

WHO grade associated downregulation of MHC class I antigen-processing machinery components in human astrocytomas: does it reflect a potential immune escape mechanism?

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Abstract Defects of major histocompatibility complex (MHC) class I antigen-processing machinery (APM) components have been shown to contribute to immune escape of malignant cells. We investigated the expression of APM components in astrocytomas without detectable defects in HLA class I antigen expression and correlated it with grade of malignancy. Quantitative immunohistochemical analysis of astrocytomas revealed reduced expression of the cytosolic proteasome subunit low molecular weight protein 2 (LMP2), the endoplasmic reticulum (ER) transporter associated with antigen processing-1 (TAP1), and the ER chaperone β 2-microglobulin (β 2m) in astrocytoma cells

when compared to astrocytes from nonpathological brain. Among human WHO grade II–IV astrocytomas, downregulation of LMP2, TAP1 and β 2m correlated with grade of malignancy. Furthermore, astrocytoma cell lines ($n = 12$) expressed all APM components analyzed at levels comparable to dendritic cells (DC), which were used for comparative purposes. However, upregulation of β 2m after stimulation with inflammatory cytokines was significantly lower in astrocytoma cell lines than in control cells. Our results support the hypothesis that coordinated downregulation or impaired upregulation of certain HLA class I APM components may serve as a mechanism for astrocytoma cells to evade the host's immune response, even if HLA class I antigen surface expression is not altered.

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Introduction

Tumor cells express antigens that enable their immunological recognition as “altered-self” cells. These so-called tumor antigens are not expressed or they are expressed much less in nonmalignant cells, or their expression is considered to be limited to certain developmental stages [41]. One hypothesis for how tumors are able to escape from immune surveillance postulates the inadequate activation of cytotoxic T lymphocytes (CTL) that recognize tumor antigens [7]. Efficient recognition and elimination of cells expressing tumor antigens by CTL is dependent on proper processing and presentation of the respective antigens by an intact major histocompatibility complex (MHC) class I APM [37].

During the last decade, various components of the MHC class I antigen-processing machinery (APM) have

been identified, and their functions in the processing of endogenous proteins have been characterized [20, 30]. After ubiquitination, proteins are catalytically degraded by the cytosolic multiproteasome complex, which comprises the constitutive subunits delta, MB1, and Z, as well as the interferon- γ -inducible subunits LMP2, LMP7, and LMP10 [13]. The newly generated peptides are subsequently transported to the endoplasmic reticulum (ER) by TAP, which consists of TAP1 and TAP2 [14]. Within the ER, the chaperones calnexin, calreticulin, tapasin, and β 2m ensure proper loading of the peptides onto MHC class I molecules [2]. Subsequently, β 2-microglobulin (β 2m) stabilizes the newly synthesized MHC class I-peptide complex during translocation to the cell surface via the Golgi compartment [26]. MHC-peptide complexes can then be recognized by the T cell receptor in the context of CD8 (trimolecular complex), eventually leading to T-cell receptor activation and cytolytic damage of the presenting target cell. A prerequisite for the “immune” elimination of transformed cells, therefore, is an intact MHC class I APM, which ensures appropriate processing of antigens expressed by tumor cells.

MHC class I APM alterations have been documented in many malignancies and proposed as a potential mechanism for tumor escape [9]. Interestingly, dysregulations of MHC class I APM components can also occur in tumor cells expressing normal levels of MHC class I molecules [36]. Intact MHC class I surface expression in combination with defective antigen processing might protect tumor cells from natural killer cell (NK cell)-mediated lysis without enabling target cell recognition by T cells [4, 10, 23].

Among nonmetastatic brain tumors, high-grade astrocytomas carry the poorest prognosis. Despite resection, radiotherapy, and chemotherapy, the median survival of glioblastomas (WHO grade IV) is approximately 12 months [42]. The malignancy of these tumors can to some extent be ascribed to their ability to effectively evade the immune system, although infiltration by lymphocytes and macrophages is frequently found within the lesions [44]. The lack of efficient immune responses can partly be attributed to tumor-derived immunosuppressive molecules such as TGF- β , HLA-G or HLA-E [44, 47, 49–51]. It has recently been reported that the grade of malignancy in human brain tumors correlates with decreased MHC class I antigen expression [8]. However, MHC class I molecules were lost and downregulated in 46.2 and 36.2%, respectively, of the glioblastomas analyzed. MHC class I expression was unaltered in 17.6% of the lesions. Furthermore, no correlation between MHC class I expression and disease-free interval and survival was found. It can therefore be hypothesized that failure of the immune system to recognize and eliminate MHC class I positive astrocytoma cells may contribute to inadequate presentation of tumor anti-

gens, as has been reported for other MHC class I positive tumors [36]. In this work we assessed the expression and regulation of various molecules of the MHC class I APM in astrocytomas positive for MHC class I *in vivo* and *in vitro*.

