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Monoclonal Antibodies Reactive with Glioma Cell Lines Derived from Experimental Brain Tumors*†

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Abstract—*The production of hybridomas between X63-Ag8.653 myeloma cells and spleen cells from female BALB/c mice immunized with 79FR-G-41 glioma cells is reported. One hundred and six hybridoma clones were obtained secreting monoclonal antibodies (McAbs) against different target cells. Specificity tests (RIA, micro-ELISA) showed that McAbs produced by three hybridoma clones (13GC1, 14BC1, 14FC3) bound 79FR-G-41 glioma cells but did not react with fibroblasts, kidney and brain cells of newborn F344 rats. From the specificity analysis of two McAbs (13GC1, 14BC1) it is evident that these did not only react with 79FR-G-41 cells but also with other glioma cell lines (78FR-G-219, 78FR-G-284, 78FR-G-299 and 78FR-G-344) established from chemically induced rat brain gliomas. These results suggest, in accordance with the findings in human neuroectodermal tumors, the expression of common reactivity antigens in different brain tumors of glial origin. However, the McAbs obtained against experimental rat glioma cells did not recognize glioma cells derived from spontaneous brain tumors of dog or man. Immunofluorescence and immunoperoxidase tests indicate that there is a remarkable heterogeneity among cells of experimental glioma lines with respect to the expression of glioma associated determinants recognized by McAbs. The fact that only a variable number instead of the totality of tumor cells in any asynchronous and uncloned tumor cell population expresses, at a certain time, recognizable antigenic determinants has to be taken into account, particularly if they are considered to be employing McAbs as carrier molecules for diagnostic and therapeutic purposes.*

INTRODUCTION

THERE is increasing evidence that human and experimental brain tumors express glioma-associated antigens [1, 2]. It is well documented that cells from nitrosamide-induced neurogenic tumors in rats are able, in a native or chemically modified condition, to induce a specific humoral and/or cell-mediated immune response in syngeneic animals [3, 4]. The serological analysis of surface antigens of methylnitrosourea-induced gliomas shows the presence of different glioma-associated antigens (GAA). Identification, characterization and isolation of GAA by means of polyclonal sera is extremely difficult due to the large number of contaminating antibody populations.

Köhler and Milstein [5] ushered in a new era in immunological research by showing that somatic cell hybridization could be used to generate continuous 'hybridoma' cell lines producing monoclonal antibodies of predefined specificity. The possibility of obtaining monoclonal antibodies against GAA provide a major practical advantage with respect to their identification, purification and characterization.

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We now report on the production of monoclonal antibodies (McAbs) against the defined glioma cell line 79FR-G-41 and the characterization of their reactivity towards a variety of human and experimental glioma cell lines and normal rat cells.

MATERIALS AND METHODS

Experimental gliomas

Brain tumors were induced post-natally in inbred Fischer rats (F 344) of both sexes over 6 months of age by weekly oral administration of 6 mg/kg *N*-methyl-*N*-nitrosourea (MNU) in the drinking water. Details of the experimental protocols have been published [3, 6].

Cell cultures

Established glioma cell lines used in the study were derived from chemically induced rat gliomas and human brain tumors. With exception of the primary rat brain tumor 79/41 and the derived cell line 79FR-G-41, details of the experimental brain tumors 78/219 (pleomorphic glioma), 78/284 and 78/344 (mixed gliomas), 78/299 (astrocytoma II) and the corresponding glioma lines 78FR-G-219, 78FR-G-284, 78FR-G-344 and 78FR-G-299 have been reported previously [3].

