Cell-Surface Ganglioside GD2 in the Immunohistochemical Detection and Differential Diagnosis of Neuroblastoma

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The expression of the disialoganglioside GD2 was analyzed in 67 solid tumors and normal tissues from children by using the GD2-specific murine monoclonal antibody 3A7 and the indirect immunoperoxidase method. GD2 was expressed in all of 28 neuroblastomas and was most abundant in stroma-poor tumors. In differentiating stroma-rich neuroblastomas, neuroblast clusters, neurofibrils, and most ganglion-like cells were positive, whereas Schwann's-cell stroma did not express GD2. In ganglioneuromas, only a few ganglion-like cells showed GD2, whereas all other structures were negative. Scattered foci of ganglioside GD2 were found in some non-neuronal tumors, such as rhabdomyosarcomas and osteosarcomas, but not in lymphomas, Askin tumors, or most Wilms' tumors. The monoclonal antibody 3A7 is a useful aid in the immunohistochemical diagnosis of neuroblastoma. In addition, the intense cell surface staining of neuroblastoma cells by this reagent makes it potentially useful for detecting residual neuroblastoma in bone marrow samples and lymph node biopsies. (Key words: Pediatric neoplasms; Soft tissue neoplasms; Neuroectodermal tumors; Sarcomas) Am J Clin Pathol 1991;96:248-252

MATERIALS AND METHODS

Materials

Case materials consisted of fresh frozen tissues from pediatric solid tumors, obtained either by surgery or biopsy...
at initial presentation or after chemotherapy. The samples were frozen in liquid nitrogen and cryopreserved at -70 °C until they were analyzed. Twenty-eight neuroblastomas (including five well-differentiated stroma-rich ganglioneuroblastomas) were analyzed. The diagnosis was based on light and electron microscopy of the tumors, immunohistochemical demonstration of neurofilaments and synaptophysin, and elevated urinary catecholamine secretion. Other solid tumors of childhood included lymphoma (N = 6), Wilms' tumor (7), renal malignant rhabdoid tumor (1), rhabdomyosarcoma (10), ganglioneuroma (3), alveolar soft part sarcoma (1), small-cell desmoplastic tumor of childhood (1), Askin's tumor (2), osteogenic sarcoma (5), synovial sarcoma (1), and anaplastic sarcoma of the urinary tract (1). Biopsies of normal brain, sympathetic ganglion, adrenal gland, striated muscle, lymph node, thyroid gland, gut, myocardium, spleen, thymus, liver, pancreas, and kidney were obtained from autopsies of neonates (2) and fetuses of 20 to 22 weeks' gestation (2).

All samples were frozen in liquid nitrogen, cut at 10 μm with a cryostat (Cambridge Instruments GmbH, Nussloch, FRG), air-dried at room temperature for 30 minutes, fixed for 10 minutes in cold (−20 °C) acetone, and stained by the indirect immunoperoxidase method, as described here.

Immunohistochemistry

The murine monoclonal antibody (MoAb) 3A7 is of the IgM class and specifically labels the cell-surface disialoganglioside GD2.18 3A7 is not commercially available and was provided by Dr. N-K.V. Cheung, the Memorial Sloan Kettering Cancer Center, New York, New York. MoAb 3A7 was used as a hybridoma supernatant. It was diluted at 1:6 in Tris-buffered saline (TBS) containing 0.1% bovine serum albumin (pH 7.4). The final concentration of the antibody was 3 μg/mL. Sections were incubated for 10 minutes in 10% normal rabbit serum, blotted, incubated with the primary antibody for 30 minutes, and washed three times for 5 minutes in TBS. The secondary antibody, a horseradish peroxidase-labeled rabbit antimeumonoglobulin (Dakopatts P-161, Copenhagen, Denmark), was diluted at 1:400 and incubated for 30 minutes at room temperature. After three further washes in TBS, sections were immersed for 20 minutes at room temperature in a freshly made solution of 3-amino-9-ethylcarbazole (40 mg) in 12 mL of N, N-di methylvformamid mixed in 200 mL of acetate buffer (0.05 M, pH 5.0) and 200 μL of hydrogen peroxide. The sections were counterstained with hematoxylin and mounted in Aquamount* (BDH Limited, Poole, UK).

In the preparation of negative controls, the primary antibody was omitted. In addition, before the 3A7 antibody incubation, some sections were fixed for 5 minutes in methanol. This solvent effectively dissolves the GD2 ganglioside. Only frozen sections of tumor or tissue were used in the study. The 3A7 antibody is not applicable to paraffin-embedded samples because gangliosides are dissolved during tissue processing.

