The Localization of Antilymphosarcoma Antibodies in the Murphy Lymphosarcoma of the Rat*

LEONHARD KORNGOLD AND DAVID PRESSMAN

(Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

INTRODUCTION

In a previous report, we have shown that antisera prepared against a mouse tumor (the Wagner osteogenic sarcoma) contained antibodies capable of localizing in vivo in the mouse tumor, while antisera prepared against normal mouse organs did not. We had already shown that antisera prepared against normal rat (2, 4, 7, 9, 10) and mouse tissues (5) contained antibodies capable of localizing in these tissues in vivo.

The present work shows that antisera prepared against the Murphy lymphosarcoma of rats contained antibodies which localized in this tumor, and that such antibodies were absent in other antitissue sera. The presence of localizing antibodies was demonstrated as follows: The globulin fractions of control sera and antisera were iodinated with iodine containing radio-iodine (I131), and the antibodies were purified by treating these radio-iodinated globulin fractions with lymphosarcoma sediment and subsequently eluting the adsorbed antibody (8). These purified antibodies were then injected into rats bearing the tumor, and the tumor and other tissues were assayed 24 hours later for their content of radioactivity. Increased localization of radioactivity in tumor from anti-tumor sera showed the presence of localizing antibodies.

MATERIALS AND METHODS

Tumor. — The Murphy lymphosarcoma (8) was grown in Sherman albino rats for these experiments. The original implant was kindly supplied by Dr. Sugiura of this Institute, who has been carrying this tumor in Sherman rats. It originated in Wistar rats.

Antigens. —

a) Lymphosarcoma sediment.—Batches of 10 gm.
of lymphosarcoma tissue were immersed in 40 ml. iced saline and homogenized for 3 minutes in a homogenizer of 50-ml. capacity driven by a motor at 16,000 r.p.m. The homogenizer had a stainless steel shaft and four blades, 0.7 cm. long. The volume was brought to 60 ml. with cold saline, and the saline-insoluble fraction was centrifuged down in a Servall SP angle centrifuge at 3,000 × g, for 10 minutes in the cold room. After the supernatant was decanted, the sediment was resuspended in 60 ml. cold saline, and recentrifuged. This procedure was repeated until the supernate was optically clear (usually seven to eight washings). The sediment thus obtained was white. Both the sediment and the cellular fraction were lyophilized and stored at 5° C. for future use. The material which was used for immunization was used up within 4 weeks, while the lymphosarcoma sediment which was used for the in vitro purification was stored for as long as 1 year without losing its ability to purify localizing antibody.

The procedures were not carried out under sterile conditions.

b) Lymphosarcoma cellular fraction.—Lymphosarcoma tissue was treated as under (a) with the exception that after homogenization by the blender the connective tissue was removed by passing the homogenate through two layers of surgical gauze. The material passing through was subsequently washed with saline as under (a) and designated as the cellular fraction. Both the sediment and the cellular fraction were lyophilized and stored for subsequent use.

c) Lymph node sediment.—A mixture of thoracic, mediastinal, and periaortic intestinal lymph nodes was homogenized in a blender and washed with saline. Sediments were prepared when needed.

Sera.—Sera are designated by the rabbit number. Normal sera were obtained from rabbits before injection.

Antilymphosarcoma sera.—Rabbits were injected intraperitoneally with either the lymphosarcoma sediment or the lymphosarcoma cellular
fraction. The dosage was 50-100 mg. in 5 ml. saline administered every other day, 3 times a week. One week after the tenth injection the animals were bled. Serum anti-RLS 645 was produced by injecting the whole sediment. Sera anti-RLS 858, 859, and 860 were prepared by injecting the cellular fraction. Serum anti-RLS 813D was obtained by injecting the cellular fraction for several courses and represents the fourth bleeding of rabbit 818.

Antilymph node sera.—Rabbit 641 was injected with lymph node sediment. After six injections of 75 mg. each the rabbit was bled, and 3 weeks later it received three more injections of 500 mg. each. Sediment in 1 ml. of borate buffer (pH 8). The mixture was shaken mechanically for 1 hour at 37° C. and centrifuged. The sediment was washed twice with 10 ml. borate buffer, before being resuspended in 7 ml. borate buffer and heated in a water bath at 60° C. for 15 minutes. The mixture was centrifuged, and the supernate, containing eluted antibody, was assayed for localizing activity. All the purifications described in this report were carried out with the lymphosarcoma sediment.

