Immune Phenomena Elicited by Transplanted Tumors

I. The Participation of the Eye and the Brain*

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The brain, the anterior chamber of the eye, and the subcutaneous tissue of guinea pigs differ in their suitability as sites for transplantation of mouse tumors. Successful heterologous transplantation is achieved in the brain and in the eye but not in the subcutaneous tissue of the adult, nonirradiated host. Transplantation of heterologous tumor to the eye is usually followed by regression after a short period of growth. By contrast, tumor growth in the brain is frequently more rapid, regression is delayed, and death of the host is not uncommon. Death in these instances is due to local factors, such as increased intracranial pressure.

When homologous transplants of mouse tumors in the eye, brain, and subcutaneous tissue of nonsusceptible strains of mice are compared to the heterologous grafts described above, basic similarities are observed. Growth occurs readily in the eye and the brain. Regression of tumor placed in the eye occurs as in heterologous transplants to guinea pigs, but often this does not take place until there has been perforation of the cornea. Possibly this is attributable to the smaller size of the mouse eye. Homologous tumor transplanted to mouse brain frequently kills the host before regression can occur. The higher mortality may also be explained by the smaller size of the cranial cavity of the mouse. In the subcutaneous site, however, transplanted tumors may grow for a short time in homologous hosts of a nonsusceptible strain before regression begins.

Regression of established transplanted tumors is associated with the development of an immune response by the host (9, 10). The factors responsible for the initial fate of a graft are not fully understood; it is not certain why the eye and the brain are more suitable sites for transplantation than the subcutaneous tissue. It has been postulated that these differences in ability to support tumor growth are dependent upon the isolation of the brain and eye from systemic immune events. Evidence for such isolation has been presented by Saphir et al. (8), by successfully transplanting rabbit tumors to the eyes of tumor-immune rabbits. Evidence to the contrary has been presented by Greene (6), Schilling et al. (9), and others (5).

The experiments of Greene and of Schilling et al. indicate that the eye can develop immunity to tumors transplanted elsewhere and that systemic immunity to tumors will follow transplantation to the eye. Less is known about the brain in this respect. According to Medawar (7), there is evidence that systemic immunity is not evoked by tissues transplanted to the brain, although the host will become resistant to tissues transplanted elsewhere.

The experiments reported below were designed to determine to what extent resistance phenomena can be evoked by the growth of tumors in the brain and in the eye, and to what extent the brain and the eye are influenced by such phenomena.

MATERIALS AND METHODS

Heterologous transplantation.—Mouse Sarcoma 37 carried in ABC mice was inoculated into stock guinea pigs fed a commercial diet supplemented with greens. The method of inoculation to the anterior chamber has been described before (1). The method of inoculation to the brain, utilizing the subdural space, has been described in a previous communication (4). Introduction of the tumor by direct visualization through the transparent dura facilitates detection of transplant sites and allows re-inoculation of the brain with subsequent identification of separate transplant sites.

A total of 223 guinea pigs was divided into eight groups of approximately equal size. One of two groups which received an initial transplant to the eye was given an inoculation in the
brain after 10 days, while the other was given an inoculation in the other eye. After 10 days one of two groups with primary brain inoculations received a secondary eye inoculation, and the other a secondary inoculation into the other cerebral hemisphere. Of the two groups with primary subcutaneous inoculations, one received a secondary eye and the other a secondary brain inoculation. The eye and the brain of previously un inoculated control groups were inoculated at the time of the secondary inoculation of the immunized groups. At the end of the second 10-day period all guinea pigs were sacrificed, and the secondary and control transplant sites were studied microscopically for the presence of growing tumors.

Homologous transplantation.—Mouse tumor E 0771 carried in mice of the C57BL strain was inoculated into strain A mice obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The method of inoculating the anterior chamber of mice has been previously described (6). The brain was inoculated by slitting the scalp in the midline, penetrating the skull paramedially with a pointed scalpel, inserting a loaded 20 trocar subdurally, and depositing the graft under direct vision through the translucent calvarium.