Material and methods

Patients and tissue specimens

Brain tumor specimens from 16 patients were examined. Diagnosis and histological grading was done by two independent neuropathologists according to the World Health Organization (WHO) grade I–IV classification [18]. Our study included four patients with WHO grade I astrocytoma (pilocytic astrocytoma): two females and two males, mean age of 26 years, range 9–38 years; four patients with WHO grade II astrocytoma (diffuse astrocytoma): one female and three males, mean age of 65 years, range 43–84 years; four patients with WHO grade III astrocytoma (anaplastic astrocytoma): two females and two males, mean age of 44 years, range 35–67 years; four patients with WHO grade IV astrocytoma (glioblastoma): four males, mean age of 56 years, range 41–73 years). Two non-pathological brain tissue specimens obtained from the brain bank of the Brain Research Institute of the University of Tübingen served as controls.

Monoclonal and polyclonal antibodies and reagents

The mAb HC-10 which recognizes a determinant expressed on β 2m-free HLA-A10, -A28, -A29, -A30, -A31, -A32 and -A33 heavy chains and on all β 2m-free HLA-B and C heavy chains [31], the mAb HCA-2 which recognizes a determinant expressed on β 2m-free HLA-A (except -A24), -B7301 and -G heavy chains [39], the β 2m-specific mAb L368 [22], the delta-specific mAb SY-5, the MB-1-specific mAb SJJ-3, the Z-specific mAb NB1, the LMP2-specific mAb SY-1, the LMP7-specific mAb HB2, the LMP10-specific mAb TO-6, the TAP1-specific mAb NOB1, the TAP2-specific mAb, NOB2, the calnexin-specific mAb TO-5, the calreticulin-specific mAb TO-11, and the tapasin-specific mAb TO-3 [1, 29, 45] were developed and characterized as described. The HLA-DR-specific PE mAb L243, the CD80-specific mAb BB-1, and the CD86-specific mAb B-T7 were purchased from BD PharMingen, Heidelberg, Germany. Biotinylated rabbit anti-mouse F(ab')₂ fragments was purchased from Dako, Glostrup, Denmark. Goat anti-mouse-phycoerythrin (PE) IgG (H + L) F(ab')₂ fragments were purchased from Dianova, Hamburg, Germany. Mouse IgG2a G155-178 was purchased from BD PharMingen, and mouse IgG1 κ -FITC and mouse IgG1 κ -PE MOPC-21 were purchased from Sigma, Deisenhofen, Germany.

Cell lines

The human glioma cell lines A172, D247MG, LN-18, LNT-229, LN-308, LN-319, LN-428, T98G, U87MG, U138MG, U251MG, and U373MG (kindly provided by Dr. N. de Tribolet, Neurosurgical Service, Centre Hospitalier Universitaire, Vaudois, Lausanne, Switzerland) were cultured in 75 cm³ Falcon plastic flasks (BD Biosciences, Heidelberg, Germany) using DMEM supplemented with 1% glutamine (Life Technologies, Paisley, UK), 10% FCS (Biochrom, Berlin, Germany), and penicillin (100 IU/ml)/streptomycin (100 µg/ml) (Life Technologies) [49].

