Inhibition of the Growth of Mouse Polyoma Tumors by Lymph Node Fragments from Specifically Immunized Rats

H. F. Jeejeebhoy and A. G. Rabbat

Department of Experimental Surgery, McGill University, Montreal, Canada

SUMMARY

Lymph node fragments from rats immunized against a transplantable mouse polyoma tumor were implanted i.p. in C57BL/6J mice previously inoculated with cells from the same tumor or, alternatively, another tumor of the same transplant generation. Significant inhibition of tumor growth was demonstrable in 7 separate experimental groups (5–8 animals each time) regardless of whether the lymph node fragments were placed in Millipore diffusion chambers or not. In other experiments tumor-bearing mice received lymph node fragments from rats immunized with normal mouse tissue or with a tumor (BW10232) antigenically unrelated to the polyoma tumor. Some tumors grew faster than the controls while most of the others were unaffected. Occasional tumors grew slower than controls, but no inhibition of tumor growth was demonstrable when untreated and treated groups were statistically compared. The results suggest that the ability of lymphoid cells from specifically immunized rats to inhibit the growth of mouse polyoma tumors is due to agents directed against tumor-specific factors and that some, at least, of these agents are diffusible humoral substances.

INTRODUCTION

Previous communications (1, 5) have shown that the growth of both primary and transplantable tumors is often slowed, with occasional decreases in tumor size, when tumor-bearing animals receive lymphoid cells from xenogeneic animals previously immunized against the tumor being treated. Though suggestive, the results obtained by these workers did not show conclusively that inhibition of tumor growth by xenogeneic immune cells was due to agents directed against tumor-specific factors.

In an initial series of experiments, we found that the growth of a transplantable mouse polyoma tumor was retarded when lymph node fragments from rats immunized against the tumor were placed in the peritoneal cavities of tumor-bearing mice. In subsequent experiments attempts were made to demonstrate that this inhibition of tumor growth was due to agents directed against tumor-specific factors and that these agents were, in part at least, diffusible humoral substances. This paper records our findings.

The rat-mouse combination is easier and more convenient to use than the sheep-rat combination used by Alexander et al. (1). In addition, transplantable mouse polyoma tumors are ideally suited for use as target tumors in experiments of this nature. They are readily passaged in syngeneic animals, and they contain tumor-specific antigens (9, 19, 20) which are relatively stable from one transplant generation to the other (21).

MATERIALS AND METHODS

Plan of Experiments. Male C57BL/6J mice (20 ± 2 gm body weight) were inoculated with cells from a transplantable C57BL/6J polyoma tumor. On the same day a rat was immunized by s.c. implantation of fragments from the same tumor or, alternatively, from another polyoma tumor of the same transplant generation. Seven days later the lymph nodes (specific immune lymph nodes) were removed from the immunized rat, cut into small fragments, and divided into 5–9 equal portions. A portion was implanted i.p. in each of a number of tumor-bearing mice (mice previously injected with tumor cells but not necessarily bearing palpable tumors). These animals constituted a single experimental group. A comparable number of mice, inoculated with polyoma cells but otherwise untreated, were used as controls. Mice in both the experimental and the control groups were inoculated at the same time from the same suspension of polyoma cells. They were randomly separated into experimental and control groups seven days later, immediately prior to treatment. Tumor diameters were measured at 2- or 3-day intervals, and the growth of tumors on treated and untreated animals was compared. On occasion, more than one experimental group was treated at the same time and shared the same controls. Each experimental group received the divided lymph nodes from one rat only.

In other experiments having a similar design, mice were inoculated with 10⁶ polyoma cells and seven days later received: (a) Specific immune rat lymph node fragments in Millipore diffusion chambers. (b) Lymph node fragments from rats immunized with normal mouse tissue. In some experiments these lymph node fragments were placed in diffusion chambers prior to their i.p. introduction; in others they were not. (c) Millipore diffusion chambers containing lymph node fragments from rats immunized with BW10232 tumor. (d) Lymph node frag-
ments from normal unimmunized rats. These fragments were not contained in diffusion chambers.

Lymph node fragments from rats immunized with either normal mouse tissue or BW10232 tumor are referred to as nonspecific immune rat lymph node fragments.

