

Role of Cytokines in the Regulation of Glioma Tumour Growth and Angiogenesis

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Abstract: Malignant glioblastomas are the most common type of primary brain tumors accounting for more than 50% of all intracranial gliomas. Due to their aggressive nature, recurrent growth, and their extremely poor prognosis for survival, research approaches have made little progress in advancing treatment of these tumours which have the ability of both modulating host immune function and neutralizing immune-based therapies. Patients with gliomas exhibit a broad suppression of cell-mediated immunity by a shift in cytokine secretion from Th1-type immune response to Th2, with an increased secretion of anti-inflammatory cytokines been observed, possibly due to release of a variety of immunosuppressive factors. Furthermore, secretion of specific cytokines stimulates angiogenic processes which contribute to the development of malignancy of these tumours. This review summarizes the role of cytokines in glioma growth and invasion, and indicates how specific cytokines may affect angiogenesis.

Key words: glioblastomas, cytokines, angiogenesis, review

INTRODUCTION

Malignant glioblastomas (gliomas), the most common primary supratentorial cerebral neoplasms in adults, are diffusely infiltrative and rapidly fatal [1]. Gliomas develop and remain within the brain, rarely metastasizing beyond its borders. However, it is the motile invading cells from these tumours, which cannot be surgically extirpated, that are responsible for tumour recurrence following radical resection [2,3]. Through unknown mechanisms, they can lead to progressive neurologic dysfunction without evidence of mass effects or recurrence of bulk disease [4]. Furthermore, while gliomas are sequestered from the majority of the immune system as a result of immune privilege, they are still able to cause broad host immunosuppression.

Immunotherapy is a theoretically attractive method of treatment since tumour cells may be selectively targeted [5,6]. However despite encouraging results in the laboratory, neither adoptive immunotherapies [7,8], nor recently-developed active immunogene therapies [9,10] have, as yet, led to successful tumour eradication. Recent observations suggest the immune system's failure to recognize tumour cells may in part be attributed to tumour associated-cytokine dysregulation [11].

Cytokines are multifunctional pleiotropic proteins that regulate differentiation [12], angiogenesis [13], locomotion [14], and a variety of other cell functions. Functionally, cytokines have been classified as being either pro-inflammatory (Th1-type, stimulatory) or anti-

inflammatory (Th2-type, inhibitory) depending on the final balance of their effects on the immune system [15].

The various cytokines affecting directly the CNS have two possible origins; cytokines which originate from peripheral immune organs and cross the blood-brain barrier and those cytokines that may be produced by the neuronal cells within the CNS [15].

Cytokines exert their effects by highly specific binding to cell membrane receptors which convey signals via tyrosine kinases and possibly other second messenger systems (such as cAMP-dependent kinase and protein kinase C) [15,16]. Neoplastic cells may downregulate or lose dependence on exogenous cytokines as a result of upregulated endogenous cytokine production, altered expression of membrane receptors, and/or alterations of secondary messenger systems [17].

Aberrations of normal cytokine-mediated cell proliferation have been extensively described for gliomas [18,19]. For example, amplification of the EGF receptor (EGFR) has been inversely correlated with length of survival for patients with malignant glioma [20].

Cytokine dysregulation in patients with gliomas:

Cytokine dysregulation plays a role in a number of cellular processes including the downregulation of a normal immune response (e.g., pregnancy) and in a variety of disease states (e.g., AIDS, cancer, lupus, *Leishmania* infection) which may facilitate T-cell apoptosis [21-23]. There is evidence that glial tumours may induce a Th1 to Th2-type cytokine shift. It is likely

that the switching of Th1/Th2 type cytokines in gliomas to the predominant expression of Th2 type cytokine genes is related to the origination of gliomas and the evasion of glioma cells from immune surveillance [11,24,25].

Firstly, secretion of the Th1-type cytokine IL-2 by mitogen-stimulated T-cells obtained from patients has been shown to be greatly diminished [26]. Secondly, *Candida albicans* MP, which induces normal lymphocytes to secrete the Th1-type cytokine IFN γ , fails to stimulate peripheral blood lymphocytes (PBL) obtained from patients to produce this cytokine [27]. This is not surprising given the fact that CD4q T-cells are the major producers of IFN γ and the CD4q T-cells in patients with gliomas are both reduced in number and less responsive to mitogens and antigens than normal CD4q T-cells [5,28]. In addition, prostaglandin E₂ (PGE₂), which is secreted by gliomas, can further decrease the production of IFN γ by the Th1 (or CD4q) subset [29]. Because both IL-2 and IFN γ are important in the generation of lymphokine-activated killer (LAK) cells and cytotoxic-T-Lymphocyte (CTL) activity, decreased cytokine production may be responsible for the impaired generation of antigen-stimulated, major histocompatibility complex (MHC)-unrestricted cytotoxicity observed in glioma patients [30]. Thirdly, PBL obtained from patients harbouring gliomas produce increased amounts of the Th2-type cytokine, IL-10, upon stimulation with the T-independent B-cell mitogen *Staphylococcus aureus* Cowen I strain (SAC) *in vitro* [31]. Conversely, PBL from these same individuals were unable to synthesize the Th1-type cytokines IL-12 (p40 and p70) and IFN γ in response to SAC stimulation [31]. Furthermore, gliomas themselves have been shown to synthesize and secrete IL-10, adding to the dysregulation of Th2-type cytokines [32]. Finally, tumour infiltrating lymphocytes (TIL) detected at the glioma site secrete predominantly Th2-type cytokines [33]. Underscoring the importance of Th1-type cytokines in immune responses to tumour cells, it has been shown in a colon carcinoma model that the presence of IL-12 at a tumour site may be critical for tumor regression [34]. Additional studies have demonstrated a similar role for IL-12 in tumour regression using several different murine models of cancer [31,35,36]. The anti-tumour effects of IL-12 appear to be mediated by IFN γ [37]. Thus, impaired IFN γ synthesis in patients harbouring gliomas may lead to a more severe Th1-type cytokine dysregulation by further impeding IL-12 activity. In summary, a Th2-type cytokine shift which would support humoral immunity at the expense of cell mediated immune responses is observed in patients with gliomas and may contribute to the inefficient anti-tumour responses generated in these patients.