Culture of dendritic cells (DC)

Dendritic cells (DC) were cultured from peripheral blood mononuclear cells (PBMC) as described previously [34]. In brief, PBMC were isolated from peripheral blood of healthy volunteers by density gradient centrifugation using PAA Separating Solution (PAA Laboratories, Cölbe, Germany). Monocytes were depleted by adhesion to plastic flasks for 1 h. Monocytes, >90% pure, as assessed by flow cytometry, were cultured in RPMI 1640 medium supplemented with 10% FCS, GM-CSF (100 ng/ml) (Leukomax, Sandoz, Germany) and IL-4 (40 ng/ml) (PeproTech EC LTD, London, UK). After a six-day incubation at 37 °C, cells exhibited an immature DC phenotype (MHC class II, CD86^{low}, CD80^{low}). Maturation was induced by incubation of immature DC with LPS (5 µg/ml, *S. typhi*, Sigma L-7261), TNF- α (10 ng/ml), and IFN- γ (1000 U/l) (both from PeproTech EC Ltd., London, UK) [48]. High levels of surface MHC class II and costimulatory molecules (CD86, CD80) identified mature DC.

Immunohistochemistry

All tissues were fixed in buffered 4% formalin (pH 7.4) and paraffin-embedded by routine methods. Washing steps, dilution of antibodies and addition of peroxidase-conjugated avidin–biotin complex were performed in Tris-buffered saline (pH 7.5) with 0.2% Tween. Samples were cut into 4-µm slices, deparaffinized with chloroform, and rehydrated with alcohol in descending order (100–70%). Antigens were retrieved by microwave treatment for 8 min at 700 W in citrate buffer (pH 6.0) followed by blockade of endogenous peroxidase with 3% H₂O₂ in methanol for 15 min at room temperature. After washing, tissue sections were preincubated with tenfold-diluted normal swine serum (Seromed, Berlin, Germany) prior to incubation with MHC class I- and APM component-specific monoclonal antibodies or control antibodies at 20 µg/ml in a humidified chamber overnight at 4°C. Tissue sections were then washed and incubated for 30 min at room temperature with biotinylated rabbit anti-mouse F(ab')₂ fragments. The peroxidase-conju-

gated avidin–biotin complex (ABC) technique (Dako) with 3,3-diaminobenzidine (DAB) (Sigma) used as chromogen was utilized to visualize the specific antigen binding. Tissue sections were counterstained with hemalaun. Two neuropathologists analyzed tissue sections independently. Quantification of immunoreactivity was performed by determining the proportion of positively stained astrocytes among 100 cells in six microscopic fields (magnification: 40 \times) in the respective areas of the brain specimen. Microglia cells and infiltrating lymphocytes have been reported to account for 0–2% of the cells in WHO grade IV astrocytomas and were excluded via histopathology [3]. All histological examinations were performed and photographic documentation obtained with an Olympus AX70 microscope with a digital camera device (Hamburg, Germany); digital photographs were adjusted for brightness and contrast with Jasc Paint Shop Pro (Corel, Minneapolis, MN, USA).

Flow cytometry

The staining procedure for DC and glioma cell lines with the APM component-specific mAbs was performed as described previously [28]. To obtain intracellular staining of APM components, cells were washed in PBS containing 1% bovine serum albumin (BSA) and subsequently fixed with 2% paraformaldehyde for 20 min at room temperature. After washing in PBS–BSA, cells were resuspended in 10 ml of PBS–BSA buffer and subjected to microwave treatment for 60 s at low power (700 W). Cells were then chilled on ice for 10 min, washed, permeabilized using 0.1% (w/v) saponin in the PBS–BSA buffer, and incubated with human IgG (Alphaglobin, Langen, Germany) at 1.25 mg/ml for 10 min at 4 °C to minimize binding to Fc receptors. After washing, unlabeled or labeled primary antibody (f.c. 10–25 µg/ml) diluted in saponin-containing buffer was placed on ice for 45 min. Monoclonal isotype control antibodies were used at the same concentration as the primary antibodies. Incubation was performed on ice for 45 min, followed by two washes. After incubation with secondary antibodies, cells were washed in saponin buffer, fixed with 1% (w/v) paraformaldehyde, and measured in a FACSCalibur cytometer (Becton Dickinson BD, Heidelberg, Germany) using CellQuestR software for analysis. Histograms of stained cells were analyzed by calculating the specific fluorescence index (SFI: geometric mean of the specific antibody divided by geometric mean of the isotype control antibody). Flow cytometry of cell surface markers was performed essentially as described in [34, 49].

Statistical analysis

Data nested in the different groups were analyzed by Wilk–Shapiro W-test and did not show any signs of non-normality.

