estrogens could cause a comparative increase in the number of Leydig cells that can be identified by the nitroblue tetrazolium staining method. As shown in Fig. 6, E_2 at a concentration of 10^{-8} M in the culture medium did not stimulate cell proliferation until day 4, suggesting that plating efficiency of these cells would not be altered by exposure to E_2. The stimulatory effect of E_2 on cell proliferation became evident, resulting in a 3-fold increase in cell number over that seen in unstimulated controls on day 14. Next, the effect of E_2 on DNA synthesis in cultured transformed cells was determined. As depicted in Fig. 7, these cells were responsive to concentrations of E_2 as low as 10^{-9} M in relation to [^{3}H]TdR incorporation into DNA. At 10^{-8} M to 10^{-7} M the magnitude of the E_2 effect was at a maximum, with some reduction at 10^{-6} M.

DISCUSSION

In our previous study performed in a cell-free condition, we showed that one of the Leydig cell tumor lines (T-124958) contains a unique unoccupied nuclear estrogen binder in the absence of a high-affinity cytosolic binder [4]. The simultaneous administration of CB-154 (ergocryptin) did not affect estrogen-induced tumor growth, suggesting that estrogen action on this particular tumor was directly mediated by this nuclear binder, not via pituitary factors such as prolactin [4]. In order to obtain a definite answer in this regard, however, *in vitro* analysis involving intact cells might be required. The following major points emerged from the present study: (1) this malignant Leydig cell contains a high-affinity nuclear binder similar to putative nuclear ER in the absence of the cytosol high-affinity binder; (2) this nuclear binder undergoes so-called nuclear processing in response to estrogen exposure, resulting in decreased binding capacity per cell; and (3) estrogens act directly on these cells.

The demonstration of specific E_2-binding sites in nuclei from cells pre-exposed to [^{3}H]E_2 at 0°C would support an idea that this transformed Leydig cell contains an unoccupied nuclear estrogen binder. Traish *et al.* [10] reported that incubation of uterine cells with [^{3}H]E_2 at low temperatures (0–4°C) resulted in gradual accumulation of ER in the nuclear fraction, reaching a plateau at 15 hr. Their results would imply that receptor activation can be achieved even at low temperatures in intact cells, translocating ER into nuclei. When compared with their observation, however, the relatively rapid association of [^{3}H]E_2 with nuclei at 0°C with the maximum value at 2–3 hr would favor the idea that there is an unoccupied nuclear binding site in transformed Leydig cell nuclei.

The loss of cellular ER following estrogen administration, called processing [11], has been proposed to be a prerequisite for estrogen actions. This conclusion has come from the experimental results that a rapid decrease in nuclear receptor content does occur 3–5 hr after estrogen administration while no loss of nuclear receptor is observed if ER bound to antiestrogen enters the nucleus [12]. However, exact correlation of nuclear processing of ER with biological effects has been reported as not always being observed [13]. Accordingly, it can be safely concluded that estrogen binder receiving 'nuclear processing' can be categorized as ER. In view of these considerations, it appears to be possible to say with certainty that this transformed Leydig cell contains unoccupied nuclear 'ER'.

Recent evidence has shown that the estrogen-responsive tumor growth can be modulated by pituitary factor(s). Leung and Shiu [14] showed dramatic enhancement of estrogen-dependent tumor growth of the T-47D human mammary tumor cell line in athymic mice by a co-implant of GH3/C 14 rat pituitary prolactin- and growth hormone-producing tumor cells. This observation has been extended by Sirbasku *et al.* [15], who have shown that pituitary extracts from E_2-treated ovariectomized rats have the factor(s) promoting the growth of rat mammary tumor cells (MTW9/pL) although its hormonal dependency remains obscure. Although these sophisticated studies reveal the important role of pituitary factors for the estrogen-induced tumor growth, a marked *in vitro* effect on E_2 on cell proliferation would strongly indicate that this transfomed Leydig cells used in the present experiments is influenced directly by estrogens in terms of tumor growth.

The cytoplasmic E_2-binding component might be worth taking into consideration. The use of unlabeled DES to calculate a non-specific binding site failed to demonstrate the estrogen-binding components in the cytoplasmic fraction. On the other hand, a cytoplasmic E_2-suppressible binding site was observed by the experiments on subcellular localization of the estrogen binders. No demonstrable increase in nuclear E_2-suppressible sites in response to the exposure of cells to E_2 would reveal that the cytoplasmic E_2 binder is unable to translocate into nuclei. These seem to be compatible with out previous observations [16]. Furthermore, it should be mentioned that this cytoplasmic estrogen binder was unable to interact with hydroxylapatite under the usual conditions.

The mechanism of estrogen-induced tumor growth has remained to elucidated. The require-

ment of high serum concentration in the culture medium has been recently reported to demonstrate the estrogen-induced enhancement of cell proliferation in MCF-7 human mammary cancer cells [17]. A relatively high concentration (10%) of charcoal-treated fetal calf serum was used in the present experiments. The lower concentrations (1-2%) failed to show any significant and reproducible effects of E_2 on cell proliferation (data not shown). Clearly, more research is required to clarify the molecular mechanism of estrogen-induced tumor growth. In this regard, it is desirable to establish the *in vitro* experimental system showing estrogen effects in terms of cell proliferation under serum-free conditions. Our current studies are directed toward establishing these experimental models.

