In Vivo and in Vitro Studies of the Action of Guinea Pig Serum against the Ascites Form of the Murphy-Sturm Lymphosarcoma*

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It has been shown that guinea pig serum has an inhibitory effect on several transplantable tumors (3, 4, 6). The degree of inhibition against different tumors varies and is most pronounced against the lymphomas and lymphosarcomas. However, even against these groups of tumors this effect differs from time to time. It requires the interaction of the host (5) and is not operative in vitro (7).

In a previous report from this laboratory (4) it was shown that the inhibitory effect of guinea pig serum was dependent in part on the length of the latent period, being more effective when the latent period was longer than 5 days. It was also demonstrated that the ascites form of this tumor can be inhibited, in spite of the fact that this form is lethal in one half to one third of the time of the solid tumor.

The present paper reports studies of the action of guinea pig serum on the host and on the tumor. In addition, some of the properties of guinea pig serum were studied and some possible mechanisms of action explored.

MATERIALS AND METHODS

Tumor and animals.—The ascites form of the Murphy-Sturm lymphosarcoma obtained from the City of Hope1 was carried in Wistar rats.2 This form of the tumor had fewer regressions. The same procedures of transplanting and treatment were employed as previously described (4). The guinea pig serum was obtained from a single strain of animals,3 which assured a consistency in the serum. Sera from animals in different age groups were compared. For the remainder of the experiments the adult guinea pigs were bled, a maximum of 3 times, allowing at least 3 weeks between bleedings.

Antigenic relationships.—Because of the possibility of common antigenic groups between the guinea pig serum and the tumor, rabbits and rats were immunized with guinea pig serum, and the antiserum was injected into animals implanted with the tumor.

Time studies.—Time studies were carried out to determine whether the guinea pig serum could prepare the host to reject the tumor or protect the host after the tumor had been established.

Incubation of tumor cells in guinea pig serum.—Tumor cells were incubated in the same volume of guinea pig serum as that injected immediately after implantation (20 million cells/3 ml. serum). The cells were then centrifuged and resuspended in the proper volume of buffered glucose Ringer’s solution (BGR) (10) and implanted into the rats.

After the above incubation, smears were made from an aliquot of the cells and fixed immediately in a solution of 95 per cent ethanol and ether (1:1). The slides were then stained and examined for any pathological changes.4

During the incubation it was observed that the cells were clumped. Agglutination tests were carried out to determine whether the guinea pig serum was agglutinating the tumor specifically and whether this agglutinating capacity could be absorbed from the serum. In addition, tests were carried out to determine whether any cor-

1 Capital S. Caviary, Buena Park, Calif.
2 The examinations were carried out by Dr. Fremont Davis, pathologist, Hollywood Presbyterian Hospital, Hollywood, Calif.
relation existed between the agglutinating capacity and the inhibitory activity. For the agglutination tests 7-day-old ascites tumors were used. The cell suspensions of the tumor were allowed to stand for 5 minutes to permit the clumps of cells to settle. The upper layer of the cells was drawn off, and the cells were diluted to a final concentration of $10^5$ cells/ml in modified BGR (calcium omitted). Rat and sheep erythrocytes were diluted to 0.2 per cent suspension with the modified BGR.

The absorbed sera were 150-ml aliquots of a common pool of frozen and thawed guinea pig serum which were incubated with $1.2 \times 10^{10}$ tumor cells, or $1.4 \times 10^8$ rat or sheep erythrocytes at $37^\circ$ C. for 1 hour. The sera were then refrozen, thawed 7 days later, and the agglutination and protection experiments were carried out.

**Chemical analysis.**—The sera from the different groups of guinea pigs and from rats and rabbits were analyzed for their albumin and globulin protein concentrations, the glycoproteins, and the seromucoid fraction. The total serum proteins were determined by Weichselbaum’s procedure (13). The globulin proteins were determined by the Weimer and Moshin modification (14) of the Pillemer and Hutchinson procedure (9). The difference between these proteins was considered the albumin. The glycoproteins and seromucoid fractions were determined by employing Weimer and Moshin’s modification of Winzler’s procedure (15).

