Immune surveillance and tumors of the nervous system

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The theory of immune surveillance postulates that one function of the immune system is to eliminate small numbers of malignant cells that arise spontaneously within the organism. Although there has been a great deal of both clinical and experimental evidence in favor of this theory as it applies to general oncology, the question of whether or not such a surveillance system would be effective for tumors arising within the nervous system has never been studied. The young of pregnant rats which had been exposed to the neurocarcinogen ethylnitrosourea (ENU) were divided into control, immunosuppressed, and immunoenhanced groups. These lifetime alterations of the immune system had no effect on the course of nervous system tumor formation. We believe that the most likely explanation for our results is that the "immunological privilege" of the brain prevents the usual interaction of the neoplasm and the immune system from occurring.

KEY WORDS □ tumor immunology □ immune surveillance □ neural neoplasia □ chemical carcinogenesis □ immunosuppression □ Calmette-Guérin bacillus

The concept of immune surveillance as it applies to general oncology, and several recent review articles have been published.

However, whether or not such a system of immune surveillance is operative with respect to tumors of the nervous system has not yet been studied. Since it has been known for the past 50 years that the relationship between the brain and immune system is unique; that is, that the brain is in some ways an "immunologically privileged" site, one might question whether such a surveillance mechanism would be effective for tumors arising within the central nervous system.

This report presents data from experiments designed to determine whether im-
mune surveillance is operative within the CNS.

Materials and Methods

Twelve pregnant inbred Fisher rats (F344) were injected intravenously with ethynitrosourea (ENU) at a dose of 20 mg/kg on Day 18 of gestation. The offspring were then randomly divided into three groups: the first group underwent no further treatment and served as controls; the second group underwent lifetime immunosuppression by means of neonatal thymectomy and treatment with antilymphocyte serum (ALS); and the third group underwent lifetime immunoenhancement with Calmette-Guérin bacillus (BCG).

At 3 days of age all offspring in the immunosuppressed group were given hypothermic anesthesia and were operated on. With the aid of a dissecting microscope, the sternum was split and the thymus removed by sharp dissection and suction. Beginning at 1 month of age, each of these thymectomized rats was treated with 0.5 ml of rabbit anti-rat lymphocyte serum intraperitoneally once a week.* In order to prevent possible immunization of rats against the rabbit serum, intraperitoneal injections of 0.5 ml of horse anti-rat ALS were substituted approximately every third week. The efficacy of immunosuppression produced by this regimen of neonatal thymectomy and repetitive injections of ALS was verified by skin grafting. At 1 year of age, six rats were randomly chosen from the control and immunosuppressed groups. Each animal was then made the recipient of a 2 × 2 cm square of skin from an adult inbred Wistar rat. The site of skin grafting was observed daily for rejection and the duration of skin allograft survival was calculated for each group of rats.

At 1 month of age, and every month thereafter, all rats in the immunoenhanced group were injected subcutaneously with 2 × 10⁷ colony-forming units of BCG.† The efficacy of immunoenhancement with BCG was monitored at 3 and 12 months by skin-testing a randomly chosen group of 12 BCG-treated and 12 control rats with 25 μg of a protein extract of the tubercle bacillus.‡ Twenty-four hours after subcutaneous injection of the protein, the area of induration produced was measured with calipers, and the average area calculated for both the treated and the control groups.

All rats were housed two in a cage for life, and given food and water ad libitum. They were observed daily for signs of neural tumor growth, such as paraparesis, lethargy, proptosis, and blindness. The time of first occurrence of symptoms was noted for each animal. When a rat was clearly moribund he was anesthetized with intraperitoneal pentobarbital, and under sterile conditions a specimen of the neural tumor was prepared for tissue culture. The remainder of the central and peripheral nervous system was fixed in formalin for at least 48 hours. A general autopsy was performed to search for thymic remnants, and the spleens of all animals were examined. In addition, a search was made for possible non-neural neoplasms or other pathology. Routine microscopic sections of the brain and spinal cord were taken to search for any "micro-tumors" that may have been overlooked during the necropsy. Microscopic sections were made of each tumor, and these were classified as to histological type. All data were placed on computer cards, and tests of significance among groups were performed by chi-squared analysis. The statistical evaluation of the death rates was performed using Peto’s guidelines.²⁸

Results

In order to properly interpret the results of these experiments, it is necessary to demonstrate that thymectomy and ALS treatment and BCG injections were effective as methods of modifying the immune responsiveness of the animals used in this study. Since the rat has been widely regarded as somewhat unsuitable for the study of reactions involving cellular immunity, we decided to use as our indices of cellular immune response two standard techniques that have

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*Serum obtained from Microbiological Associates, Bethesda, Maryland. Each batch of ALS was assayed for potency by at least a twofold prolongation of allogeneic skin graft survival compared to controls.