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Phenotyping of 60 Cultured Human Gliomas and 34 Other Neuroectodermal Tumors by Means of Monoclonal Antibodies against Glioma, Melanoma and HLA-DR Antigens

B. DE MURALT,* N. DE TRIBOLET,*† A.-C. DISERENS,* D. STAVROU,‡ J.-P. MACH§ and S. CARREL§

*Neurosurgical Service, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland, ‡Department of Functional Pathology, Institute of Animal Pathology, Ludwig—Maximilians University of Munich, F.R.G. and §Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland

Abstract—*The reactivity spectrum of three monoclonal antibodies (Mabs) to human malignant glioma, five Mabs to melanomas and one Mab anti-HLA-DR was investigated by an indirect antibody binding radioimmunoassay on a panel of cells derived from 60 glioma lines, including 47 malignant astrocytomas, 11 low-grade astrocytomas and two malignant ependymomas as well on cells from 12 melanoma, three neuroblastoma, three medulloblastoma, two schwannoma, two retinoblastoma, two choroïd plexus papilloma, ten meningioma and 12 unrelated tumor lines. The anti-glioma Mabs BF7 and GE2 reacted preferentially with gliomas, while the anti-glioma Mab CG12 reacted with gliomas, melanomas, neuroblastomas and medulloblastomas. The five anti-melanoma Mabs reacted with gliomas, neuroblastomas and medulloblastomas. The anti-HLA-DR Mab D1-12 reacted with gliomas, melanomas and some meningiomas. On the basis of the data presented, we describe three different antigenic systems; the first one is glioma-associated, the second one is related to differentiation antigens expressed on cells derived from the neuroectoderm and the third is represented by HLA-DR antigens which are expressed not only on B-lymphoblastoid cells but also on melanomas and gliomas.*

INTRODUCTION

ALTHOUGH monoclonal antibodies (Mabs) have provided a great improvement in the search for tumor-associated antigens, these antibodies so far have not allowed the identification of a truly tumor-specific antigen [1]. They have, however, identified antigens preferentially expressed on tumor cells but also on normal cells. In addition, Mabs clearly showed that tumor cells might express differentiation antigens restricted to cells of a common origin. Anti-melanoma Mabs were found to cross-react with gliomas and neuroblastomas [2-5], anti-neuroblastoma Mabs were found to react with neuroblastomas, retinoblastomas and gliomas [6] and anti-glioma Mabs with gliomas as well as with melanomas and neuroblastomas [2-7]. Since these tumors are known to derive from cells originating embryologically from the neuroectoderm, it is possible that the antigens recognized by these Mabs belong to a class of neuroectodermal differentiation antigens [8]. It has further been shown that cells from several established glioma and melanoma cell lines express well-characterized lymphoid-associated differentiation antigens such us HLA-DR, Thy-1 and CALLA [9-12].

In this report we analyzed the surface phenotype of human gliomas and other tumors of neuroectodermal origin using 3 anti-glioma and 5 anti-melanoma Mabs. The presence of HLA-DR antigens on these tumor cells was also assayed with an anti-HLA-DR Mab. In addition, the cells from the various glioma lines were also tested for the presence of glial fibrillary acidic protein (GFAP) and S-100 protein. The cellular distribution of the antigens recognized by these different Mabs was analyzed by the fluorescent activated

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†To whom requests for reprints should be addressed.

cell sorter (FACS II), and has shown a great polymorphism in the expression of these various antigens between, as well as within, different cell lines. The results of this analysis, performed on a large number of glioma cell lines and short-term cultures, as well as on several melanoma, neuroblastoma and malignant schwannoma lines, with nine different Mabs allowed the definition of three antigenic systems. The first one is glioma-associated, the second consists of differentiation antigens expressed on normal and neoplastic cells of neuroectodermal origin and the third is represented by HLA-DR antigens, which are shown to be expressed not only on hematopoietic cells but also on several tumor cell lines of neuroectodermal origin.

MATERIALS AND METHODS

Cell lines

The 109 human short-term cultures and cell lines used in this study consisted of 60 gliomas including 47 malignant astrocytomas, 11 low-grade astrocytomas and two malignant ependymomas, as well as three neuroblastomas, three medulloblastomas, two choroïd plexus papillomas, two malignant schwannomas, two retinoblastomas, 12 melonomas, ten meningiomas and 15 cell lines derived from unrelated tumors and normal tissue. All cells were grown in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal calf serum.

Monoclonal antibodies

The three anti-glioma Mabs BF7, GE2 and CG12 were obtained by Schnegg *et al.* [13]. Mabs BF7 and GE2 were selected for their preferential reactivity with gliomas and Mab CG12 for its cross-reactivity with melanomas and neuroblastomas. The five anti-melanoma Mabs were obtained by Carrel *et al.* [14]. The Mab D1-12 reacting against a monomorphic determinant present on human HLA-DR antigen(s) was obtained by Carrel *et al.* [15].

Antibody-binding radioimmunoassay (RIA)

The reactivity of the Mabs was tested by an indirect antibody-binding radioimmunoassay performed as follows: 10^5 target cells in 50 μl of phosphate-buffered saline (PBS) were incubated for 1 hr at 4°C in U-bottomed microtest plates with 100 μl of culture fluid. The plates were centrifuged and washed three times in PBS; 100 μl of ^{125}I-labeled rabbit anti-mouse (Fab')2 antibodies were then added and incubated for 1 hr at 4°C; the cells were then washed three times with PBS and transferred to tubes for gamma-counting. A binding ratio (BR) was calculated by dividing the total number of cell-bound counts by the number of cell bound-counts using the myeloma culture fluid. Values equal or greater than 3 were considered as positive.