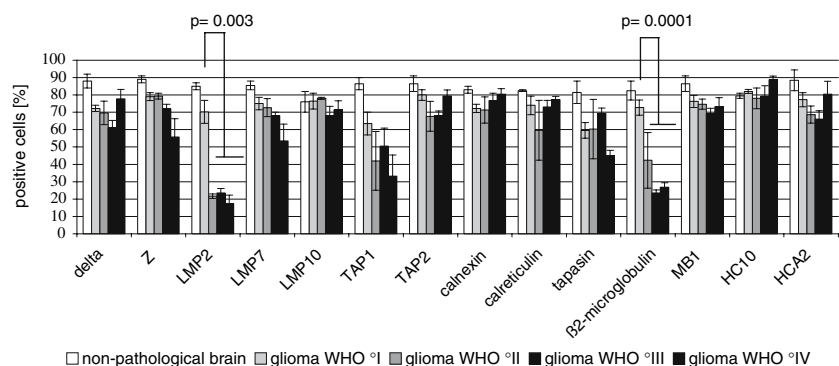
Therefore, statistical significance was assessed by paired Student's two-sided *t* test followed by Bonferroni correction in order to adjust the alpha level to minimize the type I error rate. Since 15 specific antibodies plus isotype control were measured, only a corrected *P*-value of $P < 0.05/16$ was considered to be significant.

Results

Association of WHO grade and MHC class I APM molecule downregulation in human astrocytomas

Expression of the MHC class I APM molecules delta, MB1, Z, LMP2, LMP7, LMP10, TAP1, TAP2, calnexin, calreticulin, tapasin, and $\beta 2m$ was found in astrocytes of nonpathological brain specimens (Fig. 1). MHC class I APM molecule expression in astrocytomas with different grades of malignancy was compared to values assessed in astrocytes in nonpathological brain controls. In specimens of MHC class I positive astrocytic brain tumors of WHO grade I–IV, expression of immunoreactive cells for various APM components varied considerably. In grade I astrocytomas, expression of the examined MHC class I APM molecules was comparable to that in astrocytes from nonpathological brain. In contrast to this, in grade IV glioblastomas only $17\% \pm 5$ (S.E.M.), $27\% \pm 3$ (S.E.M.), and $33\% \pm 6$ (S.E.M.) immunoreactive cells were found for LMP2 ($P = 0.0004$), $\beta 2m$ ($P = 0.00005$), and TAP1 ($P = 0.03$), whereas the expression of delta, MB1, Z, LMP7, LMP10, TAP1, TAP2, calnexin, calreticulin, and tapasin was comparable to that in grade I astrocytomas and astrocytes of nonpathological brain tissues. Interestingly, the frequencies of $\beta 2m$ - and LMP2-positive cells ($P = 0.0001$ and 0.003) were also reduced in high-grade astrocytomas compared with low-grade astrocytic tumors, therefore suggesting that alterations of these APM components correlate with the grade of malignancy (Fig. 1). Representative examples of staining patterns of LMP2, $\beta 2m$, and HLA class I heavy chains in a WHO grade IV glioblastoma and nonpathological brain tissue are illustrated in Fig. 2.

Fig. 1 Characterization of MHC class I APM molecule expression in vivo. Results from immunohistochemistry performed on tissue specimens of nonpathological brain tissue ($n = 2$) and astrocytomas (WHO grade I–IV, four samples each) are presented. Columns indicate mean percentages of positive cells, and error bars represent \pm S.E.M.



Expression and regulation properties of MHC class I APM molecules in human astrocytoma cell lines in vitro

We then investigated the APM in astrocytoma cell lines in vitro. Using flow cytometry, we assessed the expression and regulation of MHC class I APM molecules in 12 astrocytoma cell lines and compared these to DC as a paradigm for professional APC. MHC class I APM molecule expression was measured in astrocytoma cell lines cultured in the presence or absence of inflammatory cytokines, which increase the expression of MHC class I APM molecules [48]. To ensure the efficacy of the intracellular staining procedure, MHC class I APM molecule expression was determined in immature DC (iDC) and mature DC (mDC), and expression patterns with a 1.5- to 6-fold increase of expression during DC maturation were observed (data not shown). Next, the basal expression of MHC class I APM molecules was determined in astrocytoma cell lines, which were previously reported to express varying levels of MHC class I molecules [49]. All APM components were found in astrocytic cells, and the expression levels did not reveal significant differences when compared to iDC (Figs. 3 and 4). However, astrocytoma cell lines showed impaired upregulation of $\beta 2m$ upon exposure to MHC class I APM-inducing cytokine stimulation ($P = 0.003$) (Figs. 4, 5).

