Cellular immunity of patients with malignant glioma: prerequisites for dendritic cell vaccination immunotherapy

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Object. Vaccination therapy that uses dendritic cells (DCs) is a promising immunotherapeutic approach. However, it relies on intact cellular immunity and efficient generation of mature DCs, both of which can be impaired in patients with glioma. Therefore, the immune status and ex vivo generation of DC in such patients were studied.

Methods. The frequencies of white blood cell subsets and monocyte-derived, mature DCs in patients with high-grade gliomas and healthy control volunteers were analyzed using flow cytometry.

In the patients, frequencies of lymphocytes, T cells, and B cells were reduced in comparison with the volunteers in the control group, whereas frequencies of neutrophils and monocytes were increased. There were no differences between the two groups in terms of white blood cell counts or the frequency of NK cells and the major T-cell subsets. The responsiveness of T cells to lectin stimulation was normal. For monocytes, lower frequencies of CD80+ and CD86+ cells but not of CD40+ and HLA-DR+ cells were observed in patients. Ex vivo DC generation in a two-step culture protocol in autologous plasma-supplemented medium or in serum-free medium showed only minor differences in CD80 and HLA-DR expression between the patient and control groups. Frequencies of CD83+, CD1a+, CD14−, CD40+, and CD86+ cells were comparable. Overall, the serum-free medium was superior to the plasma-supplemented medium and allowed efficient ex vivo generation of CD83+, CD1a+, and CD14− mature DCs.

Conclusions. Only minor defects in the immune status of patients with glioma were observed, which probably would not hamper immunotherapy. Mature DCs can be generated successfully in normal numbers and with typical immunophenotypes from monocytes of patients with glioma, particularly under serum-free conditions.

KEY WORDS • glioma • dendritic cell • immunotherapy • tumor vaccine

The treatment of malignant gliomas has been improved in recent decades with the combination of surgery, radiotherapy, and chemotherapy. Nevertheless, the mean survival time of patients has remained unchanged. The five-year survival rate of patients with glioblastoma is still 2%. Existing treatment modalities are mainly of palliative benefit. This is particularly due to the infiltrative growth of malignant gliomas into the surrounding brain tissue, so that complete surgical removal of tumor cells would cause excessive damage to normal brain tissue, resulting in loss of function for the patient. Therefore, there is a strong need for novel therapeutic approaches in the treatment of malignant glioma.

Immunotherapy that uses autologous DCs is such an approach that might allow induction of tumor-specific T-cell responses that could kill tumor cells specifically without damaging normal tissues. Dendritic cells are specialized antigen-presenting cells that are capable of initiating and polarizing T-cell responses. They have a unique ability to capture antigens and, upon maturation, potentiate induction of tumor-specific T-cell responses, making DCs a potential tool in immunotherapy. Ex vivo, antigen-loaded DCs can be used either to elicit immune responses in vivo or to generate effector T cells ex vivo for adoptive transfer. Initial Phase I studies that have been performed in malignant glioma by using tumor antigen–loaded immature DCs or mature DCs as a vaccine have shown clear evidence of antitumor responses, but the benefits for patients remain to be demonstrated.

Vaccination therapy that uses autologous, tumor antigen–pulsed DCs requires ex vivo generation of adequate numbers of mature DCs under GMP conditions and relies on patients having functional cellular immunity. Patients who have malignant gliomas and might be candidates for vaccination therapy have already undergone radiotherapy and/or chemotherapy, and most of them take steroids to prevent tumor-associated edema. All of these factors can influence a patient’s immune status and, therefore, could hamper the effectiveness of DC vaccination therapy. In addition, the tumor cells themselves can influence the immune system, resulting in systemic immunosuppression in patients with gli-
Glioblastomas secrete various cytokines, including transforming growth factor-β, IL-6, IL-10, and prostaglandins. These tumor-derived immunoregulatory cytokines not only directly affect T-cell activation, proliferation, and effector function, but they also can interfere with DC maturation and activity. Thus, they may prevent efficient DC generation, particularly that of mature DCs. It is the mature DCs that appear to be required in vaccination therapy, rather than immature DCs, which can cause antigen-specific tolerance.