Role of cytokines in glioma development: Two major groups of signalling pathways are believed to regulate

gliomagenesis: (a) the growth factor – regulated signalling pathway and (b) the cell-cycle pathway [38]

Several cytokines are capable of regulating both pathways through direct or indirect actions. Specifically, interleukin-6 (IL-6) is expressed by human glioblastoma cell lines and patients with malignant gliomas exhibit increased IL-6 levels that correlate with the histopathological grade of the neoplasm [39].

IL-6 contributes to malignant progression and apoptosis resistance of brain tumours by: (a) controlling the final maturation of B-cells into antibody-producing cells, further stimulating immunoglobulin synthesis by plasmocytes. Autologous humoral immune responses associated with gliomas have been described and increased amounts of immune complexes have been found in patients' sera which correlated with poor prognosis; (b) by stimulating the activation of NK cells and the generation of cytotoxic T-lymphocytes and (c) by acting as a hepatocyte-stimulating factor which promotes hepatic plasma protein synthesis known as acute phase proteins [39,40]. Several reports have suggested a possible role for IL-6 in the pathogenesis of astrocytic tumours. IL-6 is mitogenic for astrocytes and together with its soluble receptor (sIL-6Ra) induces a massive reactive gliosis in IL-6/IL-6Ra double transgenic mice [39,41]. Upregulation of IL-6 in astrocytomas occurs through IL-6 gene amplification further promoting glial tumour cell growth by an auto/paracrine mechanism. Reports have shown that stimulation of astrocytoma cells by C3a, C5a and peptidic C3aR and C5aR agonists induced an increase in the IL-6 mRNA levels [42]. The production of IL-6 by normal human astrocytes stimulates also the IL-1 β secretion [40]. In a mouse model of spontaneous astrocytoma formation, it was shown that IL-6 may promote tumour development by activating STAT3 and the level of STAT3 activation was found to increase with the histopathological grade. Furthermore, abrogation of IL-6 expression suppressed astrocytic tumour formation in GFAP-v-src transgenic mice [43,44]. However, secretion of IL-6 from Rat T9 glioma clone cells and fibrosarcoma cells exhibit reduced tumorigenicity, increased immunogenicity, antiglioma immunity and decreased metastatic potential [45].

Interleukin 8 (IL-8) is another cytokine involved in glioma development with both chemotactic and angiogenic properties [46,47,48]. Its expression is initiated early in astrocytoma development through induction by inflammatory stimuli and increases later in tumor progression due to a reduced microenvironmental oxygen pressure [47]. *In vitro* hypoxic/anoxic insults on glioblastoma cells may induce an increase in IL-8 mRNA and protein expression [48]. Augmented IL-8 directly and/or indirectly promotes angiogenesis by binding to the Duffy antigen receptor for chemokines (DARC) and by inducing leucocyte infiltration and activation by binding to the chemokine receptors CXCR1 and CXCR2 [48,49].

It was also found *in vitro*, that glioblastoma cell lines constitutively expressed IL-8 mRNA and secreted IL-8 protein. *In vivo*, IL-8 protein and mRNA are presented in low grade astrocytomas, anaplastic astrocytomas, glioblastomas and in brain cyst fluids [39]. IL-8 secretion, stimulated by IL-1 β , TNF and IL-17 may be a key factor involved in the determination of the lymphoid infiltrates observed in brain tumours and the development of cerebrospinal pleocytosis in meningoencephalitis [39,50]. In addition, it has been demonstrated that higher intracavitary levels of IL-8 may be correlated with shorter adjunctive survival time [51].

Role of glioma-derived cytokines in immunosuppression: The occurrence of brain tumours is associated with the broad suppression of immune system function; however, the mechanisms involved in this impairment are not fully characterized. Although there is evidence that gliomas secrete factors capable of inhibiting immune function, there is less agreement as to the identity of these substances. It has been shown that the degree of immunosuppression observed in patients with gliomas correlates with tumour size but not with tumour location [52]. Surgical excision is followed by a partial restoration of *in vitro* T-cell function [53]. Conversely, recurrent neoplastic growth, as detected by computed tomography (CT) imaging, is associated with a return of depressed immune function [53]. Similarly, patient peripheral blood monocytes exhibit reduced MHC class II expression prior to surgical excision of the glioma, which again increases to near normal levels after surgery [54]. It is thus clear that tumour presence is associated with impaired immune function in glioma patients.

