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IMMUNOLOGICAL MARKERS OF BRAIN GLIOMAS

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Summary

Immunological markers of human gliomas have been looked for by studying the patient's immune response or by raising heteroantisera against these tumours. More recently, the hybridoma technology has allowed to produce monoclonal antibodies recognizing a single antigenic determinant.

This paper describes the production of monoclonal antibodies against human glioma-associated antigens and discusses their possible applications as diagnostic tools.

Résumé

Les marqueurs immunologiques des tumeurs gliales ont été recherchés en étudiant la réponse immune des malades porteurs de tumeurs ou en produisant des hetero-antisera contre ces tumeurs. Récemment, la technique des hybridomes a permis de produire des anticorps monoclonaux reconnaissant un seul déterminant antigénique.

Cet article décrit la production d'anticorps monoclonaux contre des antigènes associés aux gliomes humains et discute les possibilités de leur utilisation diagnostique.

Introduction

Gliomas are a group of tumours that arise mainly from neuroglial astrocytes, oligodendrocytes, and ependymal cells (1). Kernohan and Sayre's (2) system of grading glial tumours by increasing order of malignancy from I to II is widely used; it is convenient to divide these tumours into benign gliomas (grades I-II) and malignant gliomas (grades III-IV, glioblastomas). Glioblastoma multiforme, the most malignant glioma, is thought to arise from anaplastic transformation of glial cells, mostly astrocytes, and represents 55 % of all intracranial gliomas in all age groups. Mixed gliomas, composed of several gliogenous elements, are not uncommon

(1). It thus appears that on morphological grounds, gliomas represent a highly heterogenous group.

This heterogeneity of cells derived from individual gliomas has been demonstrated by Bigner et al. (3), who studied the phenotypic and genotypic characteristics of several human glioma cell lines. Gliomas share some antigens with normal brain cells. Glial fibrillary acidic protein (GFAP) is a specific marker for glial cells, especially astrocytes (4, 5). This protein is found in gliomas of astrocytic origin and some ependymomas and is useful for their diagnosis (6). However, levels of GFAP decrease with increasing malignancy (7, 8, 9). This marker is almost absent from anaplastic glial cells and it also usually disappears from tumour cells after several passages in culture (10). Another marker called S100 protein is present in normal brain glial cells and Schwann cells of the peripheral nervous system (11, 12), but S100 levels in glial tumour tissue are also inversely proportional to the degree of malignancy (13, 14). In glioma cells maintained in vitro, the presence of S100 protein has been reported by some authors (15), but was not found by others (16). Recently, S100 protein has also been found in large amounts in cultured human malignant melanomas (17), in malignant melanoma tissue (18), as well as in melanocytes and Langerhans cells of normal skin (19). α-2-glycoprotein is another protein found predominantly in oligodendrocytes (20) and associated with gliogenous tumours; its concentration also correlates inversely with the malignancy of the tumours (21).

The possible presence of tumour-associated antigens on glioma cells has been suggested by reports from several investigators. These antigens were identified either by studying the humoral and cellular immune responses of glioma patients (22-28) or by raising heteroantisera against these tumours (29-34). The major drawback of the serological methods however is the overwhelming number of contaminating antibodies in sera that have to be removed by extensive absorptions before any tumour specificity can be demonstrated. The production of somatic cell hybrids secreting monoclonal antibodies against single antigenic determinants, as described by Köhler and Milstein (35), represents a great improvement for the search of antibodies directed against tumour associated antigens. Recently, several authors have produced monoclonal antibodies against various human tumour markers or antigens (36-42).

We have analysed the specificity of monoclonal antibodies secreted by hybrids obtained from a fusion between mouse myeloma cells and spleen cells from a mouse immunized with malignant glioma cells from the LN-18 line (43).

Materials and methods

Development and characterization of established human malignant glioma lines.

All gliomas operated in the neurosurgical service are routinely cultured in our laboratory. To date, we were able to establish 15 permanent lines. HLA typing has been carried out for each line, 7 of them were studied by electron microscopy. All lines were tested for the presence of GFAP by direct immunoperoxydase using an antiserum given by Dr. L.F. Eng, Palo Alto, Califomia. One of these cell lines has been extensively characterized and published (44), and used for raising monoclonal antibodies. We also were able to obtain two clones from two different lines, which retain their capacity to synthetize GFAP after more than 50 passages.

Specificity analysis of monoclonal antibodies raised against whole human malignant glioma cells.