The 79FR-G-41 cell line was established from a cherry-stone-sized tumor (79/41) of the right cerebral hemisphere. The tumor showed extensive yellowish necrosis, pseudocysts and hemorrhage on the cut surface with a strip of glassy gray tumor tissue remaining at the tumor periphery. Microscopically the tumor was quite cellular and fairly well provided with capillaries. It consisted of pleomorphic medium-sized cells with oval to round nuclei and eosinophilic cytoplasm with occasional processes. Mitoses and giant cells were abundantly present and the tumor was classified as a pleomorphic astrocytoma (astrocytoma grade II). The cell line 79FR-G-41 is tumorigenic. Syngeneic rats were injected s.c. with 10×10^6 cells or i.c. with 1×10^6 cells of this line. After about 2–4 weeks of latency, s.c. and i.c. tumors developed at inoculation sites. Morphologically and immunologically the 79FR-G-41-cells possess all properties of pleomorphic astrocytoma cells. This has been proved by the demonstration of S-100- and GFA-proteins, using the indirect immunofluorescence and immunoperoxidase methods [3]. Moreover, three human brain tumor lines were also tested as targets. The tumor cell line 76H-M-337 was cultivated from a childhood medulloblastoma. Biopsy specimens obtained at craniotomy were minced and explanted into Nunclon tissue-culture flasks (Nunc, Roskilde, Denmark) and fed M-199 containing 20% FBS (Seromed, Munich, F.R.G.). Permanent tumor cell lines were fed DMEM containing 10% FBS. The malignant glioma line (CI-71) was provided by one of the authors (N.deT.). Details of the establishment of the astrocytoma line 79H-G-396 have been published elsewhere [7, 8]. Furthermore, the cell line 77DG-G-166 established from a spontaneously occurring dog astrocytoma [9] was also tested. Normal Fischer rat kidney and brain cells were taken from newborn animals and cultured up to the 2nd passage before use.

Immunization procedure

Adult female BALB/c mice were immunized for a secondary response. The animals were immunized intraperitoneally (i.p.) with 5×10^6 native or TNBS-modified [3] whole 79FR-G-41-cells suspended in 0.2 ml PBS-A. The mice were injected i.p. with 1×10^6 native 79FR-G-41-cells 3 months later. Immunized mice were killed 3 days after the booster dose and a spleen cell suspension was prepared.

Production of hybridomas

The fusion of immune splenocytes of two mice with the non-secreting X63-Ag8.653 mouse myeloma line [10] was performed as described by Davidson and Gerald [11]. Single-cell fusion steps are summarized in the fusion protocol (Table 1). A 50% (v/v) polyethylene glycol (PEG MW 4.000, Merck, Darmstadt, F.R.G.) solution in serum-free RPMI medium supplemented with EDTA was used as fusogen. During the fusion procedure the PEG-solution was diluted gradually without disturbing cell clumps. Before distribution, the cells could recover for 1 hr at 37°C and 5% carbon dioxide. After fusion cells were distributed in four 24-well tissue-culture Nunclon plates of 1.5 cm diameter (Nunc, Roskilde, Denmark). Hybrids between X63-Ag8.653 myeloma cells and mouse immune splenocytes were selected according to established techniques [5] in a selective medium (MEM EARLE's, Seromed, Munich, FRG) containing 10 mM hypoxanthine, 0.04 mM aminopterin, 1.6 mM thymidine (Sigma), 20% gammaglobulin-free developed FBS and double-concentrated vitamins (HAT-medium). Established hybridoma cultures were fed the same medium without HAT additives and supplemented with 10% FBS. Untreated spleen cells were the best feeder layer directly after fusion, whereas mouse peritoneal cells were well suited for cloning or any other cultivation step. Peritoneal cells originate from BALB/c mice treated with thioglycollate medium. Immunoglobulin secreting hybridomas were cloned by the limiting dilution method using 96-well Nunclon microtiter plates (Nunc, Roskilde, Denmark).

Table 1. *Cell fusion protocol*

		Cell input	Spleen cells/myeloma cells
Myeloma cell line:	X63-Ag8.653	2×10^7	10:1
BALB/c mouse ♀:	spleen cells 2×10^8		
Antigen:	glioma line 79FR-G-41		
Immunization:	priming: 5×10^6	TNBS-modified glioma cells i.p.	
	boosting: $1{:}10^6$	native glioma cells i.p.	3 months later
Fusogen	PEG, mol. wt 4000, 50%		
Working temperature:	room temperature (21°C)		
Fusion procedure:	1 min	pellet + 1 ml PEG/RPMI	
	2 min	+ 1 ml RPMI	
	3 min	+ 2 ml RPMI	
	5 min	+ 4 ml RPMI/FBS 20%	
Stabilization:	1 hr, 37°C, 5% CO_2		
Feeder layer:	mouse spleen/peritoneal cells		

The cloning procedure was performed three times consecutively and then cultures were considered as clones.