Glioma cells secrete multiple factors capable of modulating immune function. Previous studies have demonstrated that serum obtained from patients was capable of suppressing the mitogen responsiveness of autologous and homologous normal lymphocytes while serum obtained from patients with other types of brain tumours failed to do so [55-58]. In addition, tumour cyst fluid and cerebral spinal fluid from glioma patients suppressed the mitogen responsiveness of normal lymphocytes [59]. Further support for the secretion of suppressive factors by gliomas comes from the observation that glioma cell culture supernatants (GCS; freshly explanted or from tumour cell line) inhibit the function of T-cells obtained from normal individuals [60]. Taken together, these observations confirm that gliomas actively synthesize and secrete potent immunoregulatory moieties capable of inhibiting T-cell responsiveness. These factors are presented in Table 1. At present, not all of the moieties within GCS that are of an immunosuppressive nature have been fully characterized. This is exemplified by the observation that the factors inhibiting proliferative T-cell responses do not appear to be the same as those capable of

altering monocyte cytokine secretion patterns [60]. In addition, some of the activity in GCS which affects the immune functioning of monocytes and T-cells can be reversed with neutralizing antibodies (i.e., anti-IL-10 mAb or inhibitors (e.g., the PGE inhibitors, 2 naproxen, ibuprofen and indomethacin) [61].

Table 1. Glioma secreted soluble factors that inhibit T cell responsiveness

SOLUBLE FACTORS	REFERENCES
TGF- β 1, 2, 3	63-67
PGE	29
IL-10	31,32,68-73
Gangliosides (GANGs)	87,88

Although the cellular and biochemical defects induced by the addition of GCS to normal T-cells are similar to those observed in T-cells obtained from patients with gliomas, the effects of purified substances such as TGF β or IL-10 fail to replicate the immunosuppressive effect of GCS on normal T-cell function [62]. Some of the effects of those cytokines (TGF- β 1, 2, 3, IL-10, IL-2) known to be secreted by gliomas are considered in more detail below.

Gliomas synthesize and secrete TGF- β _{1, 2, 3} which downregulates monocyte surface marker expression, cytokine secretion, cytotoxicity and T-cell responsiveness [63-65]. TGF- β ₂ has been implicated as the major glioma-derived T-cell immunosuppressant. [66]. However, previous studies examining the TGF β antagonist, decorin, have indicated that factors other than TGF β appear to be responsible for glioma-mediated immunosuppression [67]. Further evidence suggesting that TGF β may not have a significant role in glioma-mediated immunosuppression stems from T-cell proliferation studies utilizing neutralizing antibodies to TGF β . Therefore, although TGF β has been implicated, its role as a significant inhibitory factor for the immune suppression observed in glioma patients is probably marginal.

Interleukin-10 (IL-10) is a 17–21-kDa cytokine produced by activated T- and B-cells, monocytes, macrophages and some cancer cell types [68,69]. Originally, it was identified as a cytokine synthesis inhibitory factor because of its ability to inhibit the synthesis of IFN γ , IL-1a, IL-1b, IL-6, IL-8, granulocyte-colony stimulating factor and granulocyte macrophage-colony stimulating factor (GM-CSF) from activated monocytes and lymphocytes [70]. IL-10 has also been shown to reduce the antigen presentation capacity of monocytes by down-modulating MHC class II expression, thus hindering antigen-stimulated proliferation of T-cells and IL-2 production [71]. IL-10 mediated suppression of human T-cell proliferation may be overcome with the addition of IL-2 or IL-4 [72]. Glioma cells synthesize and secrete IL-10 [72, 73] and expression of IL-10 mRNA increases significantly with tumour grade; 87.5% of grade III–IV astrocytomas

(gliomas) expressed IL-10 mRNA versus 4% of grade II astrocytomas^[73]. Gliomas exhibiting a high degree of brain invasiveness, express IL-10 while more localized glioma tumours do not^[32]. In addition, glioma patient monocytes, but not normal donor monocytes, produce IL-10 after *in vitro* stimulation with SAC^[31].

IL-2 is a potent stimulator of lymphocyte proliferation and augments the activity of cytotoxic T cell, NK cells and lymphokine-activated killer cells^[74] and has been demonstrated to be effective in the immunotherapy of murine and human tumours.

Tumour cells secreting IL-2 failed to grow *in vivo* despite normal *in vitro* growth. IL-2 secretion by the tumour afforded a significant survival benefit to the animal and IL-2 secreting tumour limited the growth or admixed non-secreting parental tumour^[75].

Stimulated T-cells appear to express lower levels of the IL-2 receptor and increased levels of soluble IL-2R in cell supernatants^[26]. In addition it was found that competent T cells of glioblastoma patients exhibit lower levels of tyrosine phosphorylation in response to IL-2 as compared with cells of healthy donors. The diminished response of T-cells obtained from patients with glioblastomas may thus be due to a selective defect in the production of IL-2 and in the expression of functional IL-2R due to a decreased expression of the membranal IL-2R and lower levels of tyrosine phosphorylation in response to IL-2.

Implication of glioma-derived cytokines in angiogenesis: Tumour necrosis factor- α (TNF α) is a cytokine of macrophage origin and one of the major biological response modifiers with pleiotypic biological effects. A number of biological activities of TNF α have been reported including differentiation of 3T3-L1 adipocytes *in vitro*, stimulation of granulocytes, proliferation of fibroblasts, antiviral activity, induction of bone resorption and cytotoxicity toward endothelial cells^[76].