In a final series of experiments, mice were inoculated with 10^6 BW10232 cells and seven days later received Millipore diffusion chambers containing lymph node fragments from rats previously immunized with polyoma tumor.

**Animals Used.** Male C57BL/6J mice (20 ± 2 gm body weight) and male CBA mice (20 ± 2 gm body weight) were obtained from the Jackson Laboratory, Bar Harbor, Maine. Male RVH rats (170—200 gm body weight) were used throughout this series of experiments. This is a noninbred strain of rats, and grafts are regularly rejected between members of the strain. Some of the animals were obtained from a colony maintained in our department and others from Quebec Breeding Farm, Montreal, Canada. Lymph node fragments from different rats were never mixed. This was to prevent lymphoid cells from two or more allogeneic animals from reacting against each other as in the mixed leukocyte culture reaction (4, 10).

**Preparation and Inoculation of Tumor Cell Suspensions.** The C57BL/6J polyoma tumor used was originally provided by Dr. K. Habel of the National Institute of Allergy and Infectious Diseases. The present series of experiments were performed between the 64th and 70th transplant generation of this tumor. For the preparation of a suspension of tumor cells, macroscopically viable tumor was placed on a stainless steel mesh, cut into fragments, and irrigated with TC199 (Difco). The resulting cell suspension was counted in a Neubauer hemocytometer, gently centrifuged, and then resuspended in a small volume of TC199. Animals inoculated with tumor cells were all male C57BL/6J mice (20 ± 2 gm body weight), and each mouse was injected s.c. in the midline of the dorsum with 10^6 polyoma cells in 0.1 ml of TC199. Cell suspensions were kept at room temperature. These cells seem to be readily inactivated if maintained at 4°C.

The BW10232 tumor used was obtained from the Jackson Laboratory, Bar Harbor, Maine. This tumor originally appeared as a spontaneous mammary adenocarcinoma and has been maintained by serial passage in C57BL/6J mice for the last nine years. It has growth characteristics which are similar to those of the polyoma tumor used by us. A tumor invariably appears 7—10 days after the inoculation of 10^6 BW10232 cells, and this tumor progressively grows and kills the host two to three weeks after its appearance. In addition, the results in Table 1 suggest that the BW10232 tumor is antigenically dissimilar to the polyoma tumor used by us. The preparation of tumor cell suspensions and the inoculation of animals with tumor cells were performed as described in the preceding paragraph.

**Immunization of Rats and Removal of Lymph Nodes.** Each rat received a total of 600—700 mg of minced polyoma or BW10232 tumor. Macroscopically viable tumor was cut into fragments and implanted s.c. in the cervical, anterior thoracic, and both axillary regions of the rat. Tumor was introduced through a small incision in the right axilla of the animal, and approximately equal amounts were placed in each of the five previously mentioned sites.

---

**Table 1**

<table>
<thead>
<tr>
<th>First inoculum</th>
<th>Mean tumor diameter (cm)</th>
<th>95% confidence limits of the means (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>1.35</td>
<td>1.15—1.55</td>
</tr>
<tr>
<td>10^6 BW10232 cells</td>
<td>1.56</td>
<td>1.36—1.76</td>
</tr>
<tr>
<td>10^6 polyoma cells</td>
<td>0.56</td>
<td>0.33—0.79</td>
</tr>
</tbody>
</table>

Polyma tumor growth following preimmunization with BW10232 cells. Separate groups of male C57BL/6J mice (20 ± 2 gm body weight) were either untreated or injected s.c. in the anterior part of the dorsum with 10^6 polyoma or 10^6 BW10232 cells. Seven days later all mice were inoculated s.c. in the midline of the posterior part of the dorsum with 10^6 polyoma cells. The figures relate to the size of tumors resulting from this second inoculum as measured 18 days after injection. There were nine mice in each of the three groups.

In some experiments rats were immunized with normal mouse tissue. The liver, lung, lymph nodes, spleen, thymus, heart, and some muscle were removed from normal untreated male C57BL/6J mice (20 ± 2 gm body weight) and cut into fragments. A total of 600—700 mg of minced tissue was implanted s.c. in rats as described in the preceding paragraph.