RESULTS

Binding activity of anti-glioma Mabs

The binding results obtained with the three anti-glioma Mabs BF7, GE2 and CG12 on 24 glioma cell lines are shown in Fig. 1. Both BF7 and GE2 reacted with the majority of the cell lines tested, with 83 (20/24) and 95% (23/24) positive cell lines respectively, while CG12 reacted with only 15 out of 24 cell lines (62%). Except for the MG118 cell line, the number of counts/min bound to all other cell lines was always higher for BF7 and GE2 than for CG12.

Similar binding results were obtained with the 36 short-term glioma cultures. Figure 2 shows that BF7 reacted to 75% (23/36) and GE2 to 83% of (30/36) gliomas in short-term culture while, like for long-term cell lines, CG12 bound to only 30% (11/36) of these tumors.

The reactivity of the three Mabs was further tested on ten short-term meningioma cultures (Fig. 3). Only Men-279 meningioma cells appeared to bind the three Mabs in a significant amount. When the reactivity of the anti-glioma Mabs was analyzed on a number of other tumors of neuroectodermal origin, including melanomas, neuroblastomas, medulloblastomas, schwannomas, retinoblastomas and papillomas, it was found that the most reactive antibody was CG12 (Fig. 4). As many as 9/10 melanomas and 2/3 neuroblastomas bound this particular antibody. The two other anti-glioma Mabs BF7 and GE2 bound only to 1/11 2/11 melanomas respectively and were totally non-reactive for the three neuroblastomas tested. Finally, the binding activity of the three Mabs was assayed on several cell lines from unrelated tumors such as four colon carcinomas, one endometrial carcinoma, one cervical carcinoma, one breast carcinoma and one rhabdomyosarcoma, and on four hematopoietic cell lines (Fig. 5). No significant binding was obtained for these unrelated control cell lines except for cells from the colon carcinoma line LOVO, with which the two antibodies BF7 and GE2 showed a low but reproducible binding.

Binding activity of anti-melanoma Mabs

The reactivity of five monoclonal anti-melanoma Mabs, Mel-5, Mel-14, Me3-TB7, Me4-F8 and Me5-D5, for gliomas was tested on 45 cell lines and short-term cultures (Table 1). Two of the Mabs, Mel-5 and Mel-14, bound to as much as 42 (19/45) and 48% (22/45) of the gliomas tested. Two other Mabs, Me4-F8 and Me 5-D5, reacted with 31 (14/45) and 37% (17/45) of the cultures and