†Tice strain BCG obtained from the Institute of Tuberculosis Research, Chicago, Illinois.

‡The extract was generously supplied by Dr. Sotiros Chaparas of the Division of Biological Products, Food and Drug Administration.
TABLE 1

Animals developing tumors (all types)

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Rats With Tumors</th>
<th>Rats With 1 Tumor</th>
<th>Rats &gt; 1 Tumor</th>
<th>Rats Without Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>control</td>
<td>62</td>
<td>80.5</td>
<td>59</td>
<td>95.2</td>
</tr>
<tr>
<td>ALS &amp; thymectomy</td>
<td>22</td>
<td>71.0</td>
<td>19</td>
<td>86.4</td>
</tr>
<tr>
<td>BCG</td>
<td>35</td>
<td>71.4</td>
<td>24</td>
<td>68.6</td>
</tr>
</tbody>
</table>

*ALS = antilymphocyte serum; BCG = Calmette-Guérin bacillus.
†Not significant, p = 0.169.
‡Significant, p = 0.0003.
§Not significant, p = 0.28.
||Not significant, p = 0.24.

have been shown to be effective in this species. Consequently, we used grafting of allogeneic skin to monitor immunosuppression, and delayed hypersensitivity to tubercle bacillus protein to monitor immunoenhancement.

The skin-graft tests to verify immunosuppression were performed on six randomly chosen rats from each group 12 months after the beginning of treatment. Animals in the control group rejected allogeneic grafts between 10 and 16 days, with a mean time of 12 days, whereas rats in the immunosuppressed group would continue to accept an allogeneic skin graft for as long as 6 months without any sign of rejection. For immunoenhancement, animals were tested for a delayed hypersensitivity response to tubercle bacillus protein 3 and 12 months after BCG treatment had begun. After 3 months of BCG treatment, animals had an average indurated area of 161 sq mm whereas control animals gave a response of 37 sq mm. After 12 months the average areas of skin-test response were 279 sq mm in the BCG group, and 28 sq mm in the control group. These data provide strong evidence that the designed treatments were effective in suppressing or enhancing the immune response of the animals used in these experiments.

In order to test the relative effectiveness of both treatments on tumor formation, the following observations were recorded for the three groups and compared: 1) the number of animals with tumors; 2) the number of animals with multiple tumors; 3) neural versus non-neural origin of the tumors; 4) the histology of the neural tumors; and 5) the death rates of animals due to tumors.

Table 1 presents the number of animals that developed tumors. There are no significant differences among the three treatment groups. The data for animals developing single and multiple tumors include both neural and non-neural tumors. There is no significant difference between the controls and immunosuppressed animals; however, there is a significant (p = 0.0003) increase in the number of animals with multiple tumors in the immunoenhanced group (Table 1).

Neural versus non-neural tumors among the treatment groups are compared in Table 2. There is a significant increase in the percentage of non-neural tumors in the immunosuppressed group over the other groups (p = 0.036). Four out of five of the non-neural tumors found in these immunosuppressed animals were carcinomas of the bladder. There is no significant difference between the immunoenhanced group and controls.

TABLE 2

Neural vs non-neural tumors

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Rats with Neural Tumors</th>
<th>Rats with Non-Neural Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>control</td>
<td>70</td>
<td>94.6</td>
</tr>
<tr>
<td>ALS + thymectomy</td>
<td>20</td>
<td>80.0</td>
</tr>
<tr>
<td>BCG</td>
<td>41</td>
<td>87.2</td>
</tr>
</tbody>
</table>

*ALS = antilymphocyte serum; BCG = Calmette-Guérin bacillus.
†Significant, p = 0.036.
‡Not significant, p = 0.152.
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Table 3 compares the histology of the neural tumors. Since most of the brain tumors produced were histologically variable in that they contained various areas showing a preponderance of one cell type (such as an area of oligodendroglioma and another area of ependymoma), it was decided to label as differentiated tumors only those gliomas composed entirely of one cell type, with all others simply being called "gliomas." There were no significant differences among the three treatment groups with this analysis of the data.