It is currently not well established whether such systemic effects of tumor- or treatment-related changes, which influence the generation of DCs as well as the outcome of such therapy, are present in patients with glioblastoma. For further understanding, we analyzed the immune status and the efficiency of mature DC generation in patients with malignant glioma in comparison with healthy volunteer donors. This study might also help to identify patients who could benefit from DC-based vaccination therapy.

**Clinical Material and Methods**

**Patient and Control Groups**

Peripheral blood samples from 26 patients and 23 healthy control donors were obtained after getting informed consent. The therapies and medications that were used are listed in Table 1. The median time interval between surgery and blood collection in 26 patients was 5.5 days; the mean interval between radiation therapy and blood collection in 15 patients was 7 months; and the mean interval between chemotherapy and blood sample collection in 10 patients was 6.5 months. Median tumor volumes were 42.9 ml before surgery for 15 patients (range 5.5–147.9 ml) and 3.8 ml at close to the time point of analysis in 17 patients (range 0–74.3 ml). Immunoharacterization was performed in 10 men and 11 women, who had a median age of 55 years. Glioblastomas categorized as World Health Organization Grade IV were diagnosed in 17 of these patients, and Grade III anaplastic astrocytomas were diagnosed in four. The eight men and five women in the control group had a median age of 55 years.

The generation of DCs was studied in eight men and seven women who had a median age of 51 years. Glioblastomas categorized as World Health Organization Grade IV were diagnosed in 17 of these patients, and Grade III anaplastic astrocytomas were diagnosed in four. The men and seven women in the control group had a median age of 31 years, so they were significantly younger than the patients. Although there is no evidence for the DC compartment being affected by age, this age difference may cause a bias toward a better performance of control group members compared with the patients, if it has any effect at all.

**Cytokines, mAbs, and Media**

The researchers obtained FITC-conjugated mAbs from Beckman Coulter (Krefeld, Germany). These included mAbs specific for CD3 (UCHT1), CD4 (13B8.2), CD8 (B9.11), CD14 (RM052), CD25 (B1.49.9), CD28 (CD28.2), CD45RA (ALB11), CD45R0 (UCHL1), CD80 (MAB104), CD83 (HB15a), and HLA-DR (Immu-357) as well as isotype controls; phycoerythrin-conjugated mAbs specific for CD1a (BL.6), CD3 (UCHT1), CD14 (RM052), CD19 (J4.119), CD56 (NKH-1), and CD83 (HB15a) as well as isotype controls; and nonconjugated mAbs specific for CD14 (RM052). In addition, FITC-conjugated mAbs specific for CD1a (H1149), CD40 (5C3), CD45 (2D1), and CD86 (FUN-1) and phycoerythrin-conjugated mAbs specific for CD152 (BN13) and CD154 (TRAP1) were obtained from BD Biosciences (Heidelberg, Germany).

Both TNFα and IL-4 were obtained from CellGenix (Freiburg, Germany). Two types of GM-CSF were used: Leucomax 150 (Essex Pharma, Munich, Germany) and Leukine Sargramostim (Berlex, Richmond, CA). Final concentrations of cytokines were 1000 U/ml each.

The culture medium for ex vivo DC generation was X-VIVO 15 medium (Cambrex, Verviers, Belgium) supplemented with 1.5% autologous plasma. Autologous plasma was heat inactivated (30 minutes at 56°C), sediments were removed by centrifugation (15 minutes at 1880 G and 20°C), and the plasma was filtered (0.2-μm Minisart; Sarstorius, Göttingen, Germany) before use. CellGroDC medium was obtained from CellGenix and was used without the addition of serum or plasma.

**Enrichment of CD14+ Monocytes**

Monocytes were enriched from PBMC by using LS separation columns on a VarioMACS separation unit according to the instructions of the manufacturer (Miltenyi Biotec, Bergisch Gladbach, Germany), as described previously.

