PRODUCTION OF AN ANTIRONIDASE HORSE SERUM
AND ITS ACTION ON METASTASIZATION OF
A BROWN-PEARCE CARCINOMA IN RABBITS

I. N. Maiskii, N. A. Kozlova and M. N. Nilovskii

From the Laboratory of Noninfectious Immunology (Head - Prof. I. N. Maiskii)
Institute of Experimental Biology (Director - Prof. I. N. Maiskii)
AMN SSSR, Moscow
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50
No. 11, pp 86-90, November, 1960
Original article submitted May 20, 1960

In previous work we showed depression of metastasization of a Brown-Pearce carcinoma in rabbits, after removal of the primary tumors, under the influence of an antironidase goat serum. Since the subject of the investigation is of practical as well as theoretical interest, an extension of the work in this direction is extremely important.

The object of the present investigation was to ascertain whether an antironidase serum, obtained by immunization of horses, possesses an inhibiting action on metastasization of a Brown-Pearce carcinoma.

METHOD

As donors of antironidase sera we used two horses (mare No. 11 and stallion No. 13). Immunization* of the animals was done in two cycles. The first cycle consisted of seven intramuscular injections of ronidase in doses of 1.5 to 8 g per injection. As a result of the first cycle of immunization, however, we were unable to induce a sufficiently high level of antibody production. The highest titer of antironidase antibodies in the McClean-Smirnova reaction was 1:64.

The subsequent immunization of the horses was temporarily postponed on account of the development of areas of infiltration at the sites of injection of the enzyme into the animals.

Six months later the horses were reimmunized by means of four intramuscular injections of 6, 15, 20 and 22.5 g ronidase, with intervals of 5-8 days between injections. During both immunization and reimmunization observations were made on the trend of antibody formation. At the moment of appearance of antibodies in a sufficiently high titer (on the ninth day after the last injection of ronidase) bleeding was carried out and the sera prepared.

The sera obtained were used for experiments after 2.5 months. The serum of horse No. 13, tested at this period by the McClean reaction, suppressed the activity of the homologous enzyme ronidase in a dilution of 1:4096, testicular enzyme of the rabbit and guinea pig in a dilution of 1:256, and testicular enzyme of the rat in a dilution of 1:64. The serum of horse No. 11 depressed the activity of these enzymes in the following dilutions respectively: ronidase 1:1024, testicular enzyme of the rabbit and rat 1:128 and guinea pig enzyme 1:256.

*Immunization was carried out at the Gamaleya Institute under the supervision of A. V. Ushakova, to whom we express our gratitude.
Degree of Metastasization of a Brown-Pearce Carcinoma in Rabbits after Removal of the Tumor and Injection of Horse Sera (Sacrificed on the 21st Day after Inoculation of the Tumor)

<table>
<thead>
<tr>
<th>Group of rabbits</th>
<th>Tumor</th>
<th>Serum</th>
<th>No. of rabbits</th>
<th>Mean number of affected organs per rabbit</th>
<th>Mean number of metastases* per affected organ</th>
<th>Weight of omentum (in g)</th>
<th>Weight of spermatic cord (in g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Removed</td>
<td>Antironidase</td>
<td>4</td>
<td>3.2</td>
<td>8.3</td>
<td>15.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Second</td>
<td>&quot;</td>
<td>Normal</td>
<td>4</td>
<td>5.7</td>
<td>20.1</td>
<td>25.7</td>
<td>6.0</td>
</tr>
<tr>
<td>Third</td>
<td>&quot;</td>
<td>No serum</td>
<td>4</td>
<td>3.0</td>
<td>25.1</td>
<td>37.0</td>
<td>10.1</td>
</tr>
<tr>
<td>Fourth</td>
<td>Not removed</td>
<td>The same</td>
<td>4</td>
<td>4.7</td>
<td>44.6</td>
<td>24.2</td>
<td></td>
</tr>
</tbody>
</table>

*In counting the number of metastases per affected organ, the metastases in the spermatic cord and omentum were disregarded.

Both sera were used in the experiments, which were conducted as follows: 39 male chinchilla rabbits, weighing 2.5-3.2 kg, were given an injection of 1 ml of a 25% suspension of a Brown-Pearce tumor into the left testicle. All the animals were then subsequently divided into three groups of equal weight. On the sixth day of development of the tumor, the rabbits of the first group received an intravenous injection of 6 ml of antironidase horse serum. The rabbits of the second group received an injection of the same volume of normal horse serum. The animals of the third group received no serum whatever.

On the seventh day the left testicle of 35 rabbits was removed and examined for the presence of a tumor. In four rabbits of the third group (Nos. 5, 30, 65 and 36) the testicle was not removed, and these animals then formed the fourth group.

On the next day, the eighth after transplantation of the tumor, 5 ml of the corresponding serum was injected intratesticularly into the rabbits of the first and second groups. Subsequently, at intervals of 2 days, each rabbit received a further four injections, each of 6 ml, alternately into the right and left posterior muscles of the thigh. Thus, altogether 6 injections were given, 2 intravenously and 4 intramuscularly.

On the 21st day after inoculation of the tumor, 16 rabbits, i.e., 4 from each group, were sacrificed. The remaining rabbits were sacrificed on the 36th day.

The effectiveness of the action of the sera on metastasization was estimated by the degree to which the organs were affected by metastases. As an index of the latter we used the number of organs affected by metastases, the average number of metastases in one affected organ and the weight of the omentum and the spermatic cord. The technique of counting the metastases in the organs was described in detail in the previous paper [1].

The results obtained at sacrifice of the animals on the 36th day were treated statistically by the Fisher-Student method. The differences were considered significant if the value of P was not greater than 0.05.

**RESULTS**

As the figures in Table 1 show, on the 21st day after transplantation of the tumor, in the rabbits from which the primary focus had been removed and which had received injections of normal horse serum (second group) and also in the rabbits which had received no treatment whatever (fourth group) the average number of affected organs was 5.7 and 4.7 respectively.

In the rabbits receiving antironidase serum (first group) the value of this index at this time was lower (3.2), although it did not differ from that in the group of animals not receiving serum after removal of the tumor (third group), for which it was (3.0).

Comparison of the mean indices of the number of metastases per affected organ shows that the density of metastasis formation in the organs of the experimental group (first group) was the lowest (8.3) in relation to all