Rats were killed by a blow on the head seven days after immunization. Their axillary, cervical, thoracic, and mesenteric lymph nodes were removed, moistened with a few drops of TC199, and cut into small fragments. These fragments were implanted i.p. into tumor-bearing mice through a small incision in the left anterior abdominal wall of the animal. In some experiments the minced lymph node fragments were placed in Millipore diffusion chambers before i.p. introduction. Each mouse received an approximately similar quantity of rat lymph nodes (5—8 X 10^7 cells each time). The amount of lymph nodes which could be obtained varied from rat to rat, and this factor governed the size of the experimental groups. Nodes were not used if apparently viable tumor masses were found when the rat was killed (see Discussion).

**Millipore Diffusion Chambers.** The chambers were lucite rings 2 mm in height and having an i.d. of 10 mm and an o.d. of 14 mm. Some were made in our workshop and others were obtained from the Millipore Filter Corporation. Membranes had an average pore diameter of 100 mμ (Millipore Filter Corporation No. VCWPO 1400). Membranes and cement (Formulation 1) were obtained from the Millipore Filter Corporation, and both membranes and diffusion chambers were sterilized by dry heat (60—70°C for 48 hours).

**Assessment of Tumor Growth.** At two- or three-day intervals, tumor-bearing mice were anesthetized with ether; two tumor diameters, the largest and the one at right angles to it, were measured with a pair of dividers, and mean values were calculated from these two measurements. The smallest tumor which could be measured with any accuracy was 4 mm in diameter. Smaller tumors were arbitrarily considered to be 2 mm in diameter. All measurements were performed by the same person (H. F. J.).

**Skin Grafting.** The method used and the criteria of graft rejection were both as previously described (12). Dressings
were removed on the ninth day after grafting, and grafts were inspected at daily intervals thereafter.

Statistical Analyses. Mean diameters of individual tumors in a group of animals were summed, and the mean diameter of all tumors in the group was determined from this value. To determine the ranges of the 95% confidence limits of the means, the standard error of the mean was multiplied by the Student’s 't' value for the appropriate degrees of freedom (7).

RESULTS

Mortality. Table 2 shows the number of tumor-bearing mice which died in the 7-day period following i.p. introduction of rat lymph node fragments; that is, between the seventh and fourteenth day after the mice had been inoculated with tumor cells. Death occurred in approximately 50% of the animals which received specific immune rat lymph nodes not contained in diffusion chambers. This mortality was not evenly distributed among the 6 groups of animals treated in this manner. In three groups almost all the animals died, and in the other three groups practically none. Almost identical results were obtained when six groups of tumor-bearing mice received nonspecific immune rat lymph node fragments not contained in diffusion chambers. This mortality was reduced to negligible proportions if specific or nonspecific immune rat lymph node fragments were placed in Millipore diffusion chambers prior to their i.p. introduction. Apart from involution of the thymus, which was constant, no abnormality was consistently demonstrable when animals were examined at autopsy. However, the results do indicate that the mortality noted was not due to tumor growth.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>(^a)88</td>
</tr>
<tr>
<td>Normal rat lymph nodes i.p.</td>
<td>0/21</td>
</tr>
<tr>
<td>Nonspecific immune rat lymph nodes i.p.</td>
<td>23/42</td>
</tr>
<tr>
<td>Specific immune rat lymph nodes i.p.</td>
<td>24/42</td>
</tr>
<tr>
<td>Nonspecific immune rat lymph nodes i.p. in diffusion chambers</td>
<td>4/64(^c)</td>
</tr>
<tr>
<td>Specific immune rat lymph nodes i.p. in diffusion chambers</td>
<td>0/43(^d)</td>
</tr>
</tbody>
</table>

Mortality following i.p. introduction of rat lymph node fragments. Male C57BL/6J mice (20 ± 2 gm body weight) were inoculated with \(10^6\) polyoma cells. Seven days later they received rat lymph node fragments as described in the text. “Specific immune rat lymph nodes” refers to lymph nodes from rats immunized with polyoma tumor and “nonspecific immune rat lymph nodes” to lymph nodes from rats immunized with normal mouse tissue or with BW10232 tumor. The mortality figures refer to the seven-day period following i.p. introduction of rat lymph nodes.

\(^a\)Mortality expressed as the ratio of number of dead animals/number of treated animals.

\(^b\)Number of animals which died between the seventh and fourteenth days after inoculation of the mice with tumor cells.