**Ex Vivo Generation of DCs**

Enriched CD14+ monocytes (10⁶ cells/ml) were incubated in X-VIVO 15 medium supplemented with 1.5% autologous plasma or CellGroDC medium in the presence of GM-CSF and IL-4 (1000 U/ml each) in 24-well plates (Corning Costar, Wiesbaden, Germany) at 37°C in 5% CO₂ in a humidified atmosphere. After 3 days of incubation, 1 ml of medium was replaced with 1 ml of fresh medium containing GM-CSF and IL-4 (2000 U/ml each). On Day 6 of incubation, 1 ml of medium was replaced with 1 ml of fresh medium containing GM-CSF, IL-4, and TNFα (2000 U/ml each). Cultures were continued until Day 9, when cells were harvested from the 24-well plate, washed, and analyzed.

**Cell Counts and Flow Cytometry**

Cell counts were determined using an analyzer (Abbott Cell-Dyn 3500; Abbott, Wiesbaden, Germany); viability was determined using trypan blue exclusion and microscopic evaluation.

Immunostaining of blood samples was performed as described previously. Samples were analyzed using a flow cytometer (EPICS XL-MCL or FACS Calibur; Beckman Coulter or BD Biosciences, respectively). Frequencies of leukocyte, T-lymphocyte, and monocyte subpopulations were determined using electronically gated CD45⁺ leukocytes, CD3⁺ T lymphocytes, and CD14⁺ monocytes, respectively.

**Stimulation With PHA**

To determine mitogen responsiveness, PBMC from three patients (Table 1) and two control donors (10⁶/ml, 200 μl/well) were cultured in 96-well, round-bottom plates (Grein-
Immune status of patients with glioma

**TABLE 1**

Diagnosis, therapies, and medications in 26 patients with glioma*

<table>
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<th>Case No.</th>
<th>Diagnosis</th>
<th>Previous Therapies</th>
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* AA = anaplastic astrocytoma; AED = antiepileptic drug; BB = beta-blocker; chemo = chemotherapy; D = dexamethasone; GBM = glioblastoma multiforme; H = hypothyroid medication; NA = not applicable; O = omeprazole; PCV = procarbazine, lomustine, and vincristine; RT = radiotherapy; TMZ = temozolomide; † = not specified.
‡ Tumor volumes (ml): presurgery/close to the time point of analysis.
† Months after radiotherapy or chemotherapy until blood collection.
§ Patient blood samples were used for immunocharacterization.
‖ Patient blood samples were used for DC generation.
** Patient blood samples were used for PHA stimulation.

**Results**

**Immunohistochemical Analysis of Glioma Patients and Healthy Normal Donors**

Blood cell counts, white blood cell subpopulations, and the expression of relevant immunoregulatory molecules on T cells and monocytes were studied in patients with glioma and in healthy control volunteers. Results are summarized in Fig. 1 and Table 2.

Frequencies of lymphocytes (28.6 ± 1.6% and 19.0 ± 1.7% for control and patient groups, respectively; p < 0.001), CD3+ T cells (28.9 ± 2.2% and 17.3 ± 2.6% for control and patient groups, respectively; p = 0.003), and CD19+ B cells (4.6 ± 0.7% and 2.4 ± 0.5% for control and patient groups, respectively; p = 0.011) were significantly lower in the patient group. However, frequencies of neutrophils (60.7 ± 1.9% and 70.7 ± 2.3% for control and patient groups, respectively; p = 0.005) and CD14+ monocytes (5.4 ± 0.4% and 6.9 ± 0.5% for control and patient groups, respectively; p = 0.028) were significantly higher in the patient group (Fig. 1A). There were no significant differences between the two groups in frequencies of CD56+ NK T cells and NK cells (Fig. 1). The differences in frequencies translated to a trend in patients toward lower absolute cell counts for lymphocytes and B cells (2.0 ± 0.2 × 10⁶ cells/ml compared with 1.6 ± 0.2 × 10⁶ cells/ml and 0.3 ± 0.1 × 10⁶ cells/ml compared with 0.2 ± 0.1 × 10⁶ cells/ml for control and patient groups, respectively) and a significant reduction of CD3+ T-cell numbers (2.0 ± 0.2 × 10⁶ cells/ml compared with 1.3 ± 0.2 × 10⁶ cells/ml for control and patient groups, respectively; p = 0.039). However, the total white blood cell counts and numbers of neutrophils, NK cells, and monocytes were not significantly
were analyzed. Expression of the molecules indicated on
 white blood cell counts (data not shown).