TNF α has been demonstrated to induce the death of glioma cell lines by an apoptotic pathway or stimulate tumour cell growth^[77]. For neural cells, TNF α has mitogenic action and in association with IFN γ is able to induce differentiation of a neuroblastoma cell line. Furthermore, treatment of medulloblastoma cell line with TNF α was both cytotoxic and cytostatic effects^[77]. In a glioma cell line, TNF α is capable of increasing VEGF mRNA levels^[78] which have been implicated in brain tumour angiogenesis. The expression of the VEGF gene enhanced by TNF α appeared to be mediated in part through the transcription factor SP-1 suggesting a different mechanism from that for hypoxic induced activation of the VEGF gene^[78]. In addition, it has been demonstrated that TNF α up-regulates angiogenic factor expression in malignant glioma cells and that RNA stabilization via AU-rich elements in the 3'-untranslated region, contributes to this up-regulation^[78]. Finally, intra-arterial administration of TNF α

resulted in a 20% response rate in the treatment of malignant glioma in patients with recurrent or progressive disease and an improvement of neurological symptoms in 47% of valuable cases^[79]. This improvement suggests that the response is related to selective decrease in blood flow in tumour tissue and/or to a decrease in peritumoral edema, or possibly is partially due to a decrease in tumour burden from necrosis.

Another cytokine implicated in glioma angiogenesis is IL-8. IL-8 is a chemokine with a defining CXC amino acid motif initially characterized for its leukocyte chemotactic activity, is also known to possess tumorigenic and proangiogenic properties. IL-8 is expressed and secreted in human gliomas at high levels both *in vitro* and *in vivo* and recently, experiments have suggested that it is critical to glial tumour neovascularity and progression^[47,49]. There is a significant correlation between histologic grade and levels of IL-8 in the most malignant form showed the highest expression especially in pseudopalisading cells around necrosis, suggesting that hypoxia/anoxia may stimulate expression^[49].

In addition to hypoxia/anoxia stimulation, increased IL-8 in gliomas occurs in response to numerous other factors including Fas ligation, death receptor activation, cytosolic Ca²⁺, TNF α , IL-1, other cytokines and various cellular stresses. The IL-8 promoter contains binding sites for transcription factors as AP-1 among of others. AP-1 has been demonstrated to mediate IL-8 upregulation by anoxia in gliomas^[47,49]. Furthermore, the potential tumor suppressor protein, inhibitor of growth-4 (ING4), has recently been shown to be a critical regulator of NK-kappa β -mediated IL-8 transcription and subsequent angiogenesis in gliomas^[49]. IL-8 increased significantly in response to the downregulation of ING4^[80]. Finally the tumorigenic and angiogenic properties of IL-8 are mediated by the G protein coupled receptors CXCR1 and CXCR2^[49].

A number of substances that modulate angiogenesis have been employed to treat brain tumours *in vivo*, such as fumagillin derivatives^[81,82], the combination of heparin and angiostatic steroids^[83], and antibiotics (Table 2)^[84]. O'Reilly et al^[85] has described a plasminogen-derived substance, termed angiostatin, as a highly specific inhibitor of tumour-related endothelial cell proliferation and the most potent anti-angiogenic substance so far^[85]. Angiostatin is an endogenous inhibitor purified from urine and plasma of tumour-bearing mice that is effective against a range of murine and human tumours *in vivo*, including bronchial, prostate, breast and colon carcinomas^[85]. Inhibitors of angiogenesis may become an adjunct to current chemotherapy and radiotherapy regimens for the treatment of brain tumours (Table 2)^[86]. Their apparent usefulness might prove to help with the most difficult aspect of brain tumour therapy: the local

recurrence of gliomas. Distributed glioma cells can be found throughout the edematous normal brain surrounding the solid tumour mass. Whereas only the solid tumour can be removed by the neurosurgeon, the recurrence is a result of those cells distributed in the otherwise normal parenchyma. Anti-angiogenic therapy would help to inhibit the formation of a recurrent solid tumour by limiting its size to that of an avascular microtumour.

Table 2: Angiogenic stimulators and inhibitors ^[82,85]

Angiogenic stimulators	Inhibitors of angiogenesis
Acidic FGF (fibroblast growth factor)	Interferon- α 2 ^a
Basic FGF (fibroblast growth factor)	Thalidomide
VEGF (vascular endothelial growth factor)	Glioma derived angiogenesis inhibitory factor
PDGF (platelet derived endothelial growth factor)	TNP-470 and other fumagilin analogues
TGF α/β	TGF β
Proliferin	Thrombospondin
Angiogenin	Platelet factor 4
Metalloproteinase inhibitors (TIMP 1, 2, 3)	Interleukin 12
Interleukin 8	Angiostatin (plasminogen fragment)
TNF- α	Endostatin

CONCLUSION

Despite the fact that the gliomas remain sequestered within the confines of the central nervous system, they have the capability of systemically manipulating the cell mediated immune response in the whole body. Immunosuppression observed in patients with glioma appears to result from the secretion of soluble factors (specifically cytokines) that can down regulate T-cell and monocyte function. This occurs by creating a shift in cytokine secretion to favour a decrease in Th1-type cytokines and increase in Th2 type, thus resulting in a dysregulation of cell mediated immunity. This shift further decreases the anti-tumour response of the immune system and creates potential difficulties with therapy designed to destroy the tumour. The immunological consequences of primary malignant brain tumours clearly reveal a functional link among T cells, monocytes and soluble mediators secreted by gliomas. Future elucidation of the modulatory role of glioma-derived suppressor factors and understanding of these complex interactions will greatly aid in the development of strategies to treat glioma patients

