Brief Communication

Expression by Human Neuroblastoma Cells of an Antigen Recognized by Naturally Occurring Mouse Anti-Brain Autoantibody

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SUMMARY

A number of human tumor cell lines of both neural and nonneural origin have been assayed for the expression of an interspecies brain antigen (mouse brain antigen 2), detected by naturally occurring antibodies in normal mouse sera. These experiments indicate that mouse brain antigen 2 is present on four human neuroblastoma cell lines but not on other human tumor cell lines tested, including four glial cell lines and a retinoblastoma. These findings demonstrate the value of naturally occurring antibodies in normal mouse sera for the detection and serological classification of human tumor antigens and indicate that considerable caution should be used in attempts to distinguish tumor-specific and tissue-specific antigen expression on human neuroblastoma cells.

INTRODUCTION

Normal sera of mice of a wide variety of strains including germ-free and congenitally athymic (nude) mice are cytotoxic, in the presence of rabbit complement, for the teratoma-derived murine neuroblastoma cell line NB1 (6-8). The cytotoxicity of normal serum for NB1 cells is due to naturally occurring IgM antibodies (6-8). Anti-NB1 cytotoxic antibodies in normal mouse sera can be specifically absorbed by homogenized brain and kidney tissue of mice and man and by homogenized brain tissue of rats, guinea pigs, and chickens (6). The interspecies brain antigen recognized by NOA² in mouse sera has been designated MBA-2. It is of interest to know whether the expression of MBA-2 is restricted to a subpopulation of neural cells. We have therefore tested a variety of human tumor cell lines of both neuronal and glial origin for the ability to absorb specifically anti-MBA-2 antibody activity from normal mouse serum.

MATERIALS AND METHODS

Mouse Sera. For the experiments reported in this study, sera of normal 6- to 9-month-old male C3Hf/HeN mice were used.

Murine Cell Lines. The NB1 cell line was established from a neuroblastoma adrenal metastasis of a primary ovarian teratoma of a C3Hf/HeCr fa mouse. The contact-inhibitable epithelial cell line Ter-A is 1 of several cell lines derived from the ovarian teratoma. N-T16 is a cloned cell line derived from the C1300 murine neuroblastoma (2).

Human Cell Lines. The human cell lines assayed for the expression of the MBA-2 antigen are listed in Table I.

Absorption of Mouse Sera. Aliquots of undiluted mouse sera were absorbed with a 1:3 packed volume of either tumor cells at 4° for 40 min or homogenized normal tissues at 25° for 40 min. The suspensions were centrifuged at 8000 x g for 1.5 min in a microcentrifuge (Beckman Instruments, Table 1

<table>
<thead>
<tr>
<th>Designation of cell line</th>
<th>Tissue origin of cell line</th>
<th>Passage</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>SK-N-MC</td>
<td>Neuroblastoma</td>
<td>54</td>
<td>A</td>
</tr>
<tr>
<td>SK-N-SH</td>
<td>Neuroblastoma</td>
<td>28</td>
<td>A</td>
</tr>
<tr>
<td>LANEY</td>
<td>Neuroblastoma</td>
<td>8</td>
<td>B</td>
</tr>
<tr>
<td>LANTB</td>
<td>Neuroblastoma</td>
<td>14</td>
<td>B</td>
</tr>
<tr>
<td>A172</td>
<td>Glioblastoma</td>
<td>36 + 77</td>
<td>C + D</td>
</tr>
<tr>
<td>I18 MG</td>
<td>Astrocytoma</td>
<td>215</td>
<td>C</td>
</tr>
<tr>
<td>HS0683</td>
<td>Glioma</td>
<td>13</td>
<td>C</td>
</tr>
<tr>
<td>HA207</td>
<td>Glioblastoma</td>
<td>29</td>
<td>D</td>
</tr>
<tr>
<td>Y-79</td>
<td>Retinoblastoma</td>
<td>&gt;100</td>
<td>E</td>
</tr>
<tr>
<td>FL</td>
<td>Normal amnion</td>
<td>19</td>
<td>B</td>
</tr>
<tr>
<td>MT</td>
<td>Osteogenic sarcoma</td>
<td>102</td>
<td>B</td>
</tr>
<tr>
<td>T 24</td>
<td>Bladder carcinoma</td>
<td>28</td>
<td>B</td>
</tr>
</tbody>
</table>

¹ Graduate student at University College London.
² The abbreviations used are: NOA, naturally occurring antibodies; MBA-2, mouse brain antigen 2; BSS-10, balanced salt solution containing 10% fetal calf serum; RC', rabbit complement.

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Inc., Rockville, Md.), and the absorbed sera were tested for residual cytotoxic activity. Tissue absorption does not release anticomplementary factors (6).

**51Cr Release Cytotoxicity Assay.** A 2-step complement-dependent 51Cr release cytotoxicity assay was performed as previously described (5–7). Briefly, 20 μl of a suspension of 6 × 10^4 51Cr-labeled tumor cells in BSS-10 were mixed with 20 μl of unabsorbed or absorbed mouse serum appropriately diluted. The mixture was incubated at 37° for 60 min. Cells were washed with 4 ml of BSS-10 and resuspended in 30 μl of a 1:4 dilution of mouse tissue-absorbed RC'. The mixture was incubated at 37° for 60 min, 2 ml of BSS-10 were added, and the tubes were centrifuged at 800 x g for 10 min. The radioactivity in 1 ml of supernatant (a) and that in the remaining 1 ml of supernatant plus the cell pellet (b) were measured. The absolute percentage of lysis of target cells is defined by the formula 

\[
\text{Percentage Lysis} = \frac{(2 \times a)/(a + b)}{x 100}
\]