FIG. 1. Scatterplots showing frequencies of white blood cell (A),
 T-cell (B), and monocyte (C) subpopulations in peripheral blood of
 patients with glioma and healthy control volunteers. Blood samples
 of patients (white squares) and control group members (black
 squares) were analyzed. Expression of the molecules indicated on
 T cells and monocytes for electronically gated CD3+ and CD14+
 cells, respectively, was determined using flow cytometry. Median
 values are shown as horizontal lines; statistical significance was de-
 termined according to the Mann–Whitney U-test. Lym = lympho-
 cytes; mon = monocytes; neu = neutrophils.

different between the patient and control groups (data not
shown).

The median tumor volume in 12 patients at the time of
analysis (postsurgery) was 1.9 ml. When the immune sta-
status was compared for patients with tumor volume that was
above or below the median, there were no significant dif-
f erences between the two groups in the frequencies of CD3+
T cells, CD19+ B cells, CD56+ NK and NK T cells, CD14+
monocytes, neutrophils, and lymphocytes as well as in the
white blood cell counts (data not shown).

The immunophenotypic characterization of T cells (Fig.
1B) revealed no significant differences between patients
with glioma and healthy control volunteers in the fre-
frequencies of CD4+, CD8+, CD25+, CD28+, CD45RA+, CD45RO+, CD56+, and HLA-DR+ T cells.

The characterization of monocytes (Fig. 1C) showed a
significant reduction in the frequencies of CD80+ mon-
cytes for patients (72.3 ± 5.5% and 39.8 ± 9.7% for con-
trol and patient groups, respectively; p = 0.015) as well as
CD86+ monocytes (94.8 ± 2.3% and 85.7 ± 4.7% for con-
trol and patient groups, respectively; p = 0.029). There were
no significant differences between the two groups in the fre-
frequencies of CD40+ and HLA-DR+ monocytes. When the
density of expression of these molecules on monocytes was
analyzed, no significant differences were observed between
the patient and control groups (data not shown).

Stimulation With PHA

To compare lectin responsiveness, PBMCs from three
patients with glioblastomas and two healthy controls were
Immune status of patients with glioma

stimulated with PHA, and BrdU incorporation was determined after 3 days. The PHA responsiveness was comparable for the patients and healthy controls (Fig. 2).

**Ex Vivo Generation of DCs**

Monocytes from 15 patients with malignant gliomas and 13 healthy donors were differentiated to DCs in X-VIVO 15 medium supplemented with 1.5% heat-inactivated autologous plasma in the presence of GM-CSF and IL–4 for the first 6 days of incubation, followed by an additional 3-day incubation period in the presence of TNFα, GM-CSF, and IL–4 to induce DC maturation. Following immunomagnetic enrichment on Day 0, CD14+ monocytes accounted for 97.2 ± 2.0% of all cells for 10 control samples and 98.0 ± 0.5% of all cells for 15 patient samples. The differentiation and maturation of the DCs were evaluated after 9 days of incubation by using flow cytometric enumeration of CD14, CD10, CD11a, and CD83 positive cells as well as analysis of CD40, CD80, CD86, and HLA-DR expression.

The differentiation of monocytes to DCs was associated with downregulation of the monocyte marker CD14 and upregulation of the mature DC-associated CD83 antigen (Fig. 3A and B). There were no significant differences between the control and patient groups in the frequencies of mature CD83+ DCs (24.3 ± 6.7% compared with 34.1 ± 7.3%), residual CD14+ monocytes (20.8 ± 4.6% compared with 14.1 ± 4.2%), and CD14+DCs (5.1 ± 1.7% compared with 5.5 ± 1.2%) (Fig. 4A). There were also no differences in CD40, CD86, and HLA-DR expression (Figs. 3C and 4C), all of which were positive on the majority of cells, whereas expression of CD80 was significantly higher for the 10 patient samples compared with the 13 control samples (59.8 ± 6.5% and 40.7 ± 5.8%, respectively; p = 0.044). Overall cellular recovery (Fig. 4B) was not different between the patient and control groups. In addition, no differences were observed between the two groups when the outputs of CD83+ mature DCs and residual CD14+ monocytes were calculated for an input of 10^6 CD14+ monocytes.