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REFERENCES

1. Prados, M.D., and V. Levin, 2000. Biology and treatment of malignant glioma: Semin. Oncol., 27(3):1-10
2. Hsieh, J.C., and M.S. Lesniak, 2005. Surgical management of high-grade gliomas: Expert. Rev. Neurother., 5(6):S33-9
3. Nazzaro, J.M., and E.A. Neuwelt, 1990. The role of surgery in the management of supratentorial intermediate and high-grade astrocytomas in adults: J. Neurosurg., 73: 331-344
4. Silbergeld, D.L., and M.R. Chicoine, 1997. Isolation and characterization of human malignant glioma cells from histologically normal brain: J. Neurosurg., 86(3):525-31
5. Hussain, S.F., and A.B. Heimberger, 2005. Immunotherapy for human glioma: innovative approaches and recent results. Expert. Rev. Anticancer. Ther., 5(5):777-90
6. Tjoa, B.A., and M.L. Salgaller, 2000. Progress in active-specific immunotherapy of brain malignancies: Expert. Opin. Investig. Drugs., 9(9):2093-101
7. Mitchell, D.A., P.E. Fecci, and J.H. Sampson, 2003. Adoptive immunotherapy for malignant glioma: Cancer J., 9(3):157-66
8. Fecci, P.E., and J.H. Sampson, 2002. Clinical immunotherapy for brain tumors: Neuroimaging. Clin. N. Am., 12(4):641-64
9. De Vleeschouwer, S., S.W. Van Gool, and F. Van Calenbergh, 2005. Immunotherapy for malignant gliomas: emphasis on strategies of active specific immunotherapy using autologous dendritic cells: Childs Nerv. Syst., 21(1):7-18
10. 1999. Chemotherapy and immunotherapy of malignant glioma: molecular mechanisms and clinical perspectives: Cell. Mol. Life. Sci., 56(5-6):481-506
11. Hao, C., I.F. Parney, W.H. Roa, J. Turner, K.C. Petruk, and D.A. Ramsay, 2002. Cytokine and cytokine receptor mRNA expression in human glioblastomas: evidence of Th1, Th2 and Th3 cytokine dysregulation: Acta Neuropathol. (Berl), 103(2):171-8
12. Golestaneh, N., and B. Mishra, 2005. TGF-beta, neuronal stem cells and glioblastoma: Oncogene, 24(37):5722-30
13. Gagner, J.P., M. Law, I. Fischer, E.W. Newcomb, and D. Zagzag, 2005. Angiogenesis in gliomas: imaging and experimental therapeutics: Brain Pathol., 15(4):342-63
14. Chicoine, M.R., and D.L. Silbergeld, 1997. Mitogens as motogens: J. Neurooncol., 35(3):249-57
15. Szelenyi, J., 2001. Cytokines and the central nervous system: Brain Research Bulletin, 54(4): 329-338