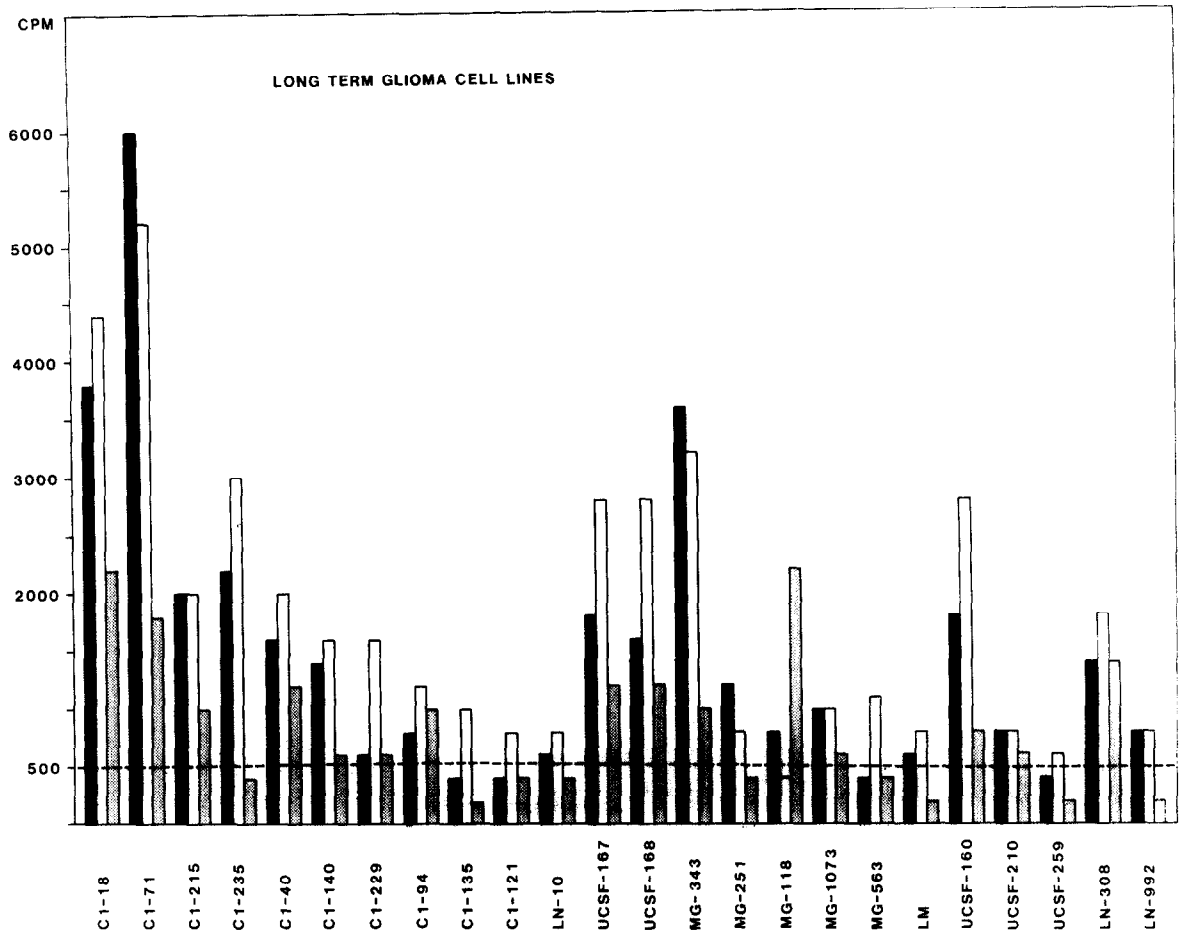

Fig. 1. Reactivity spectrum of BF7, GE2 and CG12 antiglioma monoclonal antibodies to long-term glioma cell lines. Results are expressed as counts/min *bound per* 10^5 *cells. Three bars are shown representing (left to right) BF7, GE2 and CG12 monoclonal antibodies.*

antibody Me3-TB7 bound to 24% (11/45). From the broad reactivity spectrum of these five anti-melanoma Mabs for gliomas we can conclude that each antibody must be directed against a different antigen. This has been shown previously by immunoprecipitation experiments [16]. When the five Mabs were tested on seven meningioma short-term cultures (Table 2) it appeared that only Mel-5 Mab gave a strong binding for one mengioma (Me-288) and two Mabs, Mel-14 and Me3-TB7, bound at a low level to two others. Table 3 illustrates the strong binding of the anti-melanoma Mabs for the 11 melanoma cell lines tested here. As for the anti-glioma Mabs, the five anti-melanoma Mabs were tested on ten other neuroectoderm-derived tumors including neuroblastomas, medulloblastomas, one retinoblastoma, one schwannoma and two papillomas (Table 4). Two Mabs, Mel-5 and Me5-D5, bound to 3/3 neuroblastomas, to 1/3 medulloblastomas and to schwannoma. Antibody Me3-TB7 and Me4-F8 bound to all three neuroblastomas only and antibody Mel-14 reacted with two neuroblastomas and one papilloma. As already published, none of these five anti-melanoma Mabs reacted significantly with any of the 33 non-melanoma control cell lines tested [2].

Expression of HLA-DR antigens by neuroectoderm derived tumors

The presence of HLA-DR antigens on cells from 70 tumors derived from the neuroectoderm was analyzed by the indirect antibody-binding radioimmunoassay using Mab D1-12 directed against a common epitope present on all HLA-DR molecules. The results obtained with antibody D1-12 are summarized in Table 5. About 32% (23/70) of the tumors appeared to bind the anti-HLA-DR antibody D1-12. Up to 26% of the malignant astrocytomas (9/34) and 63% (7/11) of the melanomas tested were found to be HLA-DR-positive; in addition, this antigen was also found on 3/7 meningiomas. The quantitative expression of HLA-DR on these positive cell lines appeared to be highly variable from one line to the other (data not shown).

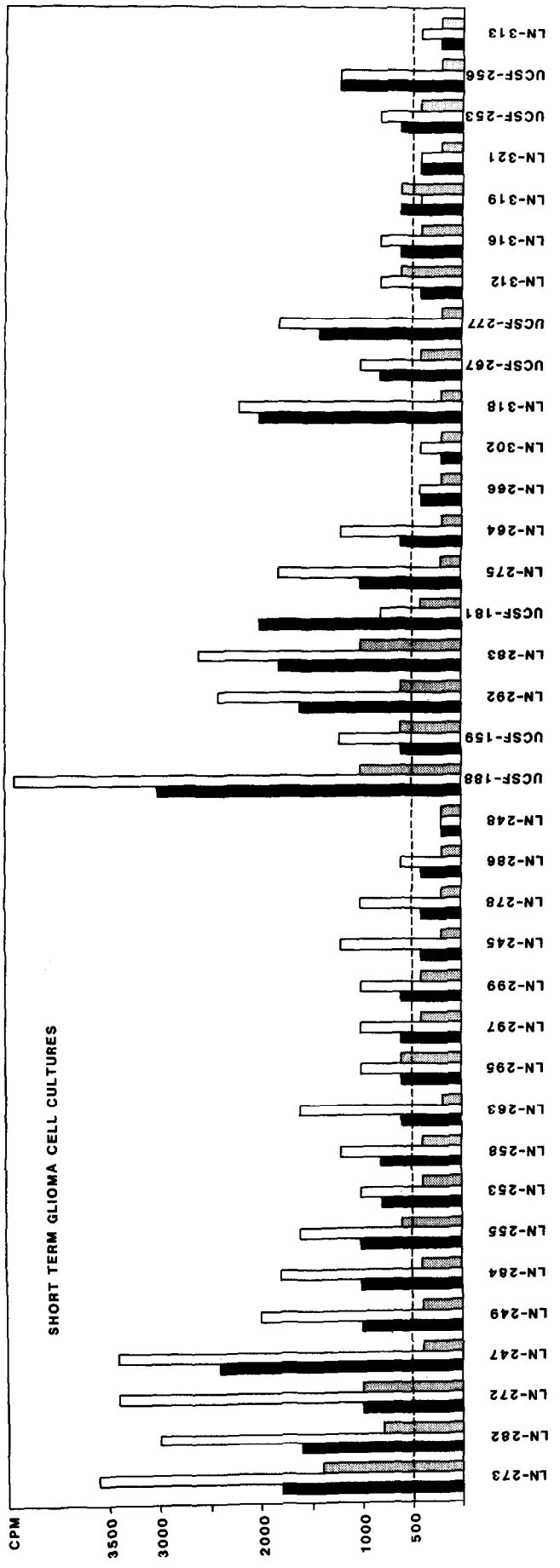

Fig. 2. Reactivity spectrum of BF7, GE2 and CG12 antiglioma monoclonal antibodies to short-term glioma cell cultures. Results are expressed as counts/min *bound per* 10^5 *cells. Three bars are shown representing (left to right) BF7, GE2 and CG12 monoclonal antibodies.*