Figures 1 and 2 illustrate two different methods for the analysis of rates of death due to both neural and non-neural tumors. The

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**Table 3**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Glioma (Cerebral)</th>
<th>Neurinoma (Peripheral)</th>
<th>Schwannoma (Vth Nerve)</th>
<th>Astrocytoma (Spinal Cord)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>control</td>
<td>35</td>
<td>47.3</td>
<td>24</td>
<td>32.4</td>
</tr>
<tr>
<td>ALS + thymectomy</td>
<td>11</td>
<td>35.5</td>
<td>4</td>
<td>12.9</td>
</tr>
<tr>
<td>BCG</td>
<td>20</td>
<td>39.2</td>
<td>13</td>
<td>25.5</td>
</tr>
</tbody>
</table>

*ALS = antilymphocyte serum; BCG = Calmette-Guérin bacillus.

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Fig. 1. Distribution of deaths in rats exposed transplacentally to ethylnitrosourea (ENU) and undergoing lifetime immunological modulation. BCG = Calmette-Guérin bacillus; ALS + THY = antilymphocyte serum and thymectomy.
Fig. 2. Percentage of rats dying over time after transplacental exposure to ethylnitrosourea (ENU) and lifetime immunological modulation. BCG = Calmette-Guérin bacillus; ALS + THY = antilymphocyte serum and thymectomy.

data presented in Fig. 1 were used for the statistical comparison of the data. Because a number of animals in the immunosuppressed group died of non-tumor causes during the first 120 days of treatment, only animals that died after 140 days (the date of the first death from tumor growth) were used in this analysis. There was no significant difference in the death rates among the groups. Figure 2 presents the data as a more conventional survival curve. Because there was more than a single risk for death on each animal during this experiment (for instance, rats in the ALS and thymectomy group were at risk from the immunosuppression as well as from the tumor growth), it was not possible to compare statistically the data presented in this form.

Discussion

The evidence pertaining to immune surveillance is both clinical and experimental. The evidence resting on clinical data is based mainly on the high incidence of cancer in immunodeficient individuals. Thus, neoplasia develops in about 10% of individuals with hereditary immunodeficiency diseases, a frequency of malignancy that is roughly $1 \times 10^4$ times greater than that of an age-matched population, and in approximately 5% to 6% of patients who have undergone immunosuppression for organ transplantation (a frequency 100 times greater than that of the corresponding general population). The other clinical evidence that is usually cited in support of immune surveillance involves the observation that the incidence of cancer is highest at the extremes of life, when there is a reduced vigor of immune response; and the fact that most cases of "spontaneous regression" of cancer occur in tumors such as neuroblastoma, choriocarcinoma, and malignant melanoma, which have been shown to elicit a strong immune response in the host.
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The situation is less clear in the experimental literature, because the results obtained have varied with the particular experimental model used. Chronic immunosuppression initiated before inoculation of tumor cell suspensions will in many cases lead to an increased percentage of successful transplants.\(^8\) Such experiments, however, may be considered largely irrelevant to the question of immune surveillance, since the tumor that arose in the original host did so by circumventing the immune system, and, furthermore, the mechanical insertion of a large number of tumor cells into a secondary host is in no way analogous to the appearance and growth of a primary autochthonous tumor, which is the situation of interest.

Carnaud, et al.,\(^7\) have shown that the number of lung metastases was significantly increased in immunologically impaired mice, and this observation has been confirmed by others.\(^16,34\) The data with respect to viral oncogenesis is quite convincing: much work has shown that immunosuppression will lead to an increased incidence and decreased latency of tumors induced by both DNA and RNA viruses in experimental animals.\(^2,12,15\)

The results of experiments studying the effect of immunosuppression on chemical carcinogenesis have been variable. Although several authors have reported that immunosuppression with ALS and/or neonatal thymectomy enhanced tumor incidence or shortened latency after chemical carcinogenesis,\(^3,18,29\) the effects shown were quite small. Furthermore, other investigators\(^14,37\) have reported that neither ALS nor thymectomy increased the frequency or reduced the latency of chemically induced tumors in mice or rats.

The data cited above were based on experiments with systemic tumors, and there is a great deal of evidence indicating that, in some respects, the relationship between the immune system and the nervous system is unique. In the 1920's, Shirai,\(^83\) and Murphy and Sturm\(^26\) showed that skin grafts could survive if the transplanted tissue was implanted into the brain rather than into the skin. In 1948, Medawar\(^22\) demonstrated that the brain does not respond with a typical “second set” reaction to skin allografts. Scheinberg, et al.,\(^30,32\) working in the 1960's, further examined this concept of the brain as an “immunologically privileged” site. Using intracerebral tumor autografts and allografts, they were able to show that the “afferent limb” (that is, the recognition phase) of the immune response was partially effective in spite of the absence of intracerebral lymphatics. In addition to showing that animals could be protected against an intracerebral tumor transplant by systemic immunization, they also showed that an initial exposure to a tumor transplant within the brain provided some degree of immunity to a subsequent tumor challenge elsewhere, although not to the same degree as would have been provided if the initial challenge had been placed in the periphery.