\(^c\)This figure includes 35 mice which received lymph nodes from rats immunized with BW10232 tumor. The remainder received lymph node fragments from rats immunized with normal mouse tissue.

\(^d\)This figure includes 16 mice previously inoculated with BW10232 instead of polyoma cells.

Effect of Rat Lymph Node Fragments on Tumor Growth. In 78 of 88 animals inoculated with \(10^6\) polyoma cells but otherwise untreated, a palpable tumor was present 7 days later. The remainder developed palpable nodules in the subsequent 5 days. Tumor size at the time of death varied from experiment to experiment as did the rate of tumor growth. These variations did not seem to affect the survival times of these mice, and with few exceptions they all died two to three weeks after the first appearance of a tumor. Tumors frequently ulcerated, but this did not affect their growth rates apart from a temporary reduction in size due to the loss of tumor substance.

Three groups of tumor-bearing mice (7 mice in each group) received lymph node fragments from normal unimmunized rats. The results are not shown, but tumor growth rates were unaffected.

Chart 1a shows that tumor growth rates were significantly retarded when one group of tumor-bearing mice received specific immune lymph node fragments not contained in diffusion chambers. Two other groups of animals were treated in a like manner, and the results are shown in Chart 1b. There was inhibition of tumor growth, but some tumors were unaffected in both groups and the results were therefore not as impressive as those shown in Chart 1a. Specific immune lymph nodes not contained in diffusion chambers were implanted i.p. in six groups of tumor-bearing mice. However, results are presented only for three of these six groups of tumor-bearing mice because in none of the other three groups did a sufficient number of animals survive for statistical analyses to be made (cf. section on Mortality).

Charts 2a and 2b show that the i.p. introduction of specific immune lymph nodes in Millipore diffusion chambers was capable of significantly retarding tumor growth in three separate groups of tumor-bearing mice. In Chart 2a one of the animals developed a tumor nodule which only began to increase in size 27 days after its first appearance. Another tumor ulcerated and then gradually decreased in size till it had almost disappeared when the animal died from pneumonia. A fourth group of 7 tumor-bearing animals also received specific immune rat lymph nodes in diffusion chambers. The results are not shown, but in this group 3 tumors grew faster than the controls, 1 was unaffected, and the growth of the remaining 3 was slowed.

Six groups of tumor-bearing mice received lymph node fragments from rats immunized with normal mouse tissue; these fragments were not contained in diffusion chambers. Almost all animals died in three groups (cf. section on Mortality), and, therefore, results could be statistically evaluated only in the other three groups. In one of these groups (Chart 3a), tumor growth rates were unaffected by the treatment. In the two other groups (Chart 3b), there was accelerated growth of many of the tumors. The control group of this latter experiment and of the experiment depicted in Chart 5b had their tumors growing at slower rates than tumors in any of the other control groups.

Three groups of tumor-bearing mice sharing the same controls received Millipore diffusion chambers containing lymph node fragments from rats immunized with normal mouse tissue. The results are shown in Chart 4. Some tumors grew rather slowly, but a significant inhibition of tumor growth was
not found when treated and control groups were statistically compared. In one group of treated animals, many tumors grew faster than the controls, and most of the animals in this group died before the control animals.

Four groups of mice previously inoculated with $10^6$ polyoma cells received diffusion chambers containing lymph node fragments from rats immunized with BW10232 tumor. In two of these groups, tumor growth was unaffected (Chart 5a). In the other two groups (Chart 5b), there was acceleration of tumor growth, and this was particularly evident in one of these two groups.

Two groups of 8 mice each were inoculated with $10^6$ BW10232 cells. Seven days later each mouse received a diffusion chamber containing lymph node fragments from rats pre-

Charts 1a, b. Growth of mouse polyoma tumors following i.p. introduction of specific immune rat lymph node fragments. Male C57BL/6J mice (20 ± 2 gm body weight) were inoculated with $10^6$ polyoma cells and on the same day rats were immunized by s.c. implantation of 600–700 mg of minced polyoma tumor. Lymph nodes were removed from the rats seven days later, cut into fragments, and introduced i.p. into the tumor-bearing mice. In Chart 1a a group of seven mice (---------) received the divided lymph nodes from one rat. Eight mice (--------) inoculated with polyoma cells but otherwise untreated were used as controls. In Chart 1b a control group of seven mice (--------) was shared by two experimental groups of seven mice each (---------) and (---------). Each of the three experimental groups received the divided lymph nodes from one immunized rat only. The closed circles represent the mean diameters of all tumors in a group of animals, and the extremities of the vertical lines represent the ranges of the 95% confidence limits of the means.
Growth Inhibition of Mouse Polyoma

