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Cytokine and cytokine receptor mRNA expression in human glioblastomas: evidence of Th1, Th2 and Th3 cytokine dysregulation

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Abstract Immunotherapies, although promising in preclinical studies, have not yet enhanced the survival of patients with glioblastomas. To further understand the immunobiology of glioblastomas in clinical settings, we examined 53 cytokine or cytokine receptor transcripts in 12 human glioblastomas and 6 human glioblastoma cell lines and correlated the findings with the degree of inflammation. Multi-probe RNase protection assays were used to examine Th1, Th2, and Th3 cytokine and cytokine receptor expression. Th2 [interleuking (IL)-6, leukemia inhibitory factor and oncostatin M] and Th3 (transforming growth factor-β1, 2, 3) cytokine and their receptor transcripts were strongly expressed in almost all glioblastomas and glioma cell lines. Two other Th2 cytokine receptor subunit transcripts (IL-4Rα and IL-13Rα) were also commonly detected. In contrast, although Th1 cytokine receptors tumor necrosis factor (TNF) RI, interferon (IFN)-γRα, IFN-γRβ, were detected, their cytokines (IFN-γ, TNF-α, lymphotoxin-α) were not. Transcripts for IL-2 family cytokine (IL-2, IL-7, IL-9, IL-15) and receptors (IL-2Rα, IL-2Rβ, γc, IL-7Rα, IL-9Rα, IL15Rα) and IL-12 family cytokine (IL-12p40) and receptors (IL-12Rβ1 and IL-12β2) were essentially absent in both tumors and cell lines. Immunohistochemical methods showed sparse T lymphocyte infiltrates and numerous microglia in the glioblastomas. This pattern indicates an 'immunosuppressive status' in glioblastomas and could account for the failure of immunotherapy in such tumors.

Keywords Cytokine · Receptor · Glioblastoma · Cell lines · Immunotherapy

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Introduction

Malignant astrocytomas (anaplastic astrocytoma, glioblastoma) are the most common and aggressive of human brain tumors. Despite intensive research, various widely employed treatment regimes for patients with these tumors have not significantly improved the prognosis. Immunotherapy is a theoretically attractive, alternative method of treatment since tumor cells can be selectively targeted [2]. However, despite encouraging results in the research laboratories, neither adoptive immunotherapies [18, 26] nor recently developed active immunogene therapies [7, 35] have yet led to successful tumor eradication. Recent observations suggest the immune system's failure to recognize tumor cells may, in part, be attributed to tumor-associated cytokine dysregulation [36, 48, 50].

Our understanding of cytokine regulation has been facilitated by the Th1/Th2 model in which different T helper (Th) lymphocyte subsets secrete cytokines whose properties vary with the nature of the immune response generated [29]. Th1 (proinflammatory) cytokines include interferon-γ (IFN-γ), interleukin-2 (IL-2), IL-12, IL-15, lymphotoxin (LT), and tumor necrosis factor-α (TNF-α). Since these molecules promote cell-mediated immune responses they have the potential to exert anti-tumor effects. In contrast, Th2 (immunosuppressive) cytokines such as IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 stimulate humoral immune responses and thus down-regulate tumor-specific immunity. Also strongly immunosuppressive are cytokines, referred to as 'Th3', which include members of the transforming growth factor (TGF)-β family [4].

Glioblastoma cells appear to secrete Th2 (IL-6, IL-10) [13, 20] and Th3 (TGF-β) cytokines [5, 34], whose immunosuppressive properties may abrogate cytotoxic anti-

tumor immune responses. Evidence on the expression of these cytokine receptors suggests that these cytokines may also have some effects on the target tumor cells [13, 22]. Paradoxically, these glioma cells have also been reported to produce the potentially cytotoxic Th1 cytokines such as IFN-γ and TNF-α [32] and to express their receptors [43]. To explore further the possible interactions of these cytokines and their receptors in human glioblastomas, we performed experiments using a multi-probe RNase protection assay (RPA) to determine the simultaneous mRNA transcript expression in gliomas of Th1 and Th2/Th3 cytokines and their corresponding receptors. Expression of the colony-stimulating factor (CSF) family cytokines and their receptors was also investigated. In addition, we correlated the nature of the inflammatory cell infiltrates, determined using immunohistochemical methods, with the tumor cytokine/cytokine receptor expression pattern.