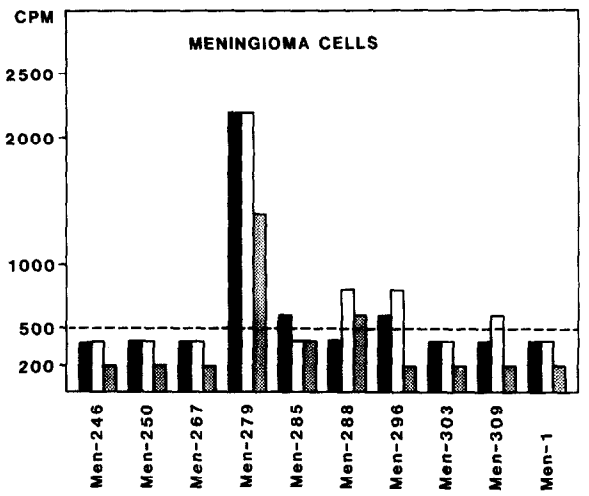

Fig. 3. Reactivity spectrum of BF7, GE2 and CG12 antiglioma monoclonal antibodies to short-term meningioma cell lines. Results are expressed as counts/min *bound per* 10^5 *cells. Three bars are shown representing (left to right) BF7, GE2 and CG12 monoclonal antibodies.*

Cellular distribution of the antigens recognized by the anti-glioma Mabs

The quantitative expression on cultured glioma cells of the antigens recognized by the anti-glioma Mabs BF7, GE2 and CG12 as well as by the anti-HLA-DR Mab D1-12 has been analyzed by the fluorescent activated cell sorter (FACS II, Becton Dickinson, Los Angeles, CA). Figure 6 shows the fluorescence pattern obtained after incubating C1-121 glioma cells with each of the four Mabs and fluorescein-labeled anti-mouse immunoglobulin (GM/FITC). A bimodal distribution of the glioma cells was observed for both anti-glioma Mabs BF7 and GE2, showing that about 50% of these cells expressed the antigenic determinants recognized by these two antibodies. In contrast, the antigenic determinant recognized by CG12 which was not expressed significantly on these cells. Background fluorescence was obtained by incubating C1-121 glioma cells with Px63/Ag8 myeloma culture

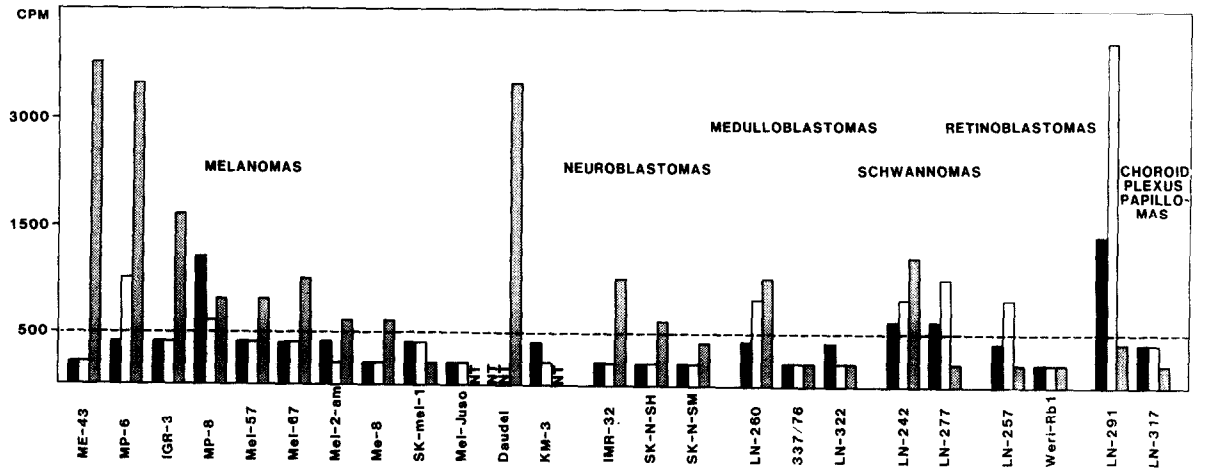

Fig. 4. Reactivity spectrum of BF7, GE2 and CG12 antiglioma monoclonal antibodies to neuroectodermal tumor cell lines. Results are expressed as counts/min *bound per* 10^5 *cells. Three bars are shown representing (left to right) BF7, GE2 and CG12 monoclonal antibodies.*

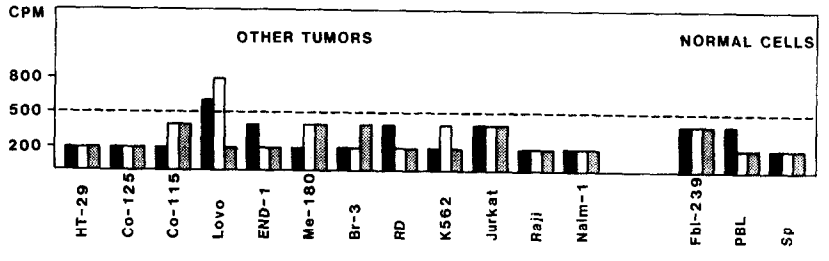

Fig. 5. Reactivity spectrum of BF7, GE2 and CG12 antiglioma monoclonal antibodies to unrelated tumor cells and normal cells. Results are expressed as counts/min *bound per* 10^5 *cells. Three bars are shown representing (left to right) BF7, GE2 and CG12 monoclonal antibodies.*

