treated; this decline tended to appear later than in unirradiated animals (Chart 1). Weight loss and anemia were similarly delayed. The possibility that local irradiation or weekly bleedings over these extended periods may have contributed, to some extent, to the decline in titer was suggested by the finding that three of thirteen similarly irradiated and bled recipients of normal kidney similarly had
significant depressions. Five of the tumor-bearers had multiple pulmonary metastases at death; however, the presence of these metastases could not be correlated with changes in serum properdin.

Zymosan administration: In several experiments in which 10-40 mg/kg of zymosan was injected intravenously or intraperitoneally at different intervals ranging from 2 days before to 22 days after tumor transplantation, no significant influence on tumor growth or host survival was observed. In one such study in which properdin assays were performed, significant depressions in their levels were confined to only one out of ten mice, despite the absence of appreciable effects on tumor growth (Chart 2). In a duplicate experiment three of sixteen mice developed low titers, thus giving a total of only four of 26 or 15.4 per cent, as compared with the usual incidence of approximately 44 per cent. Results in female MT recipients were similar. The properdin levels are given in units/ml. Each mouse is represented by a different symbol. Zymosan (10 mg/kg) was injected intraperitoneally on days shown.

DISCUSSION

These results clearly indicate that serum levels of both properdin and Shigella antibody are adversely influenced by the growth of a mammary adenocarcinoma in C57BL mice.

Zymosan was administered to MT-carrying mice in an attempt to elucidate the association between these events. This insoluble carbohydrate complex derived from yeast cell walls is purported to influence both tumor growth (3, 5, 14) and serum properdin levels in rodents (13). It is noteworthy that the transplanted tumors which are reported to be affected by zymosan have not generally been isogenic. In the present study, although properdin levels were maintained by injections of zymosan mammary tumor growth and host survival were uninfluenced (Chart 2). It would appear, therefore, that properdin, per se, has no specific or restraining influence on the mammary carcinoma. The timing of the observed impairment of immune response suggests that it is more probably a secondary manifestation of the poor condition of the host animal preceding death.

Natural antihemagglutinins have been found with some regularity in C57BL mice, although not in other strains (18). Different sublines of C57BL mice apparently differ in this respect. Immune hemagglutinins are said to be more frequently depressed during tumor growth than are natural hemagglutinins (16, 19). A decline in anti-human hemagglutinins has likewise been noted in tumor-bearing rats (1).

The possibility that serum properdin might be affected by a tumor-specific antigen has been investigated in this laboratory by injecting a large number of mice of the same subline with lyophilized mammary tumor or with alternately frozen and thawed tumor homogenates. Such preparations have been reported to retain antigenicity and to be capable of evoking a homologous response (17). No recipient of these preparations has shown a significant alteration in serum properdin. The frequency of anemia in MT-carrying mice toward the end of their survival and the accompanying marked expansion of plasma volume is another possible explanation for the results obtained. However, repeated bleedings and the production of severe anemia in otherwise normal mice have never succeeded in reproducing the effect on properdin in this laboratory.

The above results are consistent with the conclusion that the impairment of immune response as manifested by depressions in serum properdin and antibody titers to Shigella are indirectly rather than directly related to the growth of mammary tumor. The extent to which these findings apply to tumors of different origin, genotype, and histology is being investigated (15).

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