TABLE 1

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Serum used</th>
<th>No. rats</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
<th>Spleen</th>
<th>Tumor</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anti-RLS 645</td>
<td>4</td>
<td>1.9</td>
<td>0.8</td>
<td>1.0</td>
<td>2.4</td>
<td>3.1±0.5</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td>NS 469</td>
<td>4</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>1.8±0.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Anti-RLS 645</td>
<td>5</td>
<td>1.8</td>
<td>0.7</td>
<td>3.0</td>
<td>3.2±1.1</td>
<td>1.4±0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>NS 593</td>
<td>4</td>
<td>1.9</td>
<td>0.7</td>
<td>3.0</td>
<td>3.2±1.1</td>
<td>1.4±0.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Anti-RLS 858</td>
<td>5</td>
<td>1.4</td>
<td>2.1</td>
<td>2.4</td>
<td>2.8±0.7</td>
<td>2.2±0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-RLS 859</td>
<td>5</td>
<td>1.4</td>
<td>2.4</td>
<td>2.4</td>
<td>2.8±0.7</td>
<td>2.2±0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-RLS 860</td>
<td>5</td>
<td>1.4</td>
<td>2.4</td>
<td>2.4</td>
<td>2.8±0.7</td>
<td>2.2±0.6</td>
<td></td>
</tr>
</tbody>
</table>

* The antibodies were purified with lymphosarcoma sediment as described under "Materials and Methods."
† Values are the per cent of injected radioactivity present per gram of wet perfused tissue on the basis of a 100-gm. rat. Values are averages of duplicate analyses per tissue for the number of rats indicated. The average deviation from the mean was 10 per cent or less for the normal tissues. For the tumor the standard deviation is also listed.
‡ The probability levels for the difference between antitumor serum and control serum preparation as determined by "t" test (for tumor data).

The data for the localization in the tumor were analyzed statistically by the "t" test and the P values so obtained for the differences between antitumor serum and control serum preparations are listed. Table 1 shows that preparations from anti-RLS 645 localized to an appreciably greater extent than those from the other sera. The results are presented as the percentage of the injected radioactivity which localized to the basis of a 100 gm. rat (9). The experiments which were performed at the same time are grouped together for comparison.

RESULTS

The results are presented as the percentage of the injected radioactivity which localized per gram of wet tissue normalized to the basis of a 100 gm. rat (9). The experiments which were performed at the same time are grouped together for comparison.

One week after the last injection the rabbit was bled for serum anti-RLN 641A.

Other antisera.—The antikidney, antiliver, antilung, and antispleen sera were those described in previous publications (9). These sera had been prepared against tissues from Sherman rats, except in the case of the antikidney serum for which Wistar rats were used.

Radioactive preparations.—The globulin fractions of the sera used were obtained by ammonium sulfate fractionation (1). The radio-iodination procedure has been described previously (6).

In vitro purification method.—Two ml. (8 mg.) of the radio-iodinated globulin fraction of the antiserum or normal serum was added to 8 mg. of a lyophilized preparation of the lymphosarcoma sediment. The mixture was shaken mechanically for 1 hour at 37° C. and centrifuged. The sediment was washed twice with 10 ml. borate buffer, before being resuspended in 7 ml. borate buffer and heated in a water bath at 60° C. for 15 minutes. The mixture was centrifuged, and the supernate, containing eluted antibody, was assayed for localizing activity. All the purifications described in this report were carried out with the lymphosarcoma sediment.

Assay of the localizing activity.—Sherman rats were implanted in the axillary region with the lymphosarcoma. For each experiment, all rats were given implants at the same time of a single donor tumor. Seven days after implantation, 1 ml. (51,000–180,000 c.p.m.) of the radioactive eluate was injected into the tail vein of the tumor-bearing rats. The animals were anesthetized 1 day later, perfused with saline, and the concentration of the radioactivity in the tissues determined as described previously (8).
extent in the tumor than did those from the two normal sera (NS 469 and 598). That this increased localization of anti-RLS 445 was not limited to this particular antilymphosarcoma serum is indicated by the similar results obtained with three other antilymphosarcoma serum preparations—anti-RLS 858, 859, and 860 (see also anti-RLS 813D in Table 2).

From Table 2 it can be seen that the preparations from all other antitissue sera studied localized to a significantly lower degree in the tumor than did the antitumor preparation, although they did localize to the same degree or better in liver, spleen, and kidney. It is of special interest that neither the antisympathetic nor the antilymph node serum contained antibodies capable of localizing in the lymphosarcoma, since these antisera were prepared against cells which are most closely related to those in the lymphosarcoma.

In Table 3 are tabulated the averaged results obtained with 112 tumors in the course of several experiments. It can be seen from these data that the antilymphosarcoma serum preparations localized in tumors to a significantly higher extent than those for either normal serum or other antitissue preparations. There was no apparent difference between normal serum and control antitissue serum preparations with respect to tumor-localizing activity.