Materials and methods

Glioblastoma specimens

Samples of glioblastoma were provided by the London (Ontario) Brain Tumor Tissue Bank (BTTB), based in the London Health Sciences Center (LHSC), which stores frozen and paraffin-embedded tissue from patients with brain tumors who had been treated surgically at LHSC. Tissue stored between 1995 and 1999 was available from 12 cases of glioblastoma: 7 males (age range 51–82 years), 4 females (age range 44–74 years) and 1 young girl (at age 9 years). Stored tumor tissue was collected using the BTTB standard protocol. In brief, fresh tumor specimens are cut immediately after resection into strips measuring approximately 1.5×1.5× 0.5 cm. The strips are then divided into three fragments. The central fragment, for quality control purposes, is prepared for routine histological examination of paraffin-embedded, hematoxylin and eosin-stained sections. The remaining fragments are placed in cryovials, either in air or embedded in OCT, snap-frozen in liquid N_2, and stored at –80°C. Examination of sections from the central fragment allows the quality of the tumor to be categorized as 'good', 'moderate' or 'poor' (based on the extent of necrosis, hemorrhage and surgical artifact) and the location of the sample to be classed with respect to the tumor center as 'central' (i.e., pure tumor – essentially no normal host tissue is seen), 'cellular infiltrating margin', 'moderate infiltrate of white matter or gray matter', 'sparse tumor cell infiltrates', or no 'tumor cells apparent'. For the purposes of these experiments only 'good' samples from 'central' locations were used.

Immunohistochemistry

Five-micrometer-thick sections from paraffin-embedded tumor blocks were labeled by monoclonal antibodies using routine avidin-biotin conjugation (ABC) methods. Monoclonal antibodies and dilutions used were as follows: mouse anti-human HLA-DP, DQ, and DR β-chain (MHC class II) (CR3/43, Dako, 1:100); mouse anti-human CD45 (Dako-LCA, Dako, 1:100); mouse anti-human CD45R0 (UCHL1, Dako, 1:50); mouse anti-human CD20 (L26, Dako, 1:100); mouse anti-human glial fibrillary acidic protein (GFAP; 6F2, Dako, 1:300). Staining proceeded with an ABC standard detection kit (Vector Laboratories). The maximum density of positive cells was quantified in at least five fields using a grading scale from 0 to 4 as modified from Giometto et al. [12]: 0, no positive cells; 1, less than 10 positive cells/high power field (hpf) with a ×40 objective; 2, 10–20 cells/hpf; 3, 21–30 cells/hpf; and 4, >31 cells/hpf.

Human malignant glioma cell lines

The D54MG cell line was kindly provided by Dr. D. Bigner (Duke University, N.C.) and U87MG, U118MG, U138MG, U251MG and U373MG cell lines were obtained from the American Tissue Culture Collection (ATCC). All cell lines were cultured in DMED supplemented with 10% fetal bovine serum (Gibco-BRL, Ontario, Canada).

RNase protection assay

Total RNA was isolated from BTTB snap-frozen glioblastoma samples and subconfluent cultured monolayers of malignant glioma cell lines by the TRIZOL method following the manufacturer's protocol (Life Technologies, Inc.). Cytokine and receptor mRNA levels were analyzed by RPA using commercially available kits (RiboQuant Multi-Probe Template Sets, PharMingen, San Diego, Calif.) following the manufacturer's instruction. Briefly, ^{32}P-labeled antisense RNA probes were synthesized by T7 RNA polymerase from human cytokine and receptor templates. Equal total RNA aliquots (10 μg) from cell lines or tumor specimens were hybridized overnight to the ^{32}P-labeled RNA probes at 56°C. Free probe and any other single-stranded RNA fragments were then digested using RNase. The protected mRNAs were purified and resolved on a denaturing 5% urea-polyacrylamide gel followed by autoradiography for 15 h at –80°C. Cytokine and cytokine receptor transcripts were identified by the length of their respective fragments. For quantification, the gels were subjected to PhosphorImage analysis. Relative cytokine and receptor levels were calculated as a percentage by normalizing each specific cytokine or receptor RNA band to an internal control band (ribosomal RNA L32) included in the panel of probes.