Antibody binding assays

Screening for immunoglobulin production by HAT-selected mouse × mouse hybridomas was performed using the following techniques: (a) indirect immunoperoxidase stain, carried out on cells grown in Terasaki-plates with peroxidase-conjugated anti-mouse immunoglobulins [12]; (b) ^{3}H-radioimmunoassay (^{3}H-RIA) as an indirect binding method adapted to microtiter plates as described by Kummer *et al.* [13]; (c) ^{125}J-radioimmunoassay (^{125}J-RIA) according to the publications of Williams [14] and Carrel *et al.* [15]; and (d) microenzyme-linked immunosorbent assay (micro-ELISA) as described by Posner *et al.* [16]. Briefly, variable numbers of cells (10^4–5×10^5) were pipetted into 96-well flexible round-bottomed vinyl plates (Titertek/Linbro Nr. 76-364-05, Flow Laboratories, VA, U.S.A.). The plates were centrifuged at 900 *g* for 5 min, the supernatants aspirated, 20 µl of hybridoma products added and the plates incubated for 30 min at 4°C. After three washings with PBS-A, 50 µl of diluted (1:100) peroxidase-conjugated rabbit anti-mouse immunoglobulins (RAMP, Dako Laboratories, Denmark) were added to each well. After incubation for 30 min at 4°C the plates were washed three times with PBS-A/2.5% FBS and then three times with PBS-A. After the final wash 100 µl of freshly prepared substrate solution [0.1 M citrate buffer, pH 4.5, with 1 mg/ml of orthophenylendiamine (Sigma, St. Louis, MO, U.S.A.) and 4 µl/ml 3% hydrogen peroxide] was added to each well and the color change observed at 30 min. Reactions were graded from 0, the color of substrate alone without cells, to ++++, the color of substrate with 1 µl of RAMP. Furthermore, after having stopped the reaction with 25 µl of 8 N sulfuric acid a quantitative reading of the micro-ELISA reaction product was performed using a Titertek Multiscan ELISA reader (Flow Laboratories, Bonn, F.R.G.).

Moreover, the cell-binding behavior of the antibodies produced by selected hybridoma clones was determined by the indirect immunofluorescence (IIF) test as reported by Stavrou *et al.* [17] with live or acetone-, glutaraldehyde- and formalin-fixed cells.

Absorption experiments

Absorption with glia and glioma cells. Cultured cells for absorption were harvested from cultivation flasks by washing them in PBS-A and adding 2 ml lukewarm STV-solution. The flasks were placed at 37°C for 2–5 min and occasionally shaken to help loosen up the monolayer. Then 8 ml DMEM containing 10% FBS was added and the cells were suspended by repeated pipetting. Hybridoma supernatants were absorbed with cultured cells at a ratio of 5×10^6, washed and the cells packed per 0.1 ml culture fluid for 1 hr at room temperature and then overnight at 4°C. The remaining activity of the supernatants was then tested by antibody-binding tests using glioma cells as targets. For quantitative absorption experiments increasing numbers of cells (0.5–10×10^6) were suspended in aliquots of a constant dilution of culture supernatant (1:10). After incubation for 1 hr at room temperature or overnight at 4°C the cell suspension was centrifuged at 1500 *g* for 5 min to remove the cells plus bound antibody and the remaining activity on the supernatant was tested by ^{3}H-RIA.

Absorption with brain tissue. Tissue specimens of brain from newborn and adult F344-rats were mechanically desintegrated and homogenized in a 5-fold volume of PBS-A. The homogenates were centrifuged at 500 *g* and the pellets were washed 5 times with PBS. After washing the pellets from newborn and adult rat brains were separately collected and lyophilized. The absorption was

performed by using insoluble brain powder for incubation with hybridoma fluids (1 mg/ml) as described above.

Isolation and characterization of McAbs

The enrichment of McAbs from supernatants was performed by using saturated ammonium sulfate solution according to the details given by Jonak [18]. Briefly, the saturated solution of $(NH_4)_2SO_4$ was prepared at room temperature and when most of the salt was dissolved the solution was allowed to stand at 4°C overnight, then the pH was adjusted to 7.0–7.1 with 30% NaOH. In order to precipitate the McAbs from hybridoma culture medium 50% saturation was used and the whole procedure was carried out on ice. The precipitates were collected by centrifugation at 48,000 *g* for 30 min and dissolved in a small amount of distilled water. The solution was dialysed against several changes of barbital buffer (pH 7.3) and tested for antibody titer.