**DISCUSSION**

The data presented in this paper show that antilymphosarcoma sera contain antibodies capable of localizing in the lymphosarcoma. No such antibodies were found in the other antitissue sera tested. The fact that the tumor-localizing antibodies were present only in the antilymphosarcoma sera indicates that the lymphosarcoma contains an antigen which is unique for lymphosarcoma. However, there are other possible explanations for this phenomenon, for example:

a) The lymphosarcoma contains an antigen which is characteristic of the strain in which the tumor originated (Wistar rat) and absent in the Sherman rats.

b) The tumor antigen—which is responsible for the localization of antilymphosarcoma serum preparations—is present in other tissues also, but the particular rabbits immunized with these tis-

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**TABLE 2**

**COMPARISON OF THE LOCALIZATION OF ANTILYMPHOSARCOMA ANTIBODIES WITH ANTITISSUE ANTIBODIES**

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Serum used</th>
<th>No. rats</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
<th>Spleen</th>
<th>Tumor</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Anti-RLS 813D</td>
<td>5</td>
<td>1.9</td>
<td>0.7</td>
<td>0.7</td>
<td>2.1</td>
<td>3.2±0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5</td>
<td>Anti-RL 859A</td>
<td>5</td>
<td>1.9</td>
<td>1.0</td>
<td>0.7</td>
<td>2.0</td>
<td>1.2±0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6</td>
<td>Anti-RL 455B</td>
<td>5</td>
<td>2.9</td>
<td>1.8</td>
<td>1.0</td>
<td>3.0</td>
<td>1.3±0.4</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**TABLE 3**

**COMPARISON OF THE LOCALIZATION OF PURIFIED ANTIBODIES FROM ANTILYMPHOSARCOMA SERA WITH ANTIBODIES FROM OTHER ANTITISSUE SERA**

<table>
<thead>
<tr>
<th>Serum used</th>
<th>Radioactivity</th>
<th>No. tumors in tumor</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal sera (three sera)</td>
<td>34</td>
<td>1.9±0.2</td>
<td></td>
</tr>
<tr>
<td>Antitissue sera (five sera)</td>
<td>24</td>
<td>1.3±0.4</td>
<td></td>
</tr>
<tr>
<td>Antilymphosarcoma sera</td>
<td>35</td>
<td>2.4±0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Antilymphosarcoma serum</td>
<td>19</td>
<td>2.7±1.7</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*See footnotes, Table 1.*

† The abbreviations are as follows: LS = Lymphosarcoma; Li = Liver; Lu = Lung; N = Lymph node; K = Kidney; Sp = Spleen; R signifies rat.

* The probability levels for the difference between antitumor or other antitissue serum and normal serum preparation.

† The standard deviations in this table are larger than those for the individual experiments (Tables 1 and 2), because the values were obtained by averaging the results from several experiments and there was variation from one experiment to the next.

‡ The antisera used were: antikidney, antilung, antiliver, antisympathetic, and antilymph node.
sues failed to respond with the production of antibody against this antigen.

It is doubtful that we are dealing with a strain difference, because the antikidney serum was prepared against Wistar rat kidneys and failed to localize in the tumor. If a strain-specific antigen were responsible for the localization, this antikidney-kidney serum would be expected to contain the strain-specific antibody. The possibility that different responses of the various rabbits to a given antigen mixture may have been responsible for the absence of tumor-localizing antibodies in the antitissue sera cannot be ruled out, but the fact that all five rabbits immunized with normal tissues failed to produce tumor-localizing antibodies makes it doubtful that this is the explanation for the data reported here. It seems, therefore, that the Murphy lymphosarcoma contains an antigen which is absent in the liver, kidney, lung, spleen, and lymph nodes. However, the lymphosarcoma must have antigens in common with liver and spleen, because the antilymphosarcoma sera localize in these organs. That the preparations from the antikidney and antilung sera failed to localize appreciably in the homologous organs (Table 2) is owing to the fact that tumor tissue was used for purification, and it does not contain enough of these organ antigens to yield kidney and lung-localizing preparations. Previous experiments (2) had shown that this antilung serum would localize to a high degree in lung when purified with lung cells and that the antikidney serum would localize in kidney to a high degree when purified with kidney.

SUMMARY

Antisera were prepared in rabbits by injecting them with the Murphy lymphosarcoma and were shown to contain antibodies capable of localizing in the tumors of rats bearing the Murphy lymphosarcoma. This was shown by radio-iodinating the antibodies, purifying them in vitro, and injecting them into rats, after which procedure their localization in the tumor was determined. These antisera also contained antibodies capable of localizing in normal tissues. However, it was found that antisera prepared against liver, kidney, lung, spleen, and lymph node did not contain antibodies capable of localizing in the tumor, even though they contained antibodies capable of localizing in normal tissues.

ACKNOWLEDGMENTS

Richard Clarke, Helen Gross, Rose Lipari, and Jacob Plninsek assisted with the experiments.

REFERENCES

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