The data from our experiments indicate that lifetime alteration of the immune system did not have the effects that would be anticipated if the immune surveillance theory were applicable to nervous system tumors. In fact, there were only two instances where significant differences were found among the treatment groups. In the first instance, there was a significant increase in the percentage of non-neural tumors found in the immunosuppressed group, but these were comprised mainly of carcinoma of the bladder. Denlinger, et al.,\(^11\) have reported a similar increase in bladder carcinoma in rats undergoing chronic immunosuppression. In the second instance, there was a significant increase in the number of rats in the BCG-treated group that developed multiple tumors. Wepsic, et al.,\(^28\) have used a model whereby a transplantable hepatoma was injected into BCG-treated rats and reported that such pre-immunization can lead to an increase in tumor incidence and growth rate.

There are two general interpretations that can be made of the data reported here. Either our experiments are another indication that immune surveillance does not occur, or there are particular “escape mechanisms” that are operative in the case of CNS neoplasms allowing these tumors to escape immune destruction.

An “escape mechanism” is a means by which a clone of tumor cells is able to escape destruction by the immune system of the host. The following general “escape mechanisms” from immune surveillance have been postulated: 1) immunodeficiency of the host; 2) immunoresistance; 3) lack of antigenicity; and 4) malfunctioning of the effector mechanisms.\(^21\)

1. Immunodeficiency of the Host. There are numerous examples in the experimental
and human tumor literature where escape from immune surveillance can be attributed to a deficiency in immune competence. However, it is quite unlikely that such a situation occurred in our group of animals that were treated with lifetime systemic immunoenhancement with BCG, since many reports have attested that such animals will have an increase in their immunocompetence.

2. Immunoresistance. "Immunoresistance" is the phenomenon by which a tumor-cell line that has been propagated in culture for a long period of time acquires the ability to grow across allogeneic barriers. This cannot be applicable to our case, since autochthonous tumors were used in our model.

3. Lack of Antigenicity. A lack of antigenicity of the experimental tumors being used is a common escape mechanism from immune surveillance. We did not test all tumors produced for antigenicity; however, in immunization experiments done on cell lines derived from many of these ENU-induced neural tumors, we have been able to show that most of the tumor cells are antigenic. Furthermore, similar results have been reported by others; the studies of Toh and Guli, Barbato, et al., and Cornain, et al., have used both in vivo and in vitro means to show that neural tumors produced in rats by ENU are antigenic.

4. Malfunctioning of the Effector Mechanism. We believe that a malfunctioning of the effector mechanism may be a partial explanation for the absence of immune surveillance in the case of nervous system tumors. Our hypothesis as to the complete explanation would include the following factors: first, due to the lack of lymphatics in the brain, there may well be a delay before the central immune apparatus is made aware of the growth of a small clone of antigenically different tissue growing within the CNS. Second, the CNS may not allow complete and rapid ingress of immune cells to the area of tumor growth quickly enough for the tumor to be destroyed before it reaches a critical size. Finally, the size of a CNS tumor that is required to kill the host animal is markedly smaller than in general oncology because of the confines of the cranial cavity, and thus a sufficient immune response cannot be marshalled locally before the tumor reaches a sufficient size to cause death.

There are several studies of CNS tumors with results consistent with our own. Denlinger, et al., found that chronic ALS treatment did not increase the number of brain tumors induced by methylnitrosourea (MNU). Approaching the question from the opposite standpoint, Albright, et al., using a mouse glioma model, was able to demonstrate no increased survival after repeated subcutaneous injections of BCG followed by the intratumor injection of purified protein derivative (PPD). Similarly, using an entirely different model in which suspensions of Avian sarcoma virus were injected into the brains of neonatal rats, Bigner, et al., were also unable to demonstrate any protective effect of BCG when given either intradermally or intracerebrally, or combined with tumor cells.

In conclusion, our data indicate that immune surveillance is not operative with respect to tumors of the nervous system produced in the rat by the carcinogen ENU. We believe the most likely explanation of this fact is that the "immunological privilege" of the brain prevents the usual interaction of the neoplasm and the immune system from occurring.

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References

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