**Zymosan treatment.**—Some preliminary experiments were carried out in which the host or the guinea pig serum was treated with zymosan. Zymosan was prepared by Pillemer’s procedure (8) and injected intraperitoneally into the rats immediately after implantation. The same concentration of zymosan, 20 mg/kg, was used that Bradner and co-workers (1) found effective in inhibiting the Sarcoma 180 in mice.

Guinea pig serum was treated with zymosan at $37^\circ$ C. according to the method of Wardlaw and Pillemer (11). The serum was then refrozen and thawed at the time of injection into the tumor-implanted animal.

**Complement determinations.**—Complement titrations were carried out on several sera by Wedgewood and Janeway’s procedure (12).

**RESULTS**

The anti-guinea pig serum produced in either the rat or rabbit had no effect in inhibiting the tumor. The time studies (Chart 1) also indicate that the guinea pig serum could not prepare the host to increase its resistance to the tumor but apparently must be present at the same time as the tumor to be effective. The guinea pig serum was inhibitory against this ascites form even after the tumor had started to grow.

The inhibitory activity of guinea pig serum increased greatly during a short period after birth. There was a tendency for the serum from very old guinea pigs to be more effective. A check on the effect of the number of bleedings revealed no differences in the inhibitory property of the guinea pig serum (Chart 2).

Incubation of the tumor cells in guinea pig serum prior to implantation had no effect on the course of the tumor. A microscopic examination of these cells revealed that there were no pathological changes. Agglutination tests (Table 1) revealed agglutinins to both the tumor cells and the rat erythrocytes. These agglutinins were distinct from one another, as evidenced by the fact that the agglutinins could be absorbed by the homologous cell type, but were unaffected by absorption with other cell types. Guinea pig serum also contained a hemolysin against both rat and sheep erythrocytes. This hemolytic activity could be removed by absorption with tumor cells and partially by absorption with sheep erythrocytes. The serum from newborn guinea pigs also contained these agglutinins; the hemolysins, however, were absent.

The chemical analyses (Table 2) demonstrate that the major differences between the proteins of the different groups of guinea pigs lay in the globulin fraction. The globulin polysaccharides and seromucoid fractions were markedly lower.
in the ineffective serum of the newborn animals. The seromucoid fraction was the only one that was consistently lower in all the groups with the ineffective sera: newborn guinea pig serum, rabbit, and rat.

Treatment of the host with zymosan had no effect on the course of the tumor; however, treatment of the guinea pig serum decreased the inhibitory activity of the serum (Chart 3).

The complement titers of the untreated serum from adult guinea pigs were in the vicinity of 200 units. The serum from newborn guinea pigs had a titer of about 100 units, while the adult serum that had been treated with zymosan had a titer of only 45 units.

Absorption of the guinea pig serum with either tumor cells, rat or sheep erythrocytes tended to increase the inhibiting activity of the serum (Chart 4).

**DISCUSSION**

Pretreatment of the host with guinea pig serum had no effect on the course of the tumor. The fact that treatment which was started 3 days after implantation of the tumor was effective in inhibiting the growth suggested that the guinea pig serum was acting directly on the tumor cells.

**TABLE 1**

<table>
<thead>
<tr>
<th>TREATMENT OF GUINEA PIG SERUM</th>
<th>UNABSORBED</th>
<th>Absorbed (sheep erythrocytes)</th>
<th>Absorbed (rat erythrocytes)</th>
<th>Absorbed (tumor cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum from adult:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unabsorbed</td>
<td>4+</td>
<td>1+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorbed</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Absorbed (sheep erythrocytes)</td>
<td>5+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorbed (rat erythrocytes)</td>
<td>4+</td>
<td>1+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorbed (tumor cells)</td>
<td>1+</td>
<td></td>
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</tbody>
</table>