When cultures were performed in CellGroDC serum-free medium (Fig. 5) instead of autologous plasma-supplemented X-VIVO 15 medium (Fig. 4), no significant differences were seen between patient and control groups in frequencies of CD83+, CD14+, CD11a+, CD40+, CD80+, and CD86+ cells; in the cellular outputs of CD14+ and CD83+ cells; or in overall recovery (Fig. 5). The only exception was a higher frequency of HLA-DR+ cells observed for 10 patients compared with 13 healthy controls (85.2 ± 3.3% and 95.0 ± 1.9% in control and patient groups, respectively; p = 0.026). However, when the results of ex vivo DC generation were compared between the two media for all samples from patients and healthy controls combined (Fig. 6), serum-free CellGroDC medium showed superior results compared with plasma-supplemented X-VIVO 15 medium in the generation of mature DCs, with a significantly higher frequency of CD83+ mature DCs in 28 cultures (68.4 ± 4.2% compared with 29.6 ± 5.0%; p < 0.001) and CD14+DCs in 25 cultures (44.3 ± 5.4% compared with 5.3 ± 1.0%; p < 0.001) as well as a lower frequency of residual CD14+ monocytes in 28 cultures (1.2 ± 0.4% compared with 17.2 ± 3.1%; p < 0.001).

Patients had a median tumor volume of 10 ml at close to the time point of analysis. When DC generation was compared for patients with a tumor volume that was above or below the median, there were no differences between the two groups in the frequencies of CD14+ and CD83+ cells for plasma-supplemented and serum-free culture systems (data not shown).

**Discussion**

The immune status of patients with gliomas revealed several differences when compared with that of healthy controls. Patients were lymphopenic due to a significantly reduced frequency of T cells. Relative frequencies of the major CD4+ helper and CD8+ cytotoxic T-cell subsets, however, were normal, suggesting that the reduction of T cells is more general rather than subset restricted. The occurrence of T-lymphopenia is well documented in patients with malignant glioma.14 It has been attributed to T-cell apoptosis28 or reduced thymic output,10 whereas mild neutrophilia observed in such patients may be due to glioma cells producing granulocyte colony-stimulating factor.19 In contrast to T cells, frequencies of NK cells were in a normal range, which is consistent with earlier reports.24 Recently, Dhodapkar and colleagues11 have shown that functionally competent NK T cells are also present at normal frequencies and numbers in glioma patients. Thus, immunological changes associated with malignant glioma appear mainly to affect the T-cell compartment.

In the patients, T cells showed a normal expression of CD4, CD8, CD25, CD28, CD45RA, CD45RO, CD56, and HLA-DR, again arguing for a more general depletion of T cells. Differences in CD4 and CD28 expression, which have been reported by others,13 were not evident in the patients in this study.

Functionally, T cells revealed normal responsiveness to PHA stimulation, which is in contrast to previous reports, documenting a state of anergy and reduced responses to mitogens or anti-CD3 stimulation of T cells in patients with malignant gliomas.1,5,14-16 The reason for this difference is not clear, but several studies have indicated that glioma-as-
sociated immunosuppression correlates with the size of the tumor and that surgical cytoreduction can restore T-cell responsiveness, at least partially. 6,7,29 At the time point of analysis, the patients in this study had already undergone cytoreduction to produce generally lower tumor volume (median of 3.8 ml for all patients) and there were no differences in leukocyte subsets observed among patients with a residual tumor volume that was higher or lower than the median. This not only points out the potential importance of surgery prior to immunotherapy to restore the immune system of patients with malignant glioma, but also the importance of prescreening patients to identify general immunological parameters (for example, immune status, DC differentiation, PHA responsiveness, and delayed-type hypersensitivity responses) that may affect the outcome of therapy. Furthermore, although numbers are still small, the cellular compon-
not only because of the patient’s plasma potentially containing immunosuppressive factors, but also because of the use of steroid medication in patients with glioma to prevent tumor-associated edema. Dexamethasone has been reported to interfere with DC differentiation and maturation. Alternatively, serum- and plasma-free culture systems have to be established.

