Immunohistochemical study of natural killer cells in tumor-infiltrating lymphocytes of primary intracranial germinomas

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A monoclonal antibody against the surface marker IOT-10 of natural killer (NK) cells was used to investigate the presence and distribution of these cells in a series of nine primary intracranial germinomas. In all of these tumors, IOT-10-positive NK cells were found in small numbers, mainly distributed among the tumor cells. The data obtained in the present study suggest that the presence of NK cells in primary intracranial germinomas can be influenced by factors other than the mere quantity of tumor-infiltrating lymphocytes.

**KEY WORDS**
- natural killer cell
- monoclonal antibody
- lymphocyte
- brain neoplasm
- germinomas

The development of monoclonal antibodies for leukocyte-differentiation antigens has made possible the identification of distinct phenotypes in tumor-infiltrating lymphocytes (TIL's) of intracranial tumors, showing differences between primary and secondary neoplasms. Natural killer (NK) cells in brain tumors represent a percentage of these lymphocytes with spontaneous cytotoxic capacity against tumor cells, suggesting that they play a particular role in the regulation of the proliferation and tumor surveillance. Nevertheless, few studies have been reported on the presence and significance of these cells.

Primary intracranial germinomas are peculiar tumors that characteristically show a great amount of lymphocytes when they are examined under the microscope. Previous studies have reported the phenotypic nature of TIL's in these tumors, and it has been suggested that there is a difference in host-immune response to these neoplasms as compared to other intracranial tumors. In the present report, we describe the presence and distribution of NK cells in a series of primary intracranial germinomas.

**Materials and Methods**

Paraffin-embedded samples of nine primary intracranial germinomas were processed for phenotypic identification of the surface marker IOT-10 of NK cells. The avidin-biotin-peroxidase complex (ABC) technique was employed. Specific murine monoclonal antibody* diluted 1:50 was used. After the sections were dewaxed, they were hydrated with graded alcohol series and rinsed for 5 minutes in distilled water. The sections were then incubated for 30 minutes in 0.3% H2O2 in methanol, and washed in phosphate-buffered saline (PBS) for 20 minutes. Normal horse serum (3%) was applied for 20 minutes to decrease nonspecific background staining; thereafter, the sections were incubated with primary antibody for 30 minutes in a humidified chamber. This was followed by washing in PBS for 15 minutes, repeated three times. Secondary biotinylated horse antibody against mouse immunoglobulins† was then applied for 20 minutes, followed by a 45-minute wash in PBS. Next, ABC reagent‡ was added over 20 minutes, and the sections were washed for 60 minutes with four 15-minute intervals. Following this, 3-amino-9-ethylcarbazole (AEC) was added for 5 minutes, the sections

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* Murine monoclonal antibody supplied by Immunotech, Marseille, France.
† Immunoglobulin supplied by Vector Laboratories, Burlingame, California.
‡ ABC reagent supplied by Vectastain, Vector Laboratories, Burlingame, California.
Natural killer cells in intracranial germinomas

TABLE 1
Clinical data for nine patients with germinomas studied in this series

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex, Age (yrs)</th>
<th>Location of Tumor</th>
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<tbody>
<tr>
<td>1</td>
<td>M, 14</td>
<td>pineal</td>
</tr>
<tr>
<td>2</td>
<td>M, 14</td>
<td>pineal</td>
</tr>
<tr>
<td>3</td>
<td>F, 4</td>
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<tr>
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<td>pineal</td>
</tr>
<tr>
<td>5</td>
<td>M, 15</td>
<td>pineal</td>
</tr>
<tr>
<td>6</td>
<td>F, 14</td>
<td>suprasellar</td>
</tr>
<tr>
<td>7</td>
<td>M, 9</td>
<td>suprasellar</td>
</tr>
<tr>
<td>8</td>
<td>M, 16</td>
<td>suprasellar</td>
</tr>
<tr>
<td>9</td>
<td>M, 8</td>
<td>parietal</td>
</tr>
</tbody>
</table>

FIG. 1. Photomicrographs of a primary intracranial germinoma showing stained (IOT-10-positive) cells (arrows) among large tumor cells. Avidin-biotin-peroxidase complex technique, ×500.

were washed for 5 minutes, and finally hematoxylin was added for 2 minutes. The slices were mounted with aqueous mounting medium and observed under the microscope. Positive controls consisted of human tonsil, and negative controls were obtained by incubating the tumor slices with normal mouse serum diluted to 1:50 with PBS instead of the primary antibody.

Two different observers studied at least five sections from each tumor, and immunostained cells were counted on photomicrographs with the same magnification. Table 1 shows the tumor location and significant data of the patients from whom the germinomas were obtained. Only one patient (Case 3) received radiotherapy (50 Gy) before the tumor tissue was resected. Two patients (Cases 4 and 6) died due to surgical complications. The other patients received radiotherapy after histological diagnosis, and they are alive and well now, with a follow-up period ranging between 1 and 14 years.

Results

The histological pattern was similar in each tumor studied, showing the typical features of intracranial germinomas. The appearance included large polygonal or spheroid cells and a variable number of lymphocytes that were grouped or irregularly distributed among the tumor cells. In all cases, IOT-10-positive NK cells were found; however, the number of immunostained cells was small in every case (< 1% of TIL's). Although NK cells were occasionally identified in a perivascular location or within groups of lymphocytes, they were mainly found among the tumor cells (Fig. 1).

In the tumors studied, the presence of NK cells was not related to the degree of lymphocytic infiltration, because cases with many TIL's showed a small number of immunostained cells. On the other hand, even considering the small number of cases in our series, the number and distribution of immunostained cells seems not to be related to factors such as tumor location, previous radiotherapy, or sex or age of the patients.

Discussion

The histology of intracranial germinomas is intriguing because they exhibit many lymphocytes among large tumor cells, suggesting a peculiar host-immune response to these tumors. Moreover, it has been reported recently that the TIL composition of primary intracranial germinomas differs not only from most other types of tumors, but also from seminomas, a tumor histogenetically related to intracranial germinoma. The immunohistochemical characterization of TIL's in primary intracranial germinomas showed that 70% to 80% of them were T cells and 20% to 30% were B cells. A higher proportion of T cells expressing the Leu-3 antigen (helper/inducer cells) in relation to those expressing the Leu-2a antigen (cytotoxic/suppressor cells) was found in the case studied by Paine, et al., in an abdominal metastasis from a suprasellar germinoma, and in at least two of the three primary intracranial germinomas recently reported by Saito, et al.

The present study shows, for the first time, the number and distribution of NK cells in primary intracranial germinomas. These data add to the knowledge about the peculiar immunological features of these tumors. In our series, IOT-10-positive NK cells were found in small numbers, mainly scattered among the tumor cells. This pattern is similar to that previously reported in glioblastomas, and it suggests that both glioblastomas and primary intracranial germinomas show similar features when spontaneous immunological reaction mediated by NK cells is considered; however, a greater number of TIL's are present in intracranial germinomas. This study suggests that, as in glioblastomas, and in intracranial metastasis (unpublished data), there is a
discrepancy between the degree of lymphocytic infiltration and that of NK cell infiltration in each tumor. Although further studies are necessary in order to establish the real immunological significance of NK cells in brain tumors, our data suggest that factors other than the mere quantity of TIL's determine the presence of NK cells in primary intracranial germinomas.

References


Manuscript received July 3, 1989.
The publication of this work was supported in part by the FIS, Insalud, Spain.

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