Discussion

Characterization of MHC class I APM molecule expression revealed reduced levels of three APM components in human astrocytomas (LMP2, TAP1, $\beta 2m$) compared with normal astrocytes in vivo. Importantly, APM expression levels were negatively correlated with the grade of malignancy, grade IV tumors showing the lowest levels of expression. Experiments in 12 astrocytoma cell lines in vitro partly confirmed our results in vivo: while APM components are abundantly expressed in astrocytoma cell lines, upregulation of $\beta 2m$ is significantly impaired.

Fig. 2a–h Immunoperoxidase staining of serial sections of non-pathological brain tissue (**a–d**) and one WHO grade IV astrocytoma (**e–h**). Stainings were performed for the MHC class I APM components LMP2 (**b** and **f**), β 2m (**c** and **g**), and HLA class I heavy chains (**d** and **h**). Isotype controls are shown in **a** and **e**. Most nonpathological brain astrocytes stained positive for the respective MHC class I APM components. In contrast, most cells found in WHO grade IV astrocytoma specimens were negative for LMP2 (mAb SY-1, **b** and **f**) and β 2m (mAb L368, **c** and **g**). It is worth noting that malignant cells showed immunoreactivity for MHC class I heavy chains (HC10) (**d** and **h**). Arrows indicate examples of positively stained neoplastic cells (*insets in g and h*) as well as astrocytes in nonpathological brain (*inset in d*) (magnification: 400 \times ; magnification of *insets*: 1000 \times)

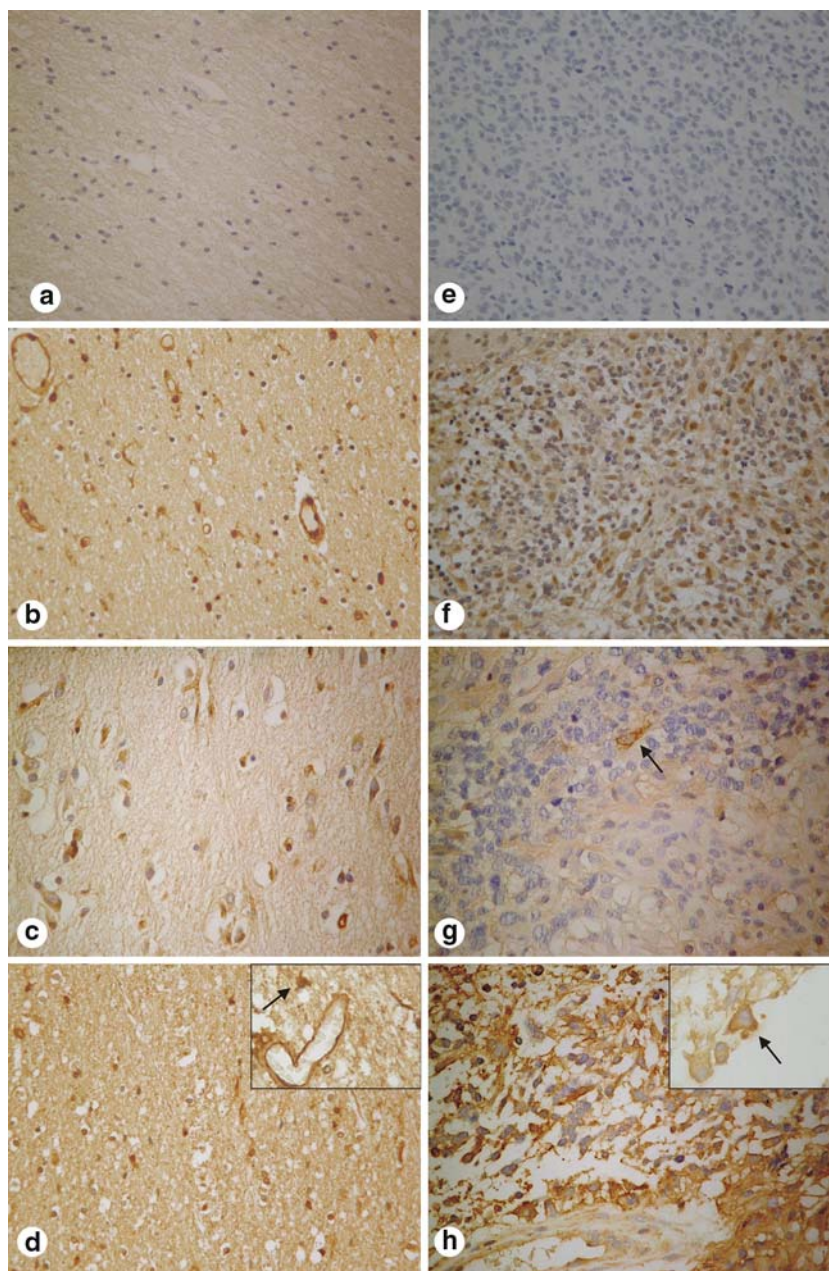
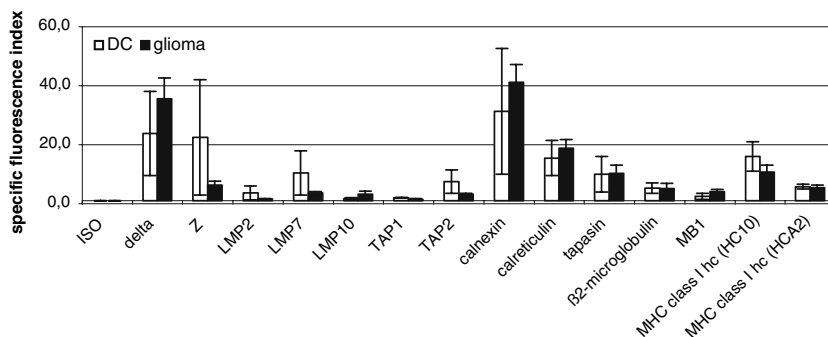