Charts 3a, b. Growth of mouse polyoma tumors following i.p. introduction of lymph node fragments from rats immunized with normal mouse tissue. Rats were immunized by s.c. implantation of 600–700 mg of minced normal C57BL/6J mouse tissue (lymph nodes, spleen, liver, lung, and muscle). Otherwise the procedures used were identical to those described in the legend to Charts 1a and 1b. In Chart 3a a group of 7 mice (-----) received the lymph nodes from one rat. Seven mice (-----) inoculated with tumor cells but otherwise untreated were used as controls. In Chart 3b a control group of 7 mice (-----) was shared by two experimental groups of 7 mice each (-------) and (--------). Closed circles represent the mean diameters of all tumors in a group of animals, and the extremities of the vertical lines represent the ranges of the 95% confidence limits of the means.

Viously immunized with polyoma tumor. The results are not shown, but tumor growth rates were unaffected in comparison with a control group of 8 mice.

Immunosuppressive Properties of Immune Rat Lymph Node Fragments. In this series of experiments, four groups of male C57BL/6J mice (20 ± 2 gm body weight) received Millipore diffusion chambers containing specific immune rat lymph node fragments, and three groups received chambers containing lymph node fragments from rats immunized with normal mouse tissue. None of the mice had been previously inoculated with tumor cells. They were grafted with skin from male CBA mice (20 ± 2 gm body weight) one day after i.p. introduction of the diffusion chambers. Table 3 shows that both procedures were capable of producing some prolongation of skin allograft survival.

DISCUSSION

Our results indicate that the i.p. introduction of lymph node fragments from specifically immunized rats can inhibit the growth of transplantable mouse polyoma tumors. These findings are similar to those previously reported by Balme et al. (5) and by Alexander et al. (1), who found that the growth of both transplantable and primary tumors was slowed when tumor-bearing animals received lymphoid cells from xenogeneic animals previously immunized against the tumor being treated. Our observations in some ways extend the findings reported by these previous workers.

Many mice previously inoculated with polyoma cells received lymph node fragments from rats immunized with normal mouse tissue or with a tumor (BW10232) which was antigenically unrelated to the polyoma tumor used. In some experiments the lymph node fragments were placed in Millipore diffusion chambers and in others they were not. When tumor-bearing mice received nonspecific immune rat lymph nodes, many tumors grew faster than the controls while most of the others were unaffected. The growth of an occasional tumor appeared to be retarded but, statistically, no inhibition of tumor growth was demonstrable when untreated and treated animals were compared. Chance variations in the responses of immunized rats might account for this failure of nonspecific immune rat lymph node fragments to inhibit tumor growth, and it is therefore possible that tumor inhibition might have been demonstrable had more experiments been performed. However, on the basis of results obtained in this study, it would seem that the ability of specific immune rat lymph node fragments to inhibit the growth of mouse polyoma tumors is due to agents directed against tumor-specific factors. These factors could be the polyoma-specific antigens described by Sjögren (19, 20) and by Habel (9).

Previous studies (13) have shown that the growth of primary benzpyrene-induced tumors in rats is often slowed when tumor-bearing animals receive Millipore diffusion chambers containing lymph node fragments from allogeneic rats previously immunized against the tumor being treated. These observations are compatible with those noted in the present study. An impressive inhibition of tumor growth was often demonstrable when tumor-bearing mice received Millipore diffusion chambers containing specific immune rat lymph node fragments. It is possible that cells do, on occasion, escape from these chambers, but the chances are slight with membranes having a mean pore diameter as small as 100 μm (6). In addition, gross leakage was never demonstrable when the chambers were examined at autopsy. Hence, the results obtained suggest that the ability of specific immune rat lymph node fragments to inhibit the growth of transplantable mouse polyoma tumors is due, in part at least, to diffusible humoral substances. The nature of these substances must for the moment remain conjectural. They could be antibodies specifically directed against polyoma tumor cells. Alternatively, the material responsible

MAY 1969

1107
for tumor inhibition could be RNA produced by the rat in response to immunization with polyoma tumor. Such a mechanism has been postulated by Alexander *et al.* (2). These workers have shown that the growth of primary benzpyrene-induced rat tumors can be slowed by injecting the tumor-bearing animals with RNA extracted from the lymphoid cells of specifically immunized sheep.