We derived hybridoma cells from a fusion between mouse P 3X 63 / Ag8 myeloma cells and spleen cells from a mouse immunized with whole cells of the human malignant glioma line LN-18. Of 345 hybrids obtained, 36 secreted antibodies that reacted with the glioma cell line used for immunization as assayed by an indirect antibody-binding radioimmunoassay. After a first screening for the absence of reactivity on two nonliogenous cell lines, 3 hybrids were selected and cloned by limiting dilution. The specificity of these monoclonal antibodies was then investigated on a panel of 47 cell lines derived from human malignant gliomas, 18 cell lines from nongliogenous neoplasms, as well as normal peripheral blood lymphocytes, normal skin fibroblasts and normal spermatozoids. The monoclonal antibodies from two positive hybrids, BF7 and GE2, reacted preferentially with glioma cells and appear to be directed against common malignant glioma antigen(s). BF7 antibodies bound to 38 and GE2 to 43 out of 47 glioma cell lines. The third monoclonal antibody, CG12 showed a broad reactivity since it bound to 23 out of 47 glioma lines, 8 out of 9 melanoma lines and 2 out of 3 neuroblastoma lines. Absorption with normal adult and fetal brain homogenates did not modify the binding capacity of BF7 and GE2 for glioma cells, while the binding of CG12 antibodies was abolished. Reciprocal binding inhibition tests using (3H)-leucine-labelled antibodies showed that BF7, GE2 and CG12 antibodies were directed against different antigenic determinants. BF7 and GE2 antibodies bound weakly to one (colon carcinoma) of 18 lines derived from nongliogenous neoplasms, whereas CG 12 antibodies bound to none of these.

Preliminary immunochemical characterization of a glioma antigen.

Cells from glioma lines were labelled either with ^{125}I by the lactoperoxydase method or with ^{35}S-methionine biosynthetically. Radiolabelled cell lysates were immunoprecipitated with the 3 monoclonal anti-glioma antibodies BF7, GE2 and CG12, using protein A-Sepharose to bind the antigen-antibody complexes, followed by analysis in SDS-PAGE and autoradiography or fluorography. The overwhole results showed that both BF7 and GE2 antibodies gave a major protein band with an apparent molecular weight of 48 KD, with glioma cell lysate labelled by either of the two isotopes, whereas CG12 did not precipitate any definite protein band in this range of molecular weight.

Use of monoclonal antibodies in the immunohistological diagnosis of fresh tumour material.

The three stage biotin-avidin-peroxydase system was used for staining fresh tumour tissue with the anti-glioma monoclonal antibodies GE2 and BF7. The monoclonal antibodies were used at the first stage, at the second stage biotinylated horse anti-mouse antiserum was added, and avidin-peroxydase conjugate at the third stage.

Fig. 1 is a section of a grade IV malignant glioma stained with monoclonal antibody GE2. An intense staining of rather well differentiated neoplastic astrocytes can be seen. The astrocytic processes are clearly visible.

Undifferentiated, multinucleated cells are also stained but with less intensity. The pattern of staining throughout the cellular body indicates that the antigen is present in large amounts in the cytoplasm and not restricted to the membrane.

Discussion

The identification of tumour-associated antigens by means of hetero-antisera is extremely difficult because of the large number of contaminating antibodies directed against antigens present on normal cells that must be removed by extensive absorption before any tumour specificity can be demonstrated. Our results show that the hybridoma technology can be used to identify glioma tumour associated antigens on glioma cells.

The malignant glioma specificity of the 2 antibodies, BF7 and GE2, directed against a putative common glioma-associated antigen was studied by a series of quantitative absorption experiments. Our first results showed that incubation with malignant glioma cells abolished the reactivity of BF7 and GE2 antibodies to malignant glioma cells, whereas nongliogenous cells were unable to reduce the binding capacity of BF7 and GE2 for gliomas. Absorptions with normal and fetal brain homogenates were also unable to reduce the binding capacity of