The identification of McAbs was performed by using the two-dimensional double immunodiffusion and immunoelectrophoresis tests.

The isotype of hybridoma products was determined by the following radioimmunoassay: 4×10^5 target cells were incubated with culture fluid in U plates. After centrifugation and washing, 100 μl of the appropriate dilution (1:100–1:1000) of goat antisera specific for either IgG1, IgG2, IgM or IgA (Meloy, Springfield, VA, U.S.A.) were added. After washing, ^{125}J-labelled rabbit anti-goat IgG was added. The cells were washed again and their bound radioactivity counted. All incubation steps, lasting 2 hr each, were done at 4°C.

RESULTS

Selection of hybridomas and characterization of hybridoma products

From the 13th and 14th fusion experiments between X63-Ag8.653 myeloma cells and 79FR-G-41-immune spleen cells, hybrids grew in 164 pots of eight 24-well plates. A screening of the supernatants of these hybridoma cultures, composed of numerous single colonies, revealed that hybrids in 54 wells were secreting antibodies binding to different rat cell targets, including the 79FR-G-41 cells used for immunization. We restricted the isolation of colonies to only one from each antibody-positive well. Immunoglobulin-secreting hybridoma cultures, their products binding glioma cells, were then cloned three times by the limiting dilution method with BALB/c mice peritoneal macrophages scavenging dead parental cells and/or serving as a feeder layer, and then considered as clones. From each original colony 3 representative stable clones were established. The stability of antibody production was judged by the screening results, which should increase to nearly 100% in the last cloning step. It is not worth cultivating clones with an average result of 75% or less.

We now aimed at finding clones which produce McAbs that bind no normal mesodermal and neuroectodermal cells but only glioma cells. Out of 106 clonal products tested we obtained 18 which did not recognize normal rat kidney cells, 21 which did not recognize normal rat brain cells and 12 which did recognize either. These 12 McAbs were studied for their reaction with 79FR-G-41 cells and 4 other cell lines derived from syngeneic nitrosamide-induced gliomas. They showed remarkable differences: whereas 6 supernatants do bind to all cell lines tested, the other 6 present different binding patterns. These McAbs did not recognize any of the other four glioma cell lines. To verify our results in another test system we applied a radioimmunoassay to the clonal products. Ten clonal products did not recognize normal rat brain cells, four did not recognize normal kidney cells and three recognized neither the former nor the latter. The specificity tests showed therefore that McAbs secreted by three hybridoma clones (13GC1, 14BC1, 14FC3) bound 79FR-G-41 glioma but did not react with normal fibroblasts, kidney and glia cells of newborn F344 rats (Table 2). Although these clones had a relatively stable antibody production (95–100%) compared to others, it nevertheless seems advisable to reclone them from time to time.

By means of immunodiffusion and immunelectrophoresis tests the McAbs were identified to be IgG. The isotype of 13GC1 and 14FC3 McAbs

Table 2. Reactivity by immunoperoxidase staining (IPS) and radioimmunoassay (RIA) of three monoclonal antibodies against 79FR-G-41-cells with glioma cell lines derived from experimental rat brain tumors

Monoclonal antibodies	Rat brain cells		Rat kidney cells		Glioma cell lines									
					79FR-G-41		78FR-G-284		78FR-F-344		78FR-G-299		78FR-G-219	
	IPS	RIA	IPS	RIA	IPS	RIA	IPS	RIA	IPS	RIA	IPS	RIA	IPS	RIA
13 GC1	-	-	-	-	+	+	+	+	+	+	+	+	+	+
14 BC1	-	-	-	-	+	+	+	+	+	+	+	+	+	+
14 FC3	-	-	-	-	+	+	+	+	-	-	-	-	+	+