There was a mortality of approximately 50% when tumor-bearing mice received either specific or nonspecific immune rat lymph node fragments not contained in diffusion chambers. The mortality was negligible if the fragments were placed in Millipore diffusion chambers prior to their i.p. introduction. These results suggest that the animals which died probably succumbed as the result of a cell-mediated toxic process which might have been akin to the one described by previous workers (8, 25) in allogeneic animals, though their autopsy findings differed from ours. It is unlikely that previous inoculation with tumor cells would account for the mortality noted in this study. On many occasions in the past (our unpublished findings), lymph node fragments from Lewis rats immunized with mouse tissue have been introduced i.p. into normal C57BL/6J mice; that is, mice not previously inoculated with tumor cells. A mortality approximately similar to that noted in this present study resulted if the lymph node fragments were not placed in diffusion chambers prior to their i.p. introduction.

Many factors might have been responsible for our failure to obtain a more impressive, prolonged, and constant inhibition of tumor growth. The pores of the membranes frequently become blocked with fibrin and other substances within a few days of i.p. introduction. Lymph nodes might have been removed from rats and introduced into mice at times which were less than optimum. Furthermore, rat lymph nodes, when not contained in diffusion chambers, are probably destroyed by an immune reaction within a few days of their i.p. introduction into mice. Cells placed in diffusion chambers can survive for prolonged periods even when placed in xenogeneic hosts (3, 24), and in our experiments histologically normal cells were found in the majority of chambers examined 20 days or more after i.p. introduction. However, normal histologic appearances do not indicate viability, and, even if the cells were viable, the nutrients available to them might still have been insufficient to allow specialized functions such as antibody production to be performed.

Two other factors merit consideration. First, in this and other experiments, some forty rats have been immunized with the polyoma tumor used. All rats were killed seven days after immunization, and on three occasions tumor masses were found. On each occasion this proliferating tumor was reimplanted in C57BL/6J mice, and each time it progressively grew and killed the mice. It is possible, though unlikely, that the

---

**Chart 4. Growth of mouse polyoma tumors following i.p. introduction of Millipore diffusion chambers containing lymph node fragments from rats immunized with normal mouse tissue.**

Rats were immunized by s.c. implantation of 600–700 mg minced normal C57BL/6J mouse tissue. Otherwise the procedures used were identical to those described in the legend to Charts 2a and 2b. A control group of 13 mice (—) inoculated with tumor cells but otherwise untreated was shared by two experimental groups of 8 mice each (-----) and (- - - - - - - - -). Animals in an experimental group received Millipore diffusion chambers containing the divided lymph nodes from one immunized rat only. The closed circles represent the mean diameters of all tumors in a group of animals, and the extremities of the vertical lines represent the ranges of the 95% confidence limits of the means.
immunogenicity of the tumor used was poor. An alternative explanation is that the tumor itself, because of its ability to proliferate rapidly, or because of some as yet unknown reason, was capable of resisting inactivation by the immune response of a rat. Over 100 rats have been immunized with normal mouse tissue in this and other unrelated experiments. We have never found mouse tissue which was even histologically normal seven days after its implantation in a rat.

The second factor relates to the immunosuppressive properties of immune rat lymph node fragments. As was shown in Table 3, immune responses of the transplantation type are depressed when Millipore diffusion chambers containing either specific or nonspecific immune rat lymph node fragments are placed in the peritoneal cavities of mice. Mouse lymphoid cells were present in the polyoma tumor and in the normal mouse tissue used to immunize the rats. Hence, the process by which immune rat lymph node fragments in diffusion chambers depress the immune responses of mice could be similar to that by which heterologous antilymphocyte serum and plasma produce immunosuppression (11, 15, 18, 23, 27).