Fig. 3 Flow cytometric analysis of basal expression of MHC class I APM components. Columns represent specific fluorescence indices (SFI) for the indicated molecules in immature DC (DC; $n = 3$) and astrocytoma cell lines ($n = 12$). Error bars illustrate mean values \pm S.E.M.



It has recently been reported that reduced expression of MHC class I molecules correlates with increasing grade of malignancy in human gliomas [8]. Nevertheless, Facoetti

et al. found that more than 50% of human glioblastomas express MHC class I molecules. Since MHC class I expression on the cell surface does not necessarily correlate with

Fig. 4 Flow cytometric analysis of the MHC class I APM components LMP2, TAP2, calnexin, and $\beta 2$ microglobulin in cells cultured in the presence and absence of LPS, TNF- α , and IFN- γ . Changes in APM component expression are illustrated as histograms comparing isotype control (*solid gray*), MHC class I APM component expression after culture with media only (*shaded fill*), and after culture with LPS, TNF- α , and IFN- γ (*open bold line*). y-axis: number of events, x-axis: fluorescence. Histograms show one representative experiment ($n = 3$)

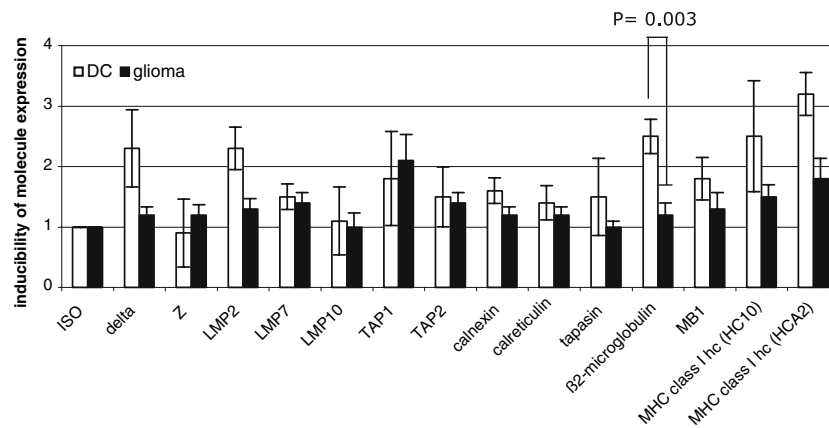
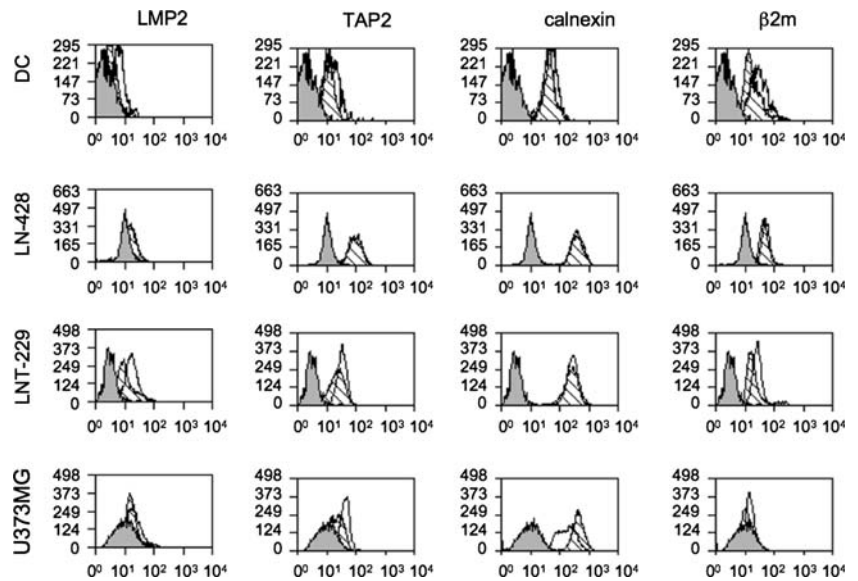


Fig. 5 Inducibility of MHC class I APM molecule expression when cultured in the presence of LPS, TNF- α , and IFN- γ was assessed in dendritic cells (DC) and in astrocytoma cell lines using flow cytometry. Columns represent the inducibility (obtained by calculating ratios of

specific fluorescence indices) of MHC class I APM molecule expression in maturing DC (DC; $n = 3$) and glioma cell lines (glioma; $n = 12$) after treatment with LPS, TNF- α , and IFN- γ for 24 h. Error bars represent \pm S.E.M.

effective antigen presentation in tumors [25], we focused here on the question of APM alteration in MHC class I molecule-positive astrocytoma specimens. These specimens were defined in terms of their MHC class I expression status by mAb HC10, which exclusively detects free HLA class I heavy chains (HC) that are not loaded with immunogenic peptides [40]. In our study, expression of MHC class I APM molecules was studied in WHO grade I–IV astrocytomas and astrocytes from nonpathological brain, which were used as controls as astrocytic progenitor cells are considered to be the key cellular source of malignant transformation in various gliomas [18].