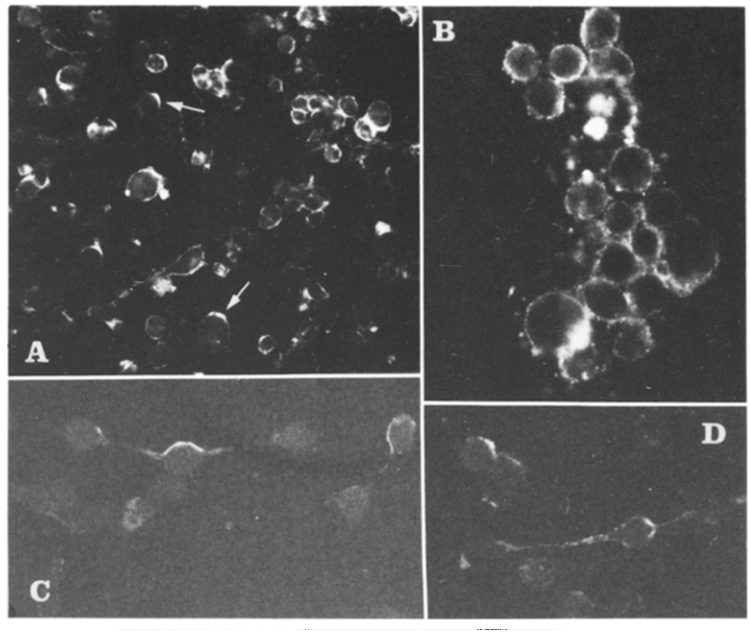

Fig. 1. *Binding capacity of* 14*FC*3 *and* 14*BC*1 *monoclonal antibodies to* 79*FR*-G-41 *cells by indirect immunofluorescence staining under different conditions. The most unfixed cells* (*A*) *show binding for* 14*FC*3 *and a 'capping' phenomenon* (*A*, ↑). *Acetone-fixed cells show granular reaction product on the cell membrane* (*B*). *Some of the glutaraldehyde-fixed cells* (*C, D*) *show binding to* 14*BC*1 *antibody. Only part of the cell membrane, including that over the cell processes, shows a positive reaction. A, C, D* ×190; *B* ×300.

was IgG1, while 14BC1 McAb was reacting with both IgG1 and IgG2 antisera, indicating that it contains two heavy chains of different IgG subclasses.

The expression and distribution of the glioma antigen(s) determined by indirect immunofluorescence staining are shown in Fig. 1. Essentially, a variable proportion of the target cells (20–95%) of all the glioma cell lines tested were marked by the three McAbs (13GC1, 14BC1, 14FC3). This pattern of binding was observed with the line 79FR-G-41, used for immunization, as well as with the remaining four glioma lines. While the antibody 13GC1 showed the lowest rate of labeling with all five lines (approx. 20–50%), the McAbs 14BC1 and 14FC3 were characterized by a high binding capacity (approx. 90–95%), which even reached 100% in some preparations. In agreement with previous observations [17], fluorescent structures were confined to the cell membrane of glioma cells bound by McAbs. Nevertheless, fluorescence is not limited to the cell membrane of the cell body but can also be detected along the cell membrane of the processes. In the positive cells fluorescent structures on the cell surface were distributed in a slightly variable way. In some cells the antigen–antibody complexes appeared to be diffusely distributed, i.e. in all regions of the cell membrane. In contrast to this distribution pattern, in the most positive cells the fluorescence complexes seemed to have a dotted appearance and a spotty arrangement on the cell surface.

Concerning the stability of glioma-associated antigen(s), we found by indirect immunofluorescence that the determinants resisted treatment with acetone as well as glutaraldehyde and formalin, and that they could be detected by all three monoclonal antibodies (Table 3).

Reactivity of McAbs against 79FR-G-41 cells with other glioma lines

The three selected clonal products with specific binding capability to glioma cells were designated as 13GC1, 14BC1 and 14FC3, corresponding to the established hybridoma clones. In the code designating a clone (13GC1, 14BC1, 14FC3) the first digits stand for the fusion number, the first letters for the positive pots and the second letters including numbers for the isolated clone. These three clones produce McAbs against 79FR-G-41 glioma cells, which also react with other glioma lines derived from experimental rat brain tumors (Table 2). Two clonal products, 13GC1 and 14BC1, recognize each of the five glioma lines, whereas the supernatant 14FC3 binds three lines out of five (Table 2). When the three McAbs produced by the hybridoma clones 13GC1, 14BC1 and 14FC3 were tested in three different assays for binding to tumor cell lines derived from spontaneous brain tumors (Table 4), they reacted only with the 79FR-G-41 cells used for immunization. The cells isolated from spontaneous brain tumors were not recognized by the McAbs against experimental glioma cells. Moreover, these monoclonal products did not bind human fibroblasts.