A total of 140 mice was divided into eight groups of about equal size and given inoculations in the same pattern used for the guinea pigs, with the following exceptions: (a) There was no group in which a primary brain inoculation was followed by a secondary brain inoculation; (b) There was a group of subcutaneously inoculated controls.

Each of the eight groups was divided into two subgroups, one of which was sacrificed 10 days after inoculation of the test tumor, while the other was left to survive (designated below as "survivors") to permit observation beyond the period of sacrifice of the other mice. The experiment was terminated 2 months after the second inoculation.

RESULTS

The results are shown in Tables 1 and 2. They indicate that the eye and the brain participate to a limited extent in immune events elicited by inoculations of eye or brain.

### TABLE 1

<table>
<thead>
<tr>
<th>First inoculation site</th>
<th>Second inoculation site (10th day of experiment)</th>
<th>Tumor growth at 8th site (90th day of experiment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye</td>
<td>Eye</td>
<td>0/27</td>
</tr>
<tr>
<td>Eye</td>
<td>Brain</td>
<td>1/25</td>
</tr>
<tr>
<td>Brain</td>
<td>Eye</td>
<td>4/90</td>
</tr>
<tr>
<td>Brain</td>
<td>Brain</td>
<td>3/94</td>
</tr>
<tr>
<td>Subcut. tissue</td>
<td>Eye</td>
<td>2/94</td>
</tr>
<tr>
<td>Subcut. tissue</td>
<td>Brain</td>
<td>9/94</td>
</tr>
</tbody>
</table>

**Controls**

Eye 29/35

Brain 39/44

Subcut. tissue 0/74*  

* Previous experiments.

Transplanted heterologous and homologous tumors. The tumors grew in the eyes or brains of 67 of 79 heterologous, and in twenty of 21 homologous controls. After inoculation of the eye, brain, or subcutaneous tissue, the second transplanted tumors grew in the eye or brain of only nineteen of 143 heterologous hosts, and of 21 of 48 homologous hosts. The differences in incidence of growth between experimental animals and controls are highly significant (P < .001, using the χ² formula with Yates modification).

### TABLE 2

<table>
<thead>
<tr>
<th>Inoculation site</th>
<th>Tumor growth at 8th site (sacrificed group)</th>
<th>Progressive growth of tumor (survivor group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye</td>
<td>Eye</td>
<td>7/10</td>
</tr>
<tr>
<td>Eye</td>
<td>Brain</td>
<td>4/9</td>
</tr>
<tr>
<td>Brain</td>
<td>Eye</td>
<td>8/9</td>
</tr>
<tr>
<td>Subcut. tissue</td>
<td>Eye</td>
<td>1/10</td>
</tr>
<tr>
<td>Subcut. tissue</td>
<td>Brain</td>
<td>1/9</td>
</tr>
</tbody>
</table>

**Heterologous Hosts**

**Controls.**—Following inoculation of Sarcoma 37 into the guinea pig eye, growing tumor (indicated by vascularization of the graft and the presence of mitotic figures in the tumor cells) was found in 31 of 38 animals after a 10-day period. Following inoculation in the brain, growing tumor was found in 38 of 44 guinea pigs. Growth of this tumor has never been observed in guinea pigs after subcutaneous inoculation (2).

**Reinoculated animals.**—Following inoculation in one eye, the other eye was susceptible in none of 27, and the brain in but one of 25 guinea pigs. Following inoculation in one cerebral hemisphere, the eye was susceptible in four of twenty, and the other hemisphere in three of 24 guinea pigs. Following subcutaneous inoculation, the eye was susceptible in two of 24, and the brain in nine of 24 guinea pigs.

It would appear, then, that immunization of heterologous hosts was more effective following inoculation in the eye (31/32) than following inoculation in the brain (37/44; difference on borderline of significance). Subcutaneous inoculation was least effective (37/48; P < .02).