Table 1. Binding of monoclonal anti-melanoma antibodies to glioma cell lines and short-term cultures

Tumor cell lines	Hybridoma product*					Counts/min bound using Px63/Ag8 culture fluid
	Mel-5	Mel-14	Me3-TB7	Me4-F8	Me5-D5	
LN-10	2	2	1	1	2	120
LN-18	9	13	1	2	3	120
C1-40	3	2	8	2	2	250
C1-71	3	3	6	3	4	245
C1-94	2	2	2	2	3	170
C1-121	16	14	5	6	8	250
C1-135	16	12	5	9	8	230
C1-140	2	2	2	1	1	150
C1-215	12	7	5	9	7	150
C1-229	10	33	5	3	2	100
C1-235	6	8	9	10	17	180
LN-247	3	5	-	-	-	140
LN-249	6	8	-	-	-	110
LN-253	4	4	-	-	-	160
LN-258	6	6	4	-	-	70
LN-286	3	2	2	3	-	400
LN-295	5	5	2	4	2	190
LN-297	4	2	1	3	4	110
LN-299	2	3	1	2	2	300
LN-308	4	2	2	2	8	190
LN-312	2	3	2	2	3	200
LN-316	2	3	3	9	6	260
LN-992	4	4	3	5	5	260
LN-319	-	4	2	3	3	300
LN-321	2	5	2	4	4	290
MG-118	2	2	1	2	2	100
MG-251	2	5	1	4	2	170
MG-343	2	1	1	2	2	240
MG-563	3	2	1	2	1	200
MG-1073	2	2	6	1	2	150
UCSF-159	9	10	-	-	-	140
UCSF-167	6	5	-	-	-	200
UCSF-168	9	11	-	-	-	90
UCSF-253	2	3	2	3	4	100
UCSF-256	3	2	4	2	6	280
UCSF-259	2	2	2	4	4	250
UCSF-160	4	7	-	-	-	250
UCSF-181	7	2	-	-	-	150
LN-302	3	2	1	2	2	270
LN-318	2	5	3	7	8	280
UCSF-210	1	2	1	2	2	300
UCSF-267	2	4	2	4	5	270
UCSF-277	2	2	2	4	5	300
LN-292	5	5	4	5	4	140
LN-313	1	3	1	1	-	230

*Results are expressed as binding ratio (BR) = total No. of cell-bound counts divided by the No. of cell-bound counts using Px63/Ag8 culture fluid; BR $\geqslant$ 3 is considered as positive.

fluid followed by GM/FITC. With the anti-HLA-DR Mab D1-12 no negative cell population was detected, suggesting that all cells of this glioma line express HLA-DR antigens. The quantitative expression of the antigens seen by two anti-glioma and one anti-melanoma Mabs was further analyzed by FACS on cells from two melanoma lines. Figure 7 shows that 68% of the cells from the melanoma line Me43 expressed the Mel-14 antigen and 40% the antigen seen by anti-glioma Mab CG12. None of the Me43 cells bound the anti-glioma Mab GE2. Likewise none of the MP-6 melanoma cells bound this antibody (Fig. 8), while 70% of the MP-6 cells bound the anti-glioma Mab CG12 and 42% the anti-melanoma Mab Mel-14. These results confirmed that the antigenic determinant recognized by the anti-glioma Mab CG12 is shared with melanoma cells. Similar experiments have also shown that the antigenic determinants recognized by the anti-melanoma Mabs were expressed on glioma cells (data not shown).

Table 2. Binding of monoclonal anti-melanoma antibodies to meningiomas

Tumor cell lines	Hybridoma product*					Counts/min bound using Px63/Ag8 culture fluid
	Mel-5	Mel-14	Me3-TB7	Me4-F8	Me5-D5	
Men-1	2	2	-	-	-	290
Men-250	2	1	-	-	-	320
Men-285	2	2	4	2	-	300
Men-288	7	4	-	-	-	280
Men-296	2	2	2	2	-	240
Men-303	2	2	1	2	1	300
Men-309	2	2	3	1	2	190

*Results are expressed as binding ratio (BR) = total No. of cell-bound counts divided by the No. of cell-bound counts using Px63/Ag8 culture fluid; BR $\geqslant$ 3 is considered as positive.

Table 3. Binding of monoclonal anti-melanoma antibodies to melanoma cell lines

Tumor cell lines	Hybridoma product*					Counts/min bound using Px63/Ag8 culture fluid
	Mel-5	Mel-14	Me3-TB7	Me4-F8	Me5-D5	
IGR-3	23	20	16	10	6	240
Me-43	20	23	22	9	7	110
Mel-57	9	11	13	12	3	250
Mel-67	10	9	7	7	9	250
Mel-2-am	12	15	18	10	5	240
Me- 8	8	13	9	6	5	150
MP-8	4	5	7	8	9	140
MP-6	13	12	6	2	5	176
SK-Mel-1	9	7	2	7	8	70
Daudel	-	41	7	6	6	40
KM-3	5	3	3	3	-	100

*Results are expressed as binding ratio (BR) = total No. of cell-bound counts divided by the No. of cell-bound counts using Px63/Ag8 culture fluid; BR $\geqslant$ 3 is considered as positive.