When monocytes from patients and healthy controls were cultured in the presence of 1.5% heat-inactivated, autologous plasma–supplemented X-VIVO 15 medium using a protocol reported previously, the ex vivo generation of DCs was comparable for patients and controls, other than a slightly increased expression of CD80 in the patients. Thus, despite the presence of plasma potentially containing immunosuppressive factors and despite a potential bias toward better performance by members of the control group due to their significantly younger age, DC differentiation and, particularly, maturation in patients were normal. When the plasma-supplemented culture system was replaced by a serum-free culture system, again, other than a slightly in-

**Fig. 4.** Scatterplots comparing DC differentiation of CD14+ monocytes obtained in patients with glioma and healthy control volunteers. Ex vivo generation of DCs from patients with malignant glioma (white squares) and healthy control volunteers (black squares) was performed in plasma-supplemented X-VIVO 15 medium. On Day 9, cells were collected and frequencies of CD14+, CD83+, and CD1a+ cells (A); cellular recovery and output of CD14+ and CD83+ cells (calculated for a cellular input of 10^6 cells [B]); and frequencies of CD40+, CD80+, CD86+, and HLA-DR+ cells (C) were determined. Median values are shown as horizontal lines; statistical significance was determined according to the Mann–Whitney U-test.
creased expression of HLA-DR, no differences between patients and controls were observed. However, differentiation and maturation of DCs under serum-free conditions using GMP-grade CellGroDC medium, which does not require any cell culture supplements other than cytokines, resulted in high purity ex vivo generation of mature CD83+ and CD14+ DCs that expressed the costimulatory molecules CD40, CD80, and CD86 as well as HLA-DR at high densities. Therefore, they should be well suited for DC-based immunotherapy for patients with malignant gliomas, even those who have had radio- or chemotherapy, are taking steroid medication, or have grim prognoses. No differences were observed among patients with residual tumor volume that was higher or lower than the median. Thus, it appears to be possible to overcome or at least to reduce tumor-induced systemic immunosuppression in malignant glioma by cytoreduction and to generate mature DC efficiently ex vivo for vaccination therapy. However, whether this will be sufficient to overcome the local immunosuppressive or immunomodulating effects of the tumor cells remains to be determined. Such effects are at least partially mediated by the production of transforming growth

Fig. 5. Scatterplots comparing DC differentiation of CD14+ monocytes obtained in patients with glioma and healthy controls. Ex vivo generation of DCs from patients with malignant glioma (white squares) and healthy controls (black squares) was performed in serum-free medium. On Day 9, cells were collected and frequencies of CD14+, CD83+, and CD1a+ cells (A); cellular recovery and output of CD14+ and CD83+ cells (calculated for a cellular input of 10^6 cells [B]); and frequencies of CD40+, CD80+, CD86+, and HLA-DR+ cells (C) were determined. Median values are shown as horizontal lines; statistical significance was determined according to the Mann–Whitney U-test.
Immune status of patients with glioma

factor–B10 and prostaglandin E2 by gliomas but may also include other immune escape strategies developed in the course of immuneediting of the tumor, all of which may hamper the effector arm of antitumor immunity in immunotherapy.

Conclusions

Following cytoreductive surgery, chemotherapy, and radiotherapy, patients with gliomas revealed only minor immune defects, which probably would not prevent the effective use of an active immunotherapy. These minor immune defects include lymphopenia, mainly due to a general reduction of all T-cell subsets, and the reduced expression of the costimulatory molecules CD80 (B7.1) and CD86 (B7.2) on monocytes. Despite these differences, monocytes of patients could be differentiated and matured in a two-step culture system and could generate mature dendritic cells in normal numbers that have immunophenotypes similar to those of healthy control volunteers. Thus, efficient ex vivo generation of mature DCs in patients with glioma is possible, particularly when serum-free medium suitable for GMP production of DCs is used. Nevertheless, all patients considered for DC vaccination therapy should be prescreened. For example, determination of their immune status, lectin and delayed-type hypersensitivity responses, and DC differentiation and maturation could help determine whether successful vaccine generation is possible and identify more general parameters that could affect the outcome of the therapy.

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References

19. Hintzen RQ, Voormolen J, Sonneveld P, van Duinen SG: Glio-

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