The eye also appeared to become more readily immunized than the brain. The only group in
which there was immunization of all animals was that in which the eye had been the site of both the first and the second inoculation. Immunization was least apparent when subcutaneous inoculation preceded brain inoculation.

**Homologous Hosts**

**Controls.**—Following inoculation of the eye of strain A mice with mouse tumor E 0771 from the C57BL strain, growing tumor was found in all the ten animals sacrificed on the 10th day. The tumor had filled the anterior chamber, had perforated the cornea, and had invaded the sclera in most animals (Fig. 1). In all but one mouse of the survivor group the tumor regressed, usually during the 8th week. Following inoculation into the brain, growing tumor was found on the 10th day or before in ten of eleven mice. In the survivor group all but one mouse died between the 9th and the 21st day, with a mean survival time of 14 (± 3.16) days. In these, growing tumor occupied up to one-fourth of the cranial cavity (Fig. 2). The mouse of this group that did not die had already shown signs of advanced tumor growth, with general debility and bulging of a small tumor through the operative skull defect before the tumor began to regress. Following subcutaneous inoculation, growing tumor was found on the 10th day in six of nine mice. In the survivor group, the tumor failed to grow, or regressed, in all mice. In some mice the tumor reached considerable size prior to regression. In one mouse with subsequent eye inoculation the subcutaneous tumor grew slowly but progressively to cause death after 65 days.

**Reinoculated animals.**—Following inoculation of one eye, the other eye was susceptible in seven of ten, and the brain in four of ten mice. Following inoculation of the brain, the eye was susceptible in eight of nine mice. Following subcutaneous inoculation, the eye was susceptible in only one of ten, and the brain in only one of nine mice.

In the mice whose eyes or brains remained susceptible after a preceding inoculation of eye, brain, or subcutaneous tissue, the secondary tumors, in most instances, were smaller than those in the controls, and elicited a distinct fibrous reaction (Figs. 3 and 4). Except in one instance in which the subcutaneous tumor grew progressively, the eye tumors did not perforate the cornea, and the brain tumors did not kill the hosts. In the survivor group with primary brain and secondary eye inoculation, all but one mouse died from the effects of the primary brain tumor before the fate of the mouse was determined.

In guinea pigs which remained susceptible subsequent to a prior inoculation in any site, tumor growth and host response were not similarly modified.

In homologous hosts most subcutaneously inoculated mice, and almost all mice with eye or brain inoculations, showed initial growth of the immunizing tumor. Although the brain tumors were larger than the eye tumors, which in turn were somewhat larger than the subcutaneous tumors, the amounts of tumor present in the mice during the immunizing period were more nearly equal than in heterologous hosts. Therefore, the stronger immune response after subcutaneous inoculation is evidence for relative isolation of the brain and the eye as immunizing sites. The more abundant growth of the immunizing tumor in eye and brain is further evidence for this isolation.

**SUMMARY**

Experiments with mouse Sarcoma 37 transplanted heterologously to guinea pigs and with mouse tumor E 0771 transplanted homologously...
to nonsusceptible mice (Strain A) permit the following conclusions with regard to the isolation of the eye and the brain from systemic immune events:

1. Both the eye and the brain participate in immunity to transplanted heterologous and homologous tumors.

2. This participation is incomplete. There exists a state of relative isolation of these sites, particularly the brain. Not infrequently, a primary inoculation of tumor tissue to eye, brain, or subcutaneous tissue fails to immunize the eye or the brain.

3. Immunization of eye or brain is most effective, in homologous hosts, after subcutaneous inoculation. Subcutaneous inoculation is least effective in heterologous hosts. This apparent contradiction may be explained by the fact that in heterologous hosts subcutaneously inoculated tumors consistently fail to grow and are absorbed in a short period of time, while in most homologous hosts subcutaneous tumors grow sufficiently to cause immunization.

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REFERENCES


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