Table 4. Binding of monoclonal anti-melanoma antibodies to neuroectodermal tumor cells

Tumor cell lines	Hybridoma product*					Counts/min bound using Px63/Ag8 culture fluid
	Mel-5	Mel-14	Me3-TB7	Me4-F8	Me5-D5	
Neuroblastomas						
IMR-32	8	10	7	5	11	140
SK-N-SH	7	2	4	4	9	90
SK-N-SM	7	3	4	4	5	100
Medulloblastomas						
LN-260	3	1	-	-	-	380
LN-322	1	3	2	3	4	350
337/76	5	2	-	-	-	150
Schwannoma						
LN-277	4	3	2	-	5	100
Retinoblastoma						
Weri-Rb-1	1	1	1	1	1	80
Choroïd Plexus papillomas						
LN-291	3	2	3	1	-	190
LN-317	4	4	2	3	4	270

*Results are expressed as binding ratio (BR) = total No. of cell-bound counts divided by the No. of cell-bound counts using Px63/Ag8 culture fluid; BR $\geqslant$ 3 is considered as positive.

Table 5. Reactivity of a monoclonal anti-HLA-DR antibody

Target cells	Anti-HLA-DR Mab D1-12
Malignant astrocytomas	9*/34†
Low-grade astrocytomas	2/7
Malignant ependymomas	1/2
Choroïd plexus papillomas	1/2
Melanomas	7/11
Neuroblastomas	0/3
Medulloblastomas	0/2
Malignant schwannomas	0/2
Meningiomas	3/7

*No. of positive lines as detected in RIA.
†No. of lines tested.

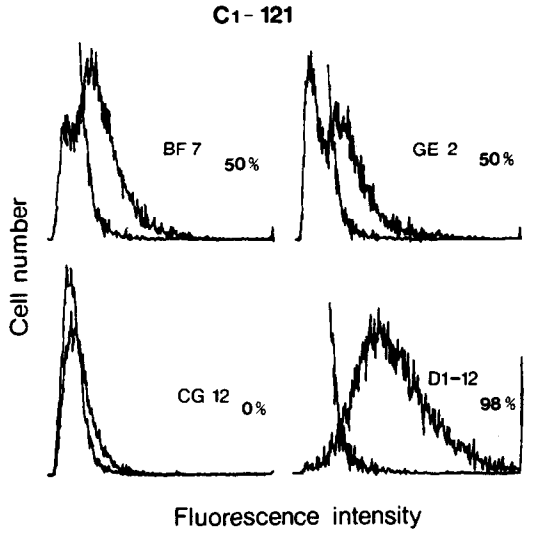

Fig. 6. Flow cytofluorometric analysis of the binding of three anti-glioma monoclonal antibodies (BF7, BF2, CG12) and one anti-HLA-DR monoclonal antibody (D1-12) on C1-121 glioma cells.

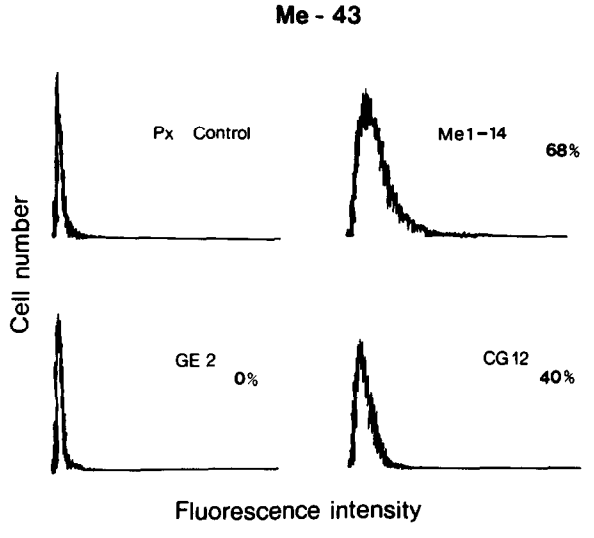

Fig. 7. Flow cytofluorometric analysis of the binding of the anti-melanoma monoclonal antibody (Mel-14) and two anti-glioma monoclonal antibodies (GE2, CG12) on Me-43 melanoma cells.

Expression of GFAP and S-100 protein by glioma cells

The presence of glial fibrillary acidic protein (GFAP) was determined by the immuno-peroxidase method on fixed and frozen cells at different passage levels using a rabbit anti-GFAP serum kindly provided by Dr L. F. Eng. Among the primary cultures analyzed, cells from 21/41 malignant astrocytomas were found to contain GFAP. The synthesis of this protein was generally observed up to the fifth passage. After more than ten passages only five cell lines out of the 21 which were originally positive still retained the capacity to produce GFAP (Table 6). There was no correlation between the expression of GFAP and the binding activity of BF7, GE2 or CG12 Mabs. LN-18 and Cl-71 glioma cells, which showed the highest binding with these Mabs, were negative for GFAP. This finding indicates that the antigen

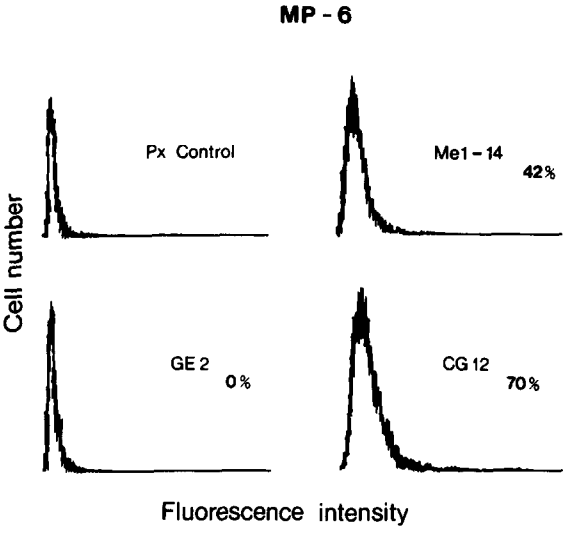

Fig. 8. Flow cytofluorometric analysis of the binding of the anti-melanoma monoclonal antibody (Mel-14) and two anti-glioma monoclonal antibodies (GE2, CG12) on MP-6 melanoma cells.