Four groups of controls were included in each experiment. The control groups were as follows: medium control (Med), initial incubation with BSS-10 and 2nd incubation with BSS-10; antibody control (Ab), initial incubation with mouse serum and 2nd incubation with BSS-10; complement control (Comp), initial incubation with BSS-10 and 2nd incubation with RC'; and maximum release (FT), initial incubation with BSS-10 followed by 3 cycles of freezing and thawing. The specific (%) lysis was calculated by the formula

\[
\frac{(\text{Absolute % lysis due to serum + RC'})}{\text{FT-Comp}}
\]

The observed values for Ab and Med controls did not differ by more than 1% lysis. The Comp value for NB1 cells averages 8.9%, for N-T16 cells it averages 13.7%, and for Ter-A cells the average is 10.8%. These values were only slightly higher than the corresponding values observed with the Med controls, i.e., the RC' used was essentially nontoxic for the cell lines tested. In each assay, duplicate determinations were performed.

**RESULTS**

Aliquots of normal mouse serum were absorbed with either human neuroblastoma or nonneural human cell lines and tested for residual cytotoxic activity. Chart 1 illustrates that absorption of normal mouse serum with each of 4 human neuroblastoma cell lines significantly reduced anti-NB1 cytotoxic activity. Included in this experiment were aliquots of the same pool of serum absorbed with either a retinoblastoma, an osteogenic sarcoma, a bladder carcinoma, or a normal amnion-derived cell line. None of the latter cell lines significantly absorbed anti-NB1 cytotoxic activity from normal sera. Chart 2 illustrates the results of an experiment in which the human neuroblastoma cell line, SK-N-MC, and 3 human glial tumor cell lines, A172, 118 MG, and HSO683, were tested for their ability to reduce anti-NB1 cytotoxic activity of mouse serum. Again, SK-N-MC cells significantly reduced anti-NB1 cytotoxic activity.

None of the glial cell lines tested, however, absorbed anti-NB1 cytotoxic NOA. In similar experiments, the glial tumor cell line HA207 was found not to absorb anti-NB1 cytotoxic activity in normal mouse serum. When sera absorbed with the neuroblastoma and glial cell lines were tested for cytotoxic activity against the murine neuroblastoma cell line N-T16, no significant reduction of cytotoxicity was observed. Chart 3 depicts results obtained in an experiment in which sera absorbed with the neuroblastoma cell lines SK-N-MC and SK-N-SH, and the glial cell lines HSO683 and A172 were tested against N-T16 target cells.
Table 2
Expression of NBI-associated antigen MBA-2 on normal brain and kidney tissue

<table>
<thead>
<tr>
<th>Tissue used for absorption</th>
<th>Residual cytotoxicity (%) of absorbed serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse brain</td>
<td>9.0</td>
</tr>
<tr>
<td>Mouse kidney</td>
<td>12.1</td>
</tr>
<tr>
<td>Mouse liver</td>
<td>105.2</td>
</tr>
<tr>
<td>Human brain</td>
<td>36.7</td>
</tr>
<tr>
<td>Human kidney</td>
<td>54.8</td>
</tr>
<tr>
<td>Human liver</td>
<td>104.2</td>
</tr>
</tbody>
</table>

* Aliquots of normal mouse serum were absorbed with a 1:3 volume of homogenized tissues, and the absorbed serum was tested for cytotoxic activity against NBI and Ter-A target cells. The results are expressed as the percentage of the specific lysis achieved against these target cells by unabsorbed serum.

DISCUSSION

The results reported in this paper indicate that an antigen detected by mouse NOA on the murine neuroblastoma cell line NBI is present on the 4 human neuroblastoma cell lines tested but not on a variety of other human tumor cell lines tested, including a retinoblastoma and 4 glial tumor cell lines. The antigen can readily be detected on normal brain and kidney tissue of both mice and man and is, therefore, a normal tissue, rather than a tumor-specific, antigen. These findings are relevant to the assumption that the common neuroblastoma antigen recognized by lymphoid cells and sera of neuroblastoma-bearing patients is tumor specific (3, 4). Thus, a lack of cross-reactivity with other tumor cell lines, even including cells of glial origin, would not necessarily exclude that the anti-neuroblastoma reactivity seen in patients might be directed against the brain-associated normal tissue antigen MBA-2.

MBA-2 is not detectable on the N-T16 neuroblastoma, and it is quite possible that some human neuroblastoma cell lines will also lack MBA-2. The nature of MBA-2 and its significance in terms of the biological behavior of neuroblastoma cells are presently obscure. By testing for the expression of MBA-2 on a wide variety of human neuroblastomas, it may be possible to correlate MBA-2 expression with a particular clinical characteristic. It is also possible that MBA-2 expression may unexpectedly occur on certain tumors that are not neuroblastomas. Thus, for example, an antigen recognized by murine NOA on the N-T16 cell line is also present on the murine glioblastoma G26 and on 1 of 2 murine lung tumor cell lines tested (unpublished data). Again, however, the detection of aberrant expression of cell surface antigens by malignant cells could be important in understanding the diverse clinical behavior of tumors of the same tissue origin. Finally, a wide variety of cell surface antigens can be detected on murine tumors using NOA (7–9). Detailed studies on antigenic cross-reactivity detected by murine NOA between murine and human tumor cell lines may provide a valuable approach to the serological classification and detection of human tumors.

ACKNOWLEDGMENTS

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Chart 3. Anti-N-T16 cytotoxicity of normal mouse serum either unabsorbed (○); absorbed with the human neuroblastoma cell lines SK-N-MC (■) and SK-N-SH (△); or absorbed with the human glial cell lines HS0683 (▲) and A172 (●).
REFERENCES


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