The three molecules (LMP2, TAP1, $\beta 2m$) found to be downregulated in high-grade astrocytomas in our study have highly important functions in the complex cascade where peptides are processed and loaded on MHC class I molecules [19]. Consequently, reduced expression or altered reg-

ulation of LMP2, TAP1, and $\beta 2m$ in astrocytoma cells expressing MHC class I affects immune recognition of tumor cells by antigen-specific T cells and at the same time promotes MHC-I-mediated inhibition of NK cell immunity. Such “non-antigen-related” peptide-free expression of MHC class I molecules could be employed by astrocytoma cells in two ways: first, it avoids activation and antigen-specific recognition by CD8⁺ cytotoxic T lymphocytes (CTLs), since T cell receptors only interact with MHC class I molecules that carry a suitable peptide in their binding cleft [11]. Second, activation of NK cells, which might potentially counteract tumor proliferation, is potentially hindered by the expression of MHC class I molecules [17, 24]. Accordingly, astrocytomas expressing “peptide-free” MHC class I molecules due to impairment of their APM may enforce immune paralysis.

Furthermore, in addition to the lowered expression of $\beta 2m$, LMP2, and TAP1 but not that of other molecules of

the MHC class I APM in human astrocytomas in vivo, we found that the alterations of APM components correlate with the grade of malignancy (Fig. 1): β 2m and LMP2 were expressed in significantly lower frequencies of cells in astrocytomas of WHO grade II–IV than in pilocytic astrocytomas of WHO grade I. This finding is remarkable, as pilocytic astrocytomas have a much better prognosis and are histogenetically not comparable to diffuse astrocytomas and their higher grade variants. In addition to lower levels of β 2m- and LMP2-positive cells, high-grade astrocytomas were also characterized by reduced levels of TAP1-positive cells when compared with nonpathological brain astrocytes. To date, only one study has correlated the alterations of MHC class I APM molecules with tumor differentiation in squamous cell carcinomas [27]. Here, we provide the first study to support the hypothesis that expression of MHC class I APM components correlates with the grade of tumor malignancy. Clearly, higher patient numbers are needed to evaluate the correlation of APM alterations with tumor grade and clinical outcomes in glioma patients as assessed in follow-up studies.