Table 6. Binding of GE2 and CG12 to glioma cells expressing GFAP or S-100 protein

Glioma lines	No. of passages	Hybridoma product* GE2	CG12	GFAP†	S-100‡
C1-18	203	+	+	-	+
C1-71	113	+	+	-	+
C1-215	71	+	+	+	-
C1-235	46	+	-	+	+
LN-308	15	+	+	+	-
LN-992	20	+	-	+	-
MG-251	287	+	-	+	+

*Scored as positive or negative.
†GFAP was determined by indirect immunoperoxidase using the anti-serum kindly provided by Dr L. F. Eng.
‡S-100 protein was determined by Prof. D. Stavrou at the University of Munich.

recognized by BF7 and GE2 and the antigen recognized by CG12 are not identical with GFAP.

S-100 protein was present in variable amounts in 6/12 gliomas. There was also no correlation between the synthesis of S-100 protein by these cells as assayed by indirect immunofluorescence and the expression of the antigens recognized by the 2 Mabs, BF7 and CG12.

DISCUSSION

The reactivity spectrum of the three anti-glioma Mabs used in this study demonstrates that none of them were directed against a true tumor-specific antigen expressed only by gliomas and not by other tumors or normal tissues. Two of the anti-glioma Mabs, GE2 and BF7, appeared to react preferentially with gliomas, suggesting the existence of antigens expressed in larger amounts on the surface of these tumor cells. It has already been shown by cross-blocking experiments that these anti-glioma Mabs were directed against two different antigenic determinants [13]. Immunochemical analysis, however, showed that they immunoprecipitated the same polypeptide chain with an apparent molecular weight of 48,000 [17]. From these results it was concluded that BF7 and GE2 recognize two different antigenic determinants present on the same molecule. Recent experiments using BF7 and GE2 for immunoperoxidase staining of frozen tissue sections have shown that the recognized antigen is expressed not only on the cell membrane but also in the cytoplasm of tumor astrocytes and of some non-tumoral reactive astrocytes [17].

The third antiglioma Mab, CG12, which reacted with only about 50% of the glioma cell lines tested but cross-reacted with 90% of the melanoma cell lines was shown to precipitate a single polypeptide chain of about 190,000.

The five anti-melanoma Mabs presented here displayed a reactivity spectrum very similar to the one observed with the anti-glioma Mab CG12. It has, however, been shown previously that these six Mabs are directed against different antigens expressed on tumors of neuroectodermal origin [2]. Thus, a second antigenic system was defined by the third anti-glioma Mab CG12 and by the five anti-melanoma Mabs. These antibodies reacted with antigenic structures common to gliomas, melanomas, neuroblastomas and several other tumors of neuroectodermal origin. It is most likely that the antigens recognized by this group of Mabs belong to a class of neuroectodermal differentiation antigens.

The expression of the molecules recognized by GE2 and BF7 on reactive astrocytes as well as on neoplastic astrocytes does not limit the application of these antibodies for immunohistology; however, it does exclude their use for *in vivo* localization in humans. In this respect the reactivity of the anti-melanoma Mabs is highly interesting since they do not bind to normal adult brain cells or other normal tissues in immunohistology (data not shown). The antibodies Mel-5 and Mel-14, which bind respectively to 42 and 48% of the glioma lines tested, may be particularly suitable for *in vivo* use. They both immunoprecipitate a molecule of 230,000 molecular weight and cross-blocking experiments suggest that they recognize two different determinants on the same molecules [Carrel *et al.*, in preparation].

The expression of the HLA-DR antigen, a product of the major histocompatibility complex restricted to B-cells, monocytes and precursors of myeloid cells by several glioma, melanoma and meningioma cell lines has been demonstrated by the use of anti-HLA-DR Mab D1-12 [9]. This antigen is known to be present on so called accessory cells and to play a major role in antigen presentation. The question arises whether the HLA-DR antigen expressed on human glioma cells is merely an abnormal gene product or whether it has a functional role. Recent studies have demonstrated that normal human brain cells also express HLA-DR and that these cells may well be astrocytes [18]. Moreover, it has been shown that mouse astrocytes do indeed present myelin basic protein to encephalitogenic T cell lines [19]. It thus becomes apparent that astrocytes may function as accessory cells in the immune response.

Finally, the high degree of variability in the cellular expression of the antigens recognized by the various Mabs was clearly illustrated by the cell sorter experiments. Even within an individual cell line there was no homogeneous distribution of one particular antigen.

In summary, the present study demonstrates that the antigens detected by the anti-glioma and anti-melanoma Mabs used were not expressed by all glioma or neuroectodermal tumor lines but showed a differential pattern of expression that defines tumor subsets on the basis of surface antigenic phenotype. Most likely such markers will provide new ways to analyze and classify neuroectodermal-derived tumors, although the relevance of such a classification awaits the results of a comparable study with fresh tumor specimens.

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