**TABLE 2**

| PROTEINS, GLYCOPROTEINS, AND SEROMUCOID FRACTIONS OF GUINEA PIG, RABBIT, AND RAT SERA |
|-------------------------------------------|-------------------------------|-----------------------------|---------------------------|
| **Animals**                              | **Animals in pool** (g.m. per cent) | **Total** (g.m. per cent) | **Globulin** (g.m. per cent) | **Albumin** (g.m. per cent) | **Total glycopolysaccharides (mg. per cent)** | **Globulin polysaccharides (mg. per cent)** | **Albumin polysaccharides (mg. per cent)** | **A/G ratio** |
| Guinea pig                                |                               |                            |                            |                            |                                              |                                              |                                              |              |
| Newborn                                    | 80                           |                             | 1.5                        | 2.7                        | 92                             | 56                             | 36                             | 0.64         |
| Rat                                         | 55                           |                             | 3.1                        | 3.1                        | 155                            | 124                            | 29                             | 0.25         |
since the cells were bathed in the guinea pig serum. This might offer an explanation for the previous report from this laboratory that the guinea pig serum was less effective against the solid form of this tumor when the latent period was less than 5 days (4). Ample time must elapse for the guinea pig serum to accumulate at the site of the tumor cells. It might also be noted that, in the final experiment (Chart 4), the tumor had become more virulent and started to kill the host at an earlier time. The normal guinea pig serum was less effective in inhibiting this tumor, even though the cells were bathed in the serum in the peritoneal cavity.

The ineffective serum from the newborn offered a means of comparing the sera in an attempt to determine what differences might be present and related to the differences in inhibitory activity. The elevated level of the seromucoid fraction in guinea pig serum, compared with other species, coupled with the lower level of this fraction in newborn guinea pig serum, suggested its possible implication in the activity. It also suggested the possibility of the properdin system's being involved. The preliminary experiments, in which rats were injected with zymosan, did not affect the course of the tumor, but, since only one dose of zymosan was administered, the results were inconclusive. However, incubation of the guinea pig serum with zymosan reduced the inhibitory action. The fact that the treated serum is still more inhibitory than serum from the newborn, although the complement titer is about half that of serum from the newborn, suggests that the C' component of hemolytic complement is not involved.

The in vitro tests demonstrated that the action of guinea pig serum on the tumor was not effective by itself and required the host to mediate its inhibitory action, as Kidd reported with a mouse lymphoma (7). Even though the guinea pig serum had no effect in vitro, under the conditions of the experiment, the presence of the agglutinins suggested that the guinea pig serum may be altering the cells in vivo in some manner, so that the natural or enhanced defenses of the host can act on the tumor.

The results of the absorption and agglutination experiments demonstrated that there were distinct agglutinins to the rat erythrocytes and tumor cells. It is interesting to note that guinea pig serum was ineffective in agglutinating the tumor cells when these cells were washed 3 times with BGR or purified by differential lysis (2) prior to incubation with the serum. Either the active sites are washed off, or a fraction in the ascitic fluid enhanced the agglutination.
The fact that guinea pig serum which had been absorbed with any of the three cell types tended to be more effective than the unabsorbed serum suggested that there might be a competition between an inhibiting and a noninhibiting fraction in guinea pig serum for the same site or sites on the tumor cells.

**SUMMARY**

Guinea pig serum has been found to be effective in inhibiting the ascites form of the Murphy-Sturm lymphosarcoma in Wistar rats. The serum from the newborn animals was relatively ineffective. Chemical analysis revealed that the seromucoid fraction, which is elevated in guinea pigs compared with other species, was low in the serum of the newborn animals. Preliminary experiments suggest that the properdin system may be involved in part. The C3 fraction of hemolytic complement does not appear to be involved in the activity. The serum must be present at the same time as the tumor cells in order to be effective. The serum is effective in inhibiting the tumor after growth has started, but even in the ascites form the guinea pig serum is not so effective if the tumor grows too rapidly. Sera both from adult and newborn animals have been found to contain agglutinins against erythrocytes, but only the serum from adults contained hemolysins. Sera from both adult and newborn animals contained agglutinins for the tumor cells. Absorption experiments indicate that the agglutinins to the erythrocytes and tumor cells are distinct from one another.

**REFERENCES**

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