Table 3. Binding of monoclonal anti-79FR-G-41 immunoglobulins to syngeneic rat cells under varying conditions by immunofluorescence

Monoclonal antibodies	Stability of antibody production	Anti-body	Targets 79FR-G-41 cells	Brain cells	Kidney cells	Stability of glioma antigen(s) with Acetone*	Glutaraldehyde†	Formalin‡
13 GC1	100%	IgG	+	-	-	+	+	+
14 BC1	100%	IgG	+	-	-	+	+	+
14 FC3	95%	IgG	+	-	-	+	+	+

*Acetone p.A., 4°C.
†Glutaraldehyde, 5.25%, 4°C.
‡Formalin, 6%, 21°C.

Table 4. Reactivity by radioimmunoassay of monoclonal antibodies against experimental rat glioma cells (79FR-G-41) with tumor cells derived from spontaneous brain tumors of dog (77DG-G-166) and man (C1-71, 76H-M-337, 79H-G-396)

Monoclonal antibodies	Target cell lines 79FR-G-41	77DG-G-166	79H-G-396	76H-M-337	C1-71
13 GC1	+	-	-	-	-
14 BC1	+	-	-	-	-
14 FC3	+	-	-	-	-

Absorption experiments

McAbs secreted by the hybridoma clones 13GC1, 14BC1 and 14FC3 did not lose their binding capability to the immunizing glioma cells 79FR-G-41 after absorption with normal brain tissue of newborn and adult F344 rats. In addition, short-term-cultured brain and kidney cells of newborn F344 rats did not reduce the binding capacity of the McAbs 13GC1, 14BC1 and 14FC3 to glioma cells. On the contrary, the quantitative absorption experiments with the immunizing line 79FR-G-41 showed that the binding activity of hybridoma supernatants as well as of ascitic fluids to glioma cells was gradually reduced, while no significant inhibition was obtained with glia cells from newborn F344-rats (Fig. 2). The remaining binding activity of the hybridoma products after every absorption step was correlated with the increasing number of glioma cells used for absorption.

DISCUSSION

Despite all research efforts in the field of tumor immunology, up until recently the very existence of tumor cell antigenicity in neural tumors, let alone the actual demonstration of antigenicity sites in cells of these neoplasms, was still controversial. Only the studies of the last few years concerning the polyclonal immune response to these tumors in several biological systems [19–30] have mustered enough evidence in support of the antigenicity of glioma cells. Circumstantial evidence along the same line was also obtained by analyzing the interaction between chemically induced glial tumors and their respective glioma-bearing hosts (see literature in [2]). In particular,

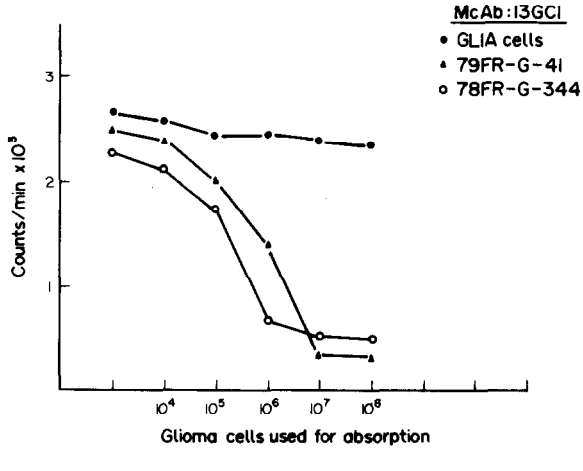

Fig. 2. Quantitative absorption of 13GC1 monoclonal product. Binding RIA using 79FR-G-41 glioma cells as targets. Each point represents the binding intensity of McAbs after absorption of 100 µl (at 1:10 dilution) culture fluid with an increasing number of normal glia cells and glioma cells (●: normal glia cells, ○: 78FR-G-344, △: 79FR-G-41, as indicated on the abscissa).

studies on chemically modified glioma cells inoculated in syngeneic animals have shown that cells of the MNU-induced gliomas share multiple glioma-associated antigens (GAA) [3].