16. Westermark, B., M. Nister, and C. Heldin, 1985. Growth factors and oncogenes in human malignant glioma: *Neurol. Clinics*, 4 (3): 185–799
17. Renner, U., M. Paez-Pereda, E. Arzt, and G.K. Stalla, 2004. Growth factors and cytokines: function and molecular regulation in pituitary adenomas: *Front. Horm. Res.*, 32:96-109
18. Hamel, W., and M. Westphal, 2000. Growth factors in gliomas revisited: *Acta Neurochir.*, 142(2):113-37
19. Goussia, A.C., N.J. Agnantis, J.S. Rao, and A.P. Kyritsis, 2000. Cytogenetic and molecular abnormalities in astrocytic gliomas: *Oncol. Rep.*, 7(2):401-12
20. Ohgaki, H., and P. Kleihues, 2005. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas: *J. Neuropathol. Exp. Neurol.*, 64(6):479-89
21. Dimitriadis, E., C.A. White, R.L. Jones, and L.A. Salamonsen, 2005. Cytokines, chemokines and growth factors in endometrium related to implantation: *Hum. Reprod. Update*, 11(6):613-30
22. Clerici, M., M. Galli, S. Bosis, C. Gervasoni, M. Moroni, and G. Norbiato, 2000. Immunoendocrinologic abnormalities in human immunodeficiency virus infection: *Ann. N Y Acad. Sci.*, 917:956-61
23. Campbell, I.L., 2005. Cytokine-mediated inflammation, tumorigenesis, and disease-associated JAK/STAT/SOCS signaling circuits in the CNS: *Brain Res. Brain. Res. Rev.*, 48(2):166-77
24. Hu, Y.S., Q.L. Zhang, Z.G. Tian, H.M. Wei, G. Li, Q. Pang, J.B. Feng, Y.Q. Xu, C.W. Wang and R. Sun, 2001. Significance of the unbalanced expression of Th1/Th2 type cytokines in human glioma: *Zhangquo Yi Xueke Xue Yaun Xue Bao.*, 23(6): 594-8
25. **Li**
26. Ashkenazi, E., M. Deutsch, R. Tisosh, A. Weinreb, A. Tsukerman, and C. Brodie, 1997. A selective impairment of the IL-2 system in lymphocytes of patients with glioblastomas. Increased level of soluble IL-2R and reduced protein tyrosine phosphorylation: *Neuroimmunomodulation.*, 4(1): 49-56
27. Urbani, F., A. Maleci, A. La Sala, R. Lande, and C.M. Ausiello, 1995. Defective expression of interferon-gamma, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor alpha, and interleukin-6 in activated peripheral blood lymphocytes from glioma patients: *J. Interferon Cytokine Res.*, 15: 421-429
28. 2004. T lymphocyte subsets and immunoglobulins in intracranial tumor patients before and after treatment, and based on histological type of tumors: *Int. J. Immunopathol. Pharmacol.*, 17(1):57-64
29. Akasaki, Y., G. Liu, N.H. Chung, M. Ehtesham, K.L. Black, and J.S. Yu, 2004. Induction of a CD4+ T regulatory type 1 response by cyclooxygenase-2-overexpressing glioma: *J. Immunol.*, 173(7):4352-9
30. Ausiello, C., A. Maleci, G.C. Spagnoli, G. Antonelli, and A. Cassone, 1988. Cell-mediated cytotoxicity in glioma-bearing patients: differential responses of peripheral blood mononuclear cells to stimulation with interleukin-2 and microbial antigen: *J. Neurooncol.*, 6:329-338
31. Zou, J.P., L.A. Morford, C. Chougnnet, A.R. Dix, A.G. Brooks, N. Torres, J.D. Shuman, J.E. Coligan, W.H. Brooks, T.L. Roszman, G.M. Shearer, 1999. Human glioma-induced immunosuppression involves soluble factor(s) that alters monocyte cytokine profile and surface markers: *J. Immunol.*, 162(8):4882-92.
32. Nitta, T., M. Hishii, K. Sato, and K. Okumura, 1994. Selective expression of interleukin-10 gene within glioblastoma multiforme: *Brain Res.*, 649:122
33. Roussel, E., M.C. Gingras, E.A. Grimm, J.M. Bruner, and R.P. Moser, 1996. Predominance of a type 2 intratumoral immune response in fresh tumor-infiltrating lymphocytes from human gliomas: *Clin. Exp. Immunol.*, 105, 344-352
34. Colombo, M.P., M.Vagliani, F. Spreafico, M. Parenza, C. Chiodoni, C.Melani, A. Stoppacciaro, 1996. Amount of interleukin 12 available at the tumor site is critical for tumor regression: *Cancer Res.*, 56: 2531-2534
35. Brunda, M.J., L. Luistro, L. Rumennik, Wright, R.B., Dvorozniak, M., Aglione, A., J.M.Wigginton, R.H. Wiltout, J.A. Hendrzak, and A.V. Palleroni, 1996. Antitumor activity of interleukin 12 in preclinical models: *Cancer Chemother. Pharmacol.*, 38 Suppl:S16-21
36. Tannenbaum, C.S., N. Wicker, D. Armstrong, R.Tubbs, J. Finke, RM. Bukowski, and T.A. Hamilton, 1996. Cytokine and chemokine expression in tumors of mice receiving systemic therapy with IL-12: *J. Immunol.*, 156: 693-699
37. Trinchieri, G., 1995. Interleukin – 12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity: *Annu. Rev. Immunol.*, 13: 251-276
38. Guha A., and J. Mukherjee, 2004. Advances in the biology of astrocytomas: *Current opinion in Neurology*, 17:655-662
39. Van Meir E., Y. Sawamura, A.C. Diserens, M.F. Hamou, N. de Tribolet, 1990. Human glioblastoma cells release interleukin 6 in vivo and in vitro: *Cancer Res.*, 20(20): 6683-8
40. Aloisi, F., A. Care, G. Borsellino, P. Gallo, S. Rosa, A. Bassani, A. Cabibbo, U. Testa, G. Levi, C. Peschle, 1992. Production of hemolymphopoietic cytokines (IL-6, IL-8, colony stimulating factors) by normal human astrocytes in response to IL-1 β and tumor necrosis factor – α : *J. Immunol.*, 149:2358-2366
41. Weissenberger, J., S. Loeffler, A. Kappeler, M. Kopf, A. Lukes, T.A. Afanasieva, A. Aguzzi, J. Weis, 2004. IL-6 is required for glioma development in a mouse model. *Oncogene*, 23(19): 3308-16