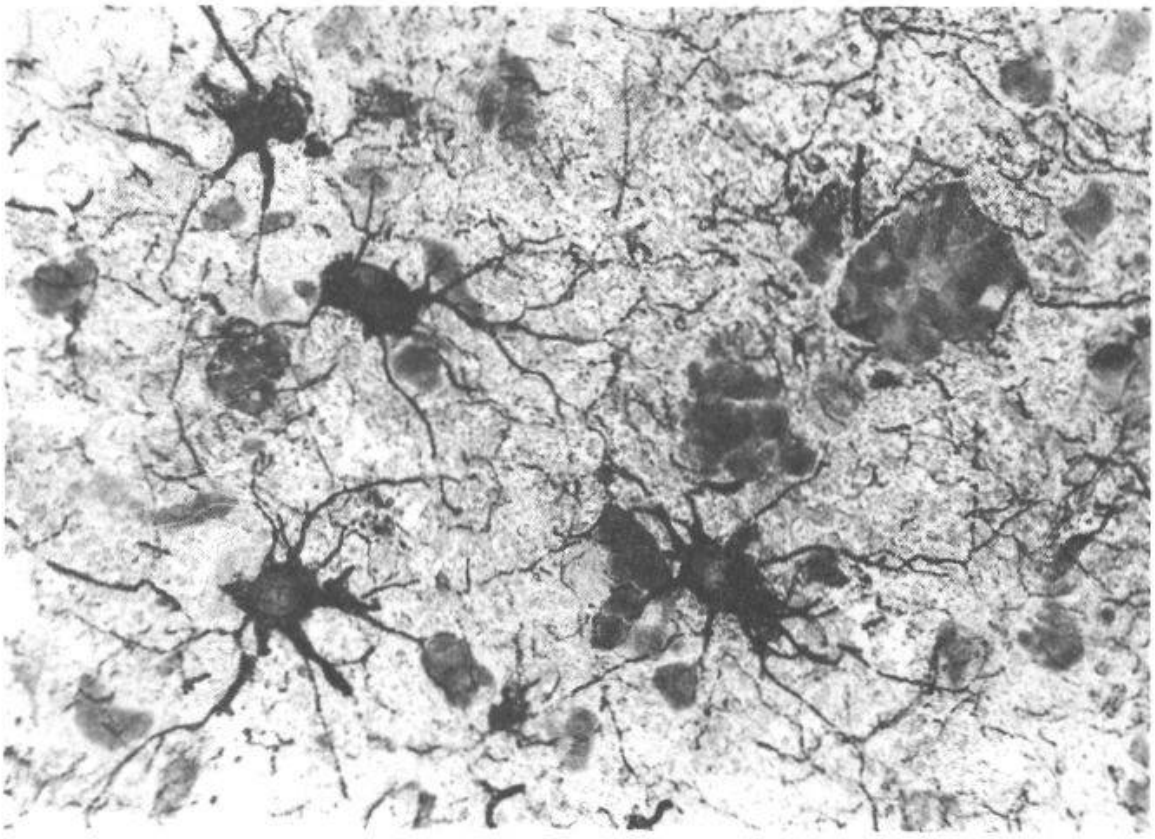

Fig. 1. Immunohistological localization of the antigen recognized by monoclonal antibody GE2 on resected glioma. Frozen section of a malignant glioma (x 400). Indirect immunoperoxydase using the Biotin-Avidin system with amino-ethyl-carbazol as chromogen. Neoplastic astrocytes, well differentiated, are stained throughout the cytoplasm and their processes are clearly visible. Anaplastic cells are stained with less intensity.

these 2 monoclonal antibodies for malignant glioma. From these experiments, we concluded that the antigenic determinants recognized by BF7 and GE2 antibodies were not expressed on normal and fetal brain cells, or at least not in an amount detectable by these absorption experiments.

Immunocytochemistry results have shown that the antigen is present in the cytoplasm of astrocytic cells and not only on their surface, as demonstrated by binding assays.

Although it has been shown by binding inhibition that BF7 and GE2 recognize two different antigenic determinants, the immunoprecipitation of radiolabelled cell lysate demonstrated that both monoclonal antibodies reacted with a similar if not identical polypeptide chain with an apparent molecular weight of 48 KD. The question then arises why these two monoclonal antibodies have different binding activities on many of the individual glioma cell lines tested, GE2 being usually more active than BF7. A possible explanation is that the determinant recognized by BF7 may be situated deeper in the cell membrane than the one

recognized by GE2 and is therefore less accessible to the antibodies. The cellular distribution and the molecular weight of this molecule are very similar to those of GFAP. It seems, however, unlikely that the 48 KD protein immunoprecipitated by GE2 and BF7 is GFAP, since the glioma cell line (C1-71) used for these experiments did not express GFAP. Furthermore, the glioma cell line (LN-18) used for immunization of the mouse which provided the lymphocytes for our fusions was also GFAP negative.

Analysis by fluorescent activated cell sorter of the antigen identified by GE2 and BF7 antibodies showed that it was expressed on a fraction (45-55 %) of the cells from the glioma line C1-121 (data not shown). It will be interesting to sort and subclone such cell lines in order to determine if one can obtain stable clones with a 100 % reactivity with GE2 and BF7 antibodies. Furthermore, the tumorogenicity in nude mice and cell division characteristics in vitro of putative positive or negative clones should be studied.

Quantitative absorption experiments performed with CG12 antibodies confirmed that they were directed against an antigenic determinant expressed on glioma as well as on melanoma cells. Absorption with normal adult and fetal brain homogenates reduced the binding capacity of CG12 culture fluid for malignant glioma as well as for melanoma. These results suggest that the antigenic determinant recognized by these antibodies has not a tumour but rather a tissue specificity for cells of a common embryological origin. Monoclonal antibodies which displayed a similar reactivity spectrum have already been reported by several authors. Kennett and Gilbert (45) raised monoclonal antineuroblastoma antibodies which cross-reacted with fetal brain cells and glioblastoma. Herlyn et al. (46) and Carrel et al. (47) raised monoclonal anti-melanoma antibodies which also cross-reacted with malignant glioma.

Thus we can conclude to the existence of at least two antigenic systems on glioma cells, the first is composed of glioma tumour associated antigens and the second of neuroectoderm differentiation antigens.

The qualitative and quantitative expression of these antigens is highly variable among different cell lines of the same tumour type and even among the cells from a single tumour line confirming the marked heterogeneity of glioma cells. Thus a panel of monoclonal antibodies recognizing tumour associated as well as differentiation antigens should be tested on histologic sections or cultured cells from each glioma in order to determine if any of these markers have a qualitative or quantitative correlation with the degree of malignancy. Monoclonal antibodies are valuable reagents for the precise identification of subpopulations of cells present in gliomas and they may help us in understanding the development of tumours of the central nervous system.

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