The hybridoma technology lately applied to produce McAbs against glioma cells has not only demonstrated beyond doubt the existence of GAA in glioma cells but it has also greatly stimulated research aimed at elucidating the very significance of the immune response to GAA. Studies using McAbs against human gliomas [30–37] have demonstrated that these neoplasms express GAA on the cell surface and that these GAA or one part of them are neuroectodermal antigens shared by gliomas, neuroblastomas and melanomas [15, 38, 39].

The results presented here indicate that a large number of hybridomas originate by fusing X63-Ag8.653 murine myeloma cells with mouse splenocytes of animals sensitized against 79FR-G-41 rat glioma cells. Some of the hybridomas obtained in this way produce no detectable antibodies or they stop producing them after a few *in vitro* passages. The majority of the remaining hybridomas, however, secrete antibodies against a large series of normal and neoplastically transformed rat cells. In addition, by all assay methods so far applied the antibodies secreted by 3 hybridoma cell lines (13GC1, 14BC1, 14FC3) bind 79FR-G-41 rat glioma cells, used to immunize BALB/c mice, but do not react with fibroblasts, kidney and brain cells of newborn F344 rats. Furthermore, McAbs produced by 13GC1 and 14BC1 cell lines cross-react with antigenic determinants of four additional glioma cell lines (78FR-G-284, 78FR-G-344, 78FR-G-299 and 78FR-G-219). On the contrary, McAbs of 14FC3 hybridoma cells react with 79FR-G-41 as well as 78FR-G-284 and 78FR-G-219 but not with 78FR-G-344 and 78FR-G-299 glioma cell lines. The glioma specificity of the three McAbs was additionally confirmed by a series of quantitative absorption experiments. These studies showed that incubation with glioma cells abolished the binding activity of 13GC1, 14BC1 and 14FC3 antibodies to glioma cells, whereas normal syngeneic brain and kidney cells were unable to inhibit the reactivity of these antibodies to gliomas.

From the binding behavior the conclusion seems justified that the McAbs obtained so far recognize different surface antigenic determinants which are expressed either in some or all glioma cell lines propagated from chemically induced rat brain tumors. Furthermore, we conclude that all chemically induced rat brain tumors tested share identical or cross-reacting antigens or antigenic determinants, all of which are invariably

recognized by the McAbs raised against these tumors. In this regard it must be stressed that a subpopulation of cells, the exact number of which varies from cell line to cell line, is not recognized by the McAbs, as shown by the cytochemical monitoring used [40]. Analogous results were obtained with McAbs raised against a human melanoma [41]. It remains to be seen to what extent the observed antigenic heterogeneity is genetically determined or otherwise conditioned. Should the expression of antigenic properties be cell-cycle-dependent, then antigenic heterogeneity of non-synchronized cells such as ours would be expected. Particularly interesting is our observation that McAbs directed against GAA of experimental rat gliomas do not recognize brain tumor cells of other species, for instance, of man (Table 4). Considering the small number of cell lines tested, this could possibly be taken to mean that GAA are species-specific.

On the whole, the results indicate that chemically induced rat gliomas share identical or cross-reacting antigenic determinants. Furthermore, the production of McAbs against antigenic structures makes it possible for us to tackle problems of more direct clinical relevance in a well-defined syngeneic system. In particular, it makes it feasible to investigate (a) the binding capability of conjugated McAbs to glioma cells *in vivo*; (b) the fate of labeled McAbs *in vivo* as they overcome the blood–brain barrier; and (c) the applicability of drug–antibody complexes (immunotoxins) aimed at destroying glioma cells *in vivo* without seriously damaging the host. The elucidation of these various problems in an experimental rat glioma model of well-known characteristics is of paramount importance. Indeed, animal studies of this kind are *conditio sine qua non* for the development of clinically relevant diagnostic tests and eventually for a therapeutic approach to the glioma disease of man.

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