42. Sayah, S., A.M. Ischenko, A. Zhakhov, A.S. Bonnard, and M. Fontaine, 1999. Expression of cytokines by human astrocytomas following stimulation by C3a and C5a anaphylatoxins: specific increase in interleukin 6 mRNA expression: *J. Neurochem* 72:2426-2436
43. Graf, M., and R.E. Merchant 1999. Interleukin-6 transduction of a rat T9 glioma clone results in attenuated tumorigenicity and induces glioma immunity in Fischer F344 rats: *J. Neuro-oncol.* 45:209-218
44. Graf, M., R.M. Prins, and R.E. Merchant, 2001. IL-6 secretion by a rat T(glioma clone induces a neutrophin dependent antitumor response with resultant cellular, anti glioma immunity: *J. Immunol.*, 166:121-129
45. Mullen, C., M.M. Coale, A.T. Levy, W.G. Settler-Stevenson, L.A. Liotta, S. Brandt, R.M. Blaese, 1992. Fibrosarcoma cells transduced with the IL-6 gene exhibit reduced tumorigenicity, increased immunogenicity and decreased metastatic potential: *Cancer Res.*, 52: 6020-6024
46. Rosenkilde, M.M., and T.W. Schwartz, 2004. The chemokine system -- a major regulator of angiogenesis in health and disease: *APMIS.*, 112(7-8):481-95
47. Desbaillets, I., A.C. Diserens, N. Tribolet, M.F. Hamou, E.G. van Meir, 1999. Regulation of interleukin-8 expression by reduced oxygen pressure in human glioblastoma: *Oncogene*, 18(7): 1447-57
48. Desbaillets, I., A.C. Diserens, N. Tribolet, M.F. Hamou, E.G. van Meir, 1997. Up regulation of interleukin 8 by oxygen deprived cells in glioblastoma suggests a role in leukocyte activation, chemotaxis and angiogenesis: *J. Exp. Med.*,
49. Brat, D.J., A.C. Bellai, E.G. van Meir, 2005. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. *Neuro-oncol.*, 7(2): 122-33
50. Kehlen, A., K. Thiele, D. Riemann, N. Rainov, J. Langner, 1999. Interleukin 17 stimulates the expression of I kappa β alpha mRNA and the secretion of IL-6 and IL-8 in glioblastoma cell lines: *J. Neuroimmunol.*, 100(1): 1-6
51. Salmaggi, A., M. Eoli, S. Frigerio, A. Sitrani, M. Gelati, E. Corsini, G. Broggi, and A. Boiardi, 2003. Intracavitary VEGF, bFGF, IL-8, IL-12 levels in primary and recurrent malignant glioma: *J. Neurooncol.*, 62(3): 207-303
52. Dix, A.R., W.H. Brooks, T.L. Roszman, and L.A. Morford, 1999. Immune defects observed in patients with primary malignant brain tumors: *J. Neuroimmunol.*, 100: 216-232
53. Brooks, W.H., R.B. Latta, M.S. Mahaley, T.L. Roszman, L. Dudka, and C. Skaggs, 1981. Immunobiology of primary intracranial tumors: Part 5: correlation of a lymphocyte index and clinical status. *J. Neurosurg.*, 54: 331-337
54. Woiciechowsky, C., K. Asadullah, D. Nestler, B. Schoning, F. Glockner, W.D. Döcke, H.D. Volk, 1998. Diminished monocytic HLA-DR expression and ex vivo cytokine secretion capacity in patients with glioblastoma: effect of tumor extirpation: *J. Neuroimmunol.* 84: 164-171
55. Neuwelt, E.A., K. Kikuchi, S. Hill, P. Lipsky, E.P. Frenkel, 1983. Immune responses in patients with brain tumors. Factors such as anti-convulsants that may contribute to impaired cell-mediated immunity: *Cancer*, 51(2):248-55
56. Toh, B.H., and M.N. Cauchi, 1974. Brain-associated tumor antigens demonstrated by immunofluorescence: *Nature*, 250:597-598
57. Levy, N.L., 1978. Cell mediated cytotoxicity and serum mediated blocking: evidence that their associated determinants on human tumor cells are different: *J. Immunol.*, 121:916-922
58. Young, H.F., R. Sakalas, and A.M. Kaplan, 1976. Inhibition of cell-mediated immunity in patients with brain tumors: *Surg. Neurol.*, 5: 19-23
59. Tada, M., A.C. Diserens, I. Desbaillets, and N. de Tribolet, 1994. Analysis of cytokine receptor messenger RNA expression in human glioblastoma cells and normal astrocytes by reverse-transcription polymerase chain reaction: *J. Neurosurg.*, 80: 1063-1073
60. Elliott, L.H., W.H. Brooks, T.L. Roszman, 1992. Suppression of high affinity IL-2 receptors on mitogen activated lymphocytes by glioma derived suppressor factor: *J. Neurooncol.* 14: 1-7
61. Taniguchi, Y., K. Ono, S. Yoshida, R. Tanaka, 2000. Antigen-presenting capability of glial cells under glioma-harboring conditions and the effect of glioma-derived factors on antigen presentation: *J. Neuroimmunol.*, 111(1-2):177-85
62. Roszman, T.L., W.H. Brooks, and L.H. Elliott, 1987. Inhibition of lymphocyte responsiveness by a glial tumor cell derived suppressive factor: *J. Neurosurg.*, 67: 874-879
63. Golestaneh, N., and B. Mishra, 2005. TGF-beta, neuronal stem cells and glioblastoma: *Oncogene*, 24(37):5722-30
64. Kaminska, B., Wesolowska, A., and M. Daniliewicz, 2005. TGF beta signalling and its role in tumour pathogenesis: *Acta Biochim. Pol.*, 52(2):329-37
65. Bodmer, S., K. Strommer, K. Frei, C. Siepl, N. de Tribolet, I. Heid, and A. Fontana, 1989. Immunosuppression and transforming growth factor beta in glioblastoma. Preferential production of transforming growth factor beta 2: *J. Immunol.*, 143:3222-3229
66. Strege, R.J., C. Godt, A.M. Stark, H.H. Hugo, H.M. Mehdorn, 2004. Protein expression of Fas, Fas ligand, Bcl-2 and TGFbeta2 and correlation with survival in initial and recurrent human gliomas: *J. Neurooncol.*, 67(1-2):29-39
67. Ammirato, M., S. Rao, G. Granger, 2001. Detection of TNF inhibitors (soluble receptors) in the sera and tumor cyst fluid of patients with malignant astrocytomas of the brain: *Front. Biosci.*, 6:B17-24
68. Mocellin, S., F. Marincola, C.R. Rossi, D. Nitti, M. Lise, 2004. The multifaceted