Furthermore, simultaneous downregulation of β 2m and LMP2 in tumors has not been reported so far. This is of special interest, as the expressions of these substantial players of the MHC class I APM, which are located in different cellular compartments, are regulated independent of each other. This observation illustrates the complex changes that occur in gene expression profiles during the malignant transformation of astrocytomas. Downregulation of the inducible immunoproteasome subunit LMP2 inhibits the processing of antigens at an early or initial point in the cytoplasm, whereas downregulation of β 2m and TAP1 impedes proper loading of MHC molecules in the ER. With respect to the immune inhibitory capabilities of astrocytoma cells, the simultaneous downregulation of two APM molecules involved in the loading of MHC molecules in the ER is noteworthy. Since malignant cells are capable of presenting immunogenic antigens such as the Lass5 protein, even if the function of TAP is disturbed [43], multiple alterations or the “coordinated” downregulation of the APM in astrocytomas underline their potential to interfere with alternative forms of antigen presentation.

The finding that all components of the MHC class I APM investigated (delta, Z, LMP2, LMP7, LMP10, TAP1, TAP2, calnexin, calreticulin, tapasin, β 2m, MB1, and HLA class I heavy chains) were expressed in the majority of nonpathological brain astrocytes in vivo is noteworthy in view of the potential of glial cells to process and present antigens. The brain has long been considered an immunologically privileged site. However, numerous studies in infectious, autoimmune, and tumor models have challenged this view by showing that potent immune reactions can and do occur in the CNS. Recently, Höftberger et al. provided evidence that various CNS cells can express MHC class I molecules under basal or inflammatory conditions in vivo,

indicating that all cells of the CNS are potential targets for class I MHC restricted cytotoxic T cells [15]. Our data underline this notion, although we detected a considerably higher proportion of astrocytes staining positive for MHC class I molecules, and are thus in line with the findings of Facoetti et al. [8]. This discrepancy may have methodological explanations. We and Facoetti et al. used different monoclonal antibodies than Höftberger et al., and the latter only considered cells with simultaneous immunoreactivity for β 2m and α -chain as being positive for MHC class I. Our data thus extend these findings by providing evidence of the principal possibility that astrocytes are able to process and present endogenous antigens, a potential function of astrocytes that has probably been underestimated [46]. This may underline the notion of the brain as being immunoprivileged in the way that immunological activity is not absent but rather well controlled [32].

Our in vivo observations with respect to MHC class I APM molecule downregulation in human astrocytomas are substantiated by our in vitro data on the expression and regulation of APM MHC class I components in 12 astrocytoma cell lines. Comparison with the behavior of MHC class I APM in DC provided further evidence for substantial dysregulation of APM components in astrocytoma cells. Upon adequate stimulation with the proinflammatory cytokines TNF- α , IFN- γ , and LPS, upregulation of β 2m, LMP2, and delta was reduced in astrocytoma cell lines as compared with DC (Figs. 4, 5). Our results are in accordance with earlier findings showing altered MHC class I APM expression in various malignant cell lines and tumors (breast cancer, renal cancer, bladder cancer, lung cancer, or melanoma) [5, 6, 9, 12, 16, 21, 33, 35, 38]. However, a coincident study correlating the status of MHC class I APM in vivo and in vitro has not yet been performed.

Taken together, our results extend existing data attributing immunological alterations to the immune-deviating nature of malignant astrocytomas [10]. Further studies are required to investigate the extent to which alterations in MHC class I APM may correlate with patient outcome and response to therapy. In view of the concepts considered for immunoselective therapeutic approaches in future glioma therapy (e.g., tumor vaccination), the problem of altered MHC class I APM molecule expression as a potential mechanism for immune escape must be taken into account. The assessment of MHC class I and APM expression may provide valuable information for the selection of patients that are likely to benefit from these strategies.

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