The prolongation of allograft survival noted was not impressive in terms of days, but this degree of immunosuppression might be enough to depress the immune response evoked in mice by weak antigens such as the polyoma-specific antigens. To conclusively demonstrate this point, it might have been more appropriate to perform some experiments based on a variation of the "neutralization" test described by Winn (26) and others (16, 22). This was not done because of the large number of animals that would have been needed. With this tumor, syngeneic immune cells have to be mixed with tumor cells in a ratio of 250:1 to 500:1 to obtain a significant inhibition of tumor growth (our unpublished results). This means that, in the control groups, cells from the lymph nodes of approximately five syngeneic mice previously inoculated with tumor cells would need to be mixed with every 10^6 polyoma cells to obtain tumor inhibition. In the experimental groups each of the syngeneic tumor-bearing mice would, in addition, need to be treated with immune rat lymph node fragments in diffusion chambers. The results obtained with the neutraliz-

---

**Table 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Graft survival times (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 or less</td>
</tr>
<tr>
<td>No treatment</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Normal rat lymph nodes i.p.</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>in diffusion chambers*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-specific immune rat lymph nodes i.p. in diffusion chambers:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Specific immune rat lymph nodes i.p. in diffusion chambers:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

*Skin allograft survival times in mice following i.p. introduction of rat lymph node fragments in Millipore diffusion chambers. RHV rats were immunized with either polyoma tumor or normal mouse tissue as described in the text. They were killed seven days later and their lymph nodes were removed, cut into fragments, and placed in Millipore diffusion chambers. One chamber was placed in the peritoneal cavity of each of the C57BL/6J mice (male, 20 ± 2 gm body weight) in the experimental groups. On the next day these mice were grafted with skin from male CBA mice (20 ± 2 gm body weight). "Specific immune rat lymph nodes" refers to lymph nodes from rats immunized with polyoma tumor and "nonspecific immune rat lymph nodes" to lymph nodes from rats immunized with normal mouse tissue. Animals in an experimental group received the divided lymph node fragments from one immunized rat only. Animals that died with intact healthy grafts have been excluded.

*Lymph node fragments from normal unimmunized Lewis rats.

---

**Growth Inhibition of Mouse Polyoma**

Charts 5a, b. Growth of mouse polyoma tumors following l.p. introduction of Millipore diffusion chambers containing lymph node fragments from rats immunized with BW10232 tumor. Rats were immunized by s.c. implantation of 600–700 mg minced BW10232 tumor. Otherwise the procedures used were identical to those described in the legend to Charts 2a and 2b. In Chart 5a a control group of 13 mice (— — —) inoculated with tumor cells but otherwise untreated was shared by two experimental groups of 10 (———) and 9 (———) mice respectively. In Chart 5b there were two experimental groups of 8 mice each (———) and (———) and a control group of 8 mice (———). The closed circles represent the mean diameters of all tumors in a group of animals, and the extremities of the vertical lines the 95% confidence limits of the means.
tion test (see above) do, however, suggest that the polyoma antigen is weak in terms of immunogenicity. In addition, they confirm previously reported findings (9, 19, 20) which showed that the injection of polyoma cells into mice evokes an immune response specifically directed against the tumor cells, one which can retard tumor growth.

Immunologic enhancement of tumor growth has been attributed to a specific suppression of the immune response by humoral antibody of appropriate specificity (14, 17, 22). Such a process might account, in part, for those instances where acceleration of tumor growth followed the i.p. introduction of specific immune rat lymph node fragments into tumor-bearing mice. However, it would not explain why many tumors grew faster than the controls when tumor-bearing mice received nonspecific immune rat lymph node fragments.

Skin allograft survival was frequently prolonged when mice received diffusion chambers containing either specific or nonspecific immune rat lymph node fragments. Many of our findings would be understandable if it were accepted that, by virtue of their immunosuppressive properties, both specific and nonspecific immune rat lymph node fragments depress the immune responses which are evoked in mice by injections of polyoma tumor cells. Such a hypothesis would explain why many tumors grew faster than the controls when tumor-bearing animals received nonspecific immune rat lymph node fragments. Also, whether and to what degree the growth of a tumor is retarded by lymphoid cells from specifically immunized xenogeneic animals might depend on the balance between the tumor-inhibiting and immunosuppressive properties possessed by these cells.

ACKNOWLEDGMENTS

The authors are indebted to Mrs. B. Mazurewicz and Mr. L. Elder for technical assistance. We are also extremely grateful to Dr. K. Habel who originally provided us with the tumor used.

REFERENCES