- relationship between IL-10 and adaptive immunity: putting together the pieces of a puzzle: *Cytokine Growth Factor Rev.*, 15(1):61-76
69. Mocellin, S., F.M. Marincola, and H.A. Young, 2005. Interleukin-10 and the immune response against cancer: a counterpoint: *J. Leukoc. Biol.*, 78(5):1043-51
70. Cassatela, M.A., L.Meda, S.Bonora, M. Ceska, G. Constantin, 1993. Interleukin 10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumor necrosis factor and IL-1 beta in mediating the production of IL-8 triggered by lipopolysaccharide: *J. Exp. Med.*, 178:2207-2211
71. Hishii, M., T. Nitta, H. Ishida, M. Ebato, A. Kurosu, H. Yagita, K. Sato., and K. Okumura, 1995. Human glioma-derived interleukin-10 inhibits antitumor immune responses in vitro. *Neurosurgery*, 37: 1160-1166
72. Wagner, S., S. Czub, M. Greif, G.H. Vince, N. Suss, S. Kerkau, P. Rieckmann, W. Roggendorf, K. Roosen, and J.C. Tonn, 1999. Microglial/macrophage expression of interleukin 10 in human glioblastomas: *Int J Cancer.*, 82(1):12-6
73. Huettner, C., S. Czub, S.Kerkau, W.Roggendorf, and J.C. Tonn, 1997. Interleukin 10 is expressed in human gliomas in vivo and increases glioma cell proliferation and motility in vitro: *Anticancer Res.*, 17: 3217-3224
74. Mirandola, P., C. Ponti, G. Gobbi, I. Sponzilli, E. Melloni, and M. Vitale, 2004. The response of human natural killer cells to interleukin-2: *J. Endocrinol. Invest.*, 27(6 Suppl):146-50
75. Karp, S.E., A. Farber, J.C. Salo, P.Hwu, J. Gitie, AL. Asher, E. Shiloni, N.P. Restifo, J.J. Mule, and S.A. Rosenberg, 1993. Cytokine secretion by genetically modified nonimmunogenic murine fibrosarcoma. Tumor inhibition by IL-2 but not tumor necrosis factor: *J. Immunol.*, 150:896-908
76. Yoshida, J., T.Wakabayashi, M.Mizuno, K. Sugita, T.Yoshida, S.Hori, T.Mori, T.Sato, A.Karashima, K.Kurisu, K.Kiya, and T.Uozumi, 1992. Clinical effect of intra-arterial tumor necrosis factor - α for malignant glioma: *J. Neurosurg.*, 77:78-83
77. Dufay, N., A. Reboul, F.Touraine-Moulin, MF.Belin, and P.Girowdon, 1999. Soluble factors, including TNF-alpha, secreted by T cells are both cytotoxic and cytostatic for medulloblastoma cells: *J. Neurooncol.*, 43(2): 115-26
78. Ryuto, M., M.Ono, H. Izumi, S.Yoshida, H.A.Weich, K.Khno, M.Kuwano, 1996. Induction of vascular endothelial growth factor by tumor necrosis factor alpha in human glioma cells. Possible roles of SP-1: *J. Biol.Chem.*, 271(45):28220-8
79. Niida, H., S.Takechui, R.Tanaka, and T.Minakawa, 1995. Angiogenesis in microvascular endothelial cells induced by glioma cells and inhibited by tumor necrosis factor in vitro: *Neurol. Med. Chir.*, 35(4): 209-14
80. Gargavtsev, I., SV.Kozin, O.Chernova, L.Xu, F.Winkler, E.Brown, GH.Barnett, and R.K.Jain, 2004. The candidate tumour suppressor protein ING4 regulates brain tumor growth and angiogenesis: *Nature* 428(6980): 328-32
81. Kirsch, M., G.Schackert, and P.M.Black, 2000. Anti-angiogenic treatment strategies for malignant brain tumors: *J. Neurooncol.* 50(1-2):149-63
82. Takamiya, Y., H.Brem, J. Ojeifo, T. Mineta, and R.L.Martuza, 1994.AGM-1470 inhibits the growth of human glioblastoma cells in vitro and in vivo: *Neurosurgery*, 34:869-75
83. Sipos, E.P., and H.Brem, 2000. Local anti-angiogenic brain tumor therapies. *J Neurooncol.*, 50(1-2):181-8
84. Weingart, J.D., E.P. Sipos, and H. Brem, 1995. The role of minocycline in the treatment of intracranial 9L glioma: *J. Neurosurg.*, 82:635 - 40
85. O'Reilly, M.S., L. Holmgren, C. Chen, and J.Folkman, 1996. Angiostatin induces and sustains dormancy of human primary tumors in mice: *Nat. Med.*, 2:689-92
86. De Bouard, S., J.S. Guillamo, C. Christov, N. Lefevre, P. Brugieres, E. Gola, P. Devanz, S. Indraccolo, and M.Peschanski, 2003. Antiangiogenic therapy against experimental glioblastoma using genetically engineered cells producing interferon-alpha, angiostatin, or endostatin; *Hum. Gene Ther.*, 14(9):883-95
87. Wikstrand, C.J., P.Fredman, L.Svennerholm, and D.D.Bigner, 1994. Detection of glioma associated gangliosides GM2, GD2, GD3, 3'-isoLM1 and 3'6'-isoLD1 in central nervous system tumors in vitro and in vivo using epitope defined monoclonal antibodies: *Prog. Brain Res.*, 101: 213-223
88. Fredman, P., J.E.Mansson, B.Delhenden, K.Bonstrom, and H.von Holst, 1999. Expression of the GM1-species [NeuN]-GM1 in a case of human glioma: *Neurochem. Res.*, 24: 275-279