RESULTS

The use of MoAb 3A7 in immunohistochemical studies demonstrated that all 28 neuroblastomas expressed the GD2 ganglioside (Fig. 1A). 3A7 reacted strongly with the membranes of the neuroblastoma cells. Although all neuroblastomas were labeled with 3A7, staining intensity was dependent on the differentiation of the tumor cells. The strongest staining reaction was obtained in stroma-poor undifferentiated neuroblastomas, in which all tumor cells evenly expressed GD2. In differentiating neuroblastomas, neurofilaments were regularly stained, whereas most mature ganglion-like cells in ganglioneuroblastomas and the Schwann's-cell stroma in stroma-rich neuroblastomas were negative for GD2. Little if any GD2 was seen in ganglioneuromas, in which only a few ganglion-like cells bound 3A7 (Fig. 1B). The reactivity of neuroblastoma cells did not decrease during cryopreservation (up to 72 months). Methanol fixation for 5 minutes abolished the staining reaction.

Other "small, round, blue-cell tumors" of childhood, such as lymphoma and Askin's tumor, did not express GD2. Scattered small foci of positive cells were found in rhabdomyosarcomas and Wilms' tumors, in which occasional positive areas were found in the stroma. GD2-positive cells were not seen in blastemal or tubular areas of Wilms' tumors. The undifferentiated cellular areas of most osteogenic sarcomas were labeled by 3A7 (Fig. 2). These results are summarized in Figure 3.

In normal neonatal and embryonic tissues (20–22 weeks' gestation), binding of 3A7 was found in the capsule of the parathyroid gland and the thymus; neuroendocrine cells of the lung; and the peripheral nerves. The adrenal gland, brain, liver, kidney, pancreas, striated and smooth muscle, gut, myocardium, aorta, and sympathetic ganglia were negative.

DISCUSSION

The monoclonal antibody 3A7 appears to be a sensitive marker for neuroblastoma and is a good adjunct in the differential diagnosis of "small round-cell tumors" of childhood. All 28 neuroblastomas in this series were positive by immunoperoxidase staining with this antibody. The ganglioside GD2 was abundant in undifferentiated neuroblastic areas, but it was not expressed in well-differentiated, stroma-rich areas of neuroblastomas. Ganglioneuromas generally were negative for GD2; only a
Fig. 1. Binding of the GD2-specific antibody 3A7 to (A) stroma-poor, undifferentiated neuroblastoma and (B) ganglioneuroma. Note that a ganglion-like cell marked by an arrow shows expression of GD2. A and B, indirect immunoperoxidase staining of frozen sections (×250).

Fig. 2. Binding of the 3A7 antibody to osteogenic sarcoma. Primitive cellular areas are positive, whereas more mature foci do not bind the antibody. Indirect immunoperoxidase staining of frozen sections (×250).

Few ganglion-like cells were labeled by 3A7. This finding is analogous to the normal developmental shift in the brain from synthesis of mono- and disialated gangliosides to the more complex multisialated gangliosides.\textsuperscript{27,28} Our results suggest that the immunoperoxidase technique with MoAb 3A7 may be more sensitive than thin-layer chromatography\textsuperscript{19} in the detection of ganglioside GD2 in tumor samples.

The ganglioneuromas we studied generally were negative for GD2. In neuroblastomas, neurofibrils were labeled by 3A7. Therefore, the loss of ganglioside GD2 may not be explained solely through neuronal differentiation of the tumor cells but may reflect the amount of Schwann's-cell stroma in such tumors. The antibody 3A7 also may be a useful aid in assessing the tumors according to the classification of Chatten and colleagues,\textsuperscript{29} which is based in part on the level of stromal differentiation.

Some other pediatric solid tumors, such as osteogenic sarcomas, some rhabdomyosarcomas, and one of seven Wilms' tumors, showed scattered foci of GD2-reactivity. This is in accord with the findings of Cheung and colleagues,\textsuperscript{18} who noted reactivity with 3A7 in three of six
We conclude that immunohistochemical studies with the anti-GD2 monoclonal antibody 3A7 are helpful in the detection and differential diagnosis of neuroblastoma. In addition, antibodies against GD2 have proven to be useful therapeutically in antibody-mediated binding of radioactive molecules to neuroblastoma cells and in detecting residual neuroblastoma in bone marrow aspirates. The role of the ganglioside GD2 as a differentiation-related antigen during neuronal maturation merits emphasis and further study.

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**REFERENCES**