Results

Inflammatory cell infiltrates in glioblastomas

Immunohistochemical treatment of the paraffin-embedded sections revealed, in all cases, GFAP immunopositivity in the tumor cells, confirming their astrocytic nature (Table 1). Common lymphocyte marker CD45 detected a few positive cells in all the samples (Table 1). These cells tended to occur in the perivascular spaces or as clusters in the tumors. They were predominantly CD45RO immuno-

Table 1 Inflammatory cell infiltration in 12 glioblastoma tumors

BTTB	197	335	353	356	398	564	256	270	337	437	439	450
GFAP	4	3	4	2	4	3	3	4	2	4	4	3
CR3/43	2	4	4	1	4	3	4	4	4	4	3	4
CD20	0	0	0	0	0	0	0	0	0	0	0	0
CD45R0	2	2	1	0	1	1	2	1	1	1	1	1

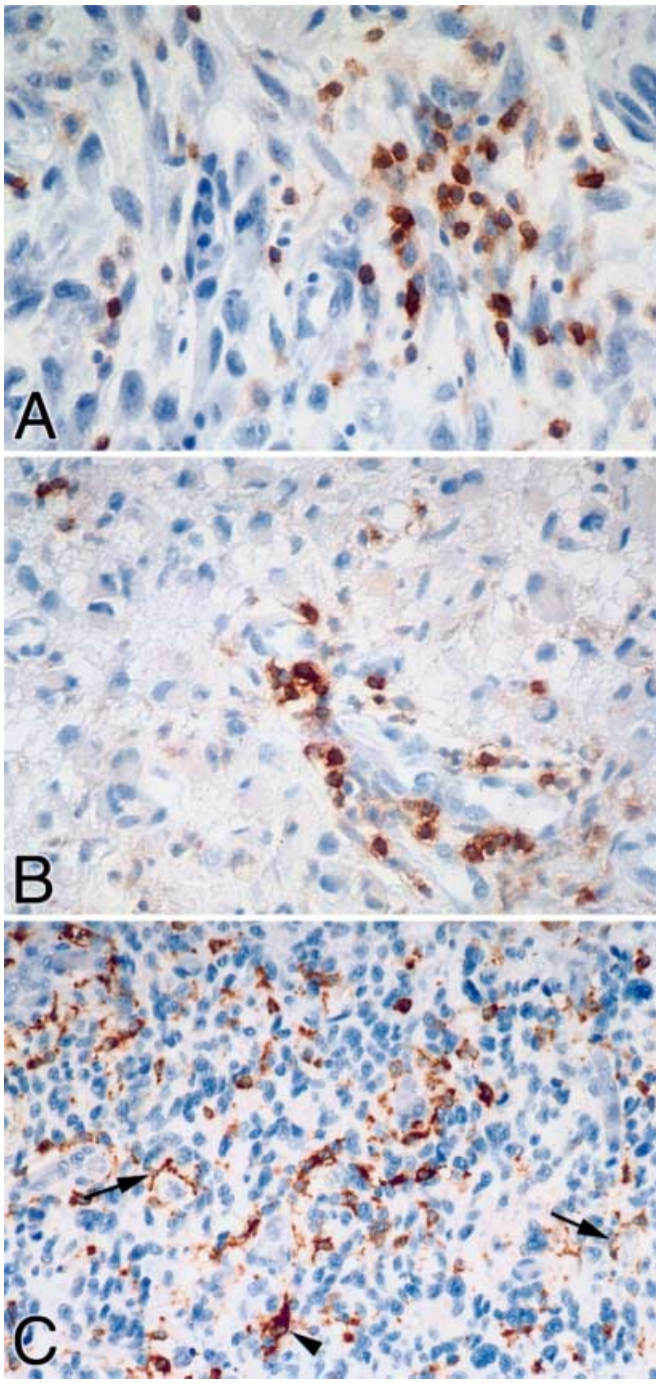

Fig. 1A–C Immunohistochemistry of the glioblastoma tumors. CD45-positive T lymphocytes are seen in a cluster (**A**) or a perivascular space (**B**). Diffusely infiltrating MHC class II-positive rod-like microglia (*arrows*) and neoplastic cells (*arrowheads*) are seen in a glioblastoma (**C**)

positive, indicating T lymphocytes (Fig. 1A, B). In contrast, no CD20-immunopositive B lymphocytes were seen in any specimens (Table 1). Major histocompatibility complex (MHC) class II (HLA-DP, DQ, and DR β-chain) -positive cells were detected in all the samples by CR3/43 immunostaining (Table 1). Frequent infiltrating CR3/43-positive rod-like microglia (Fig. 1C) were seen diffusely infiltrating through the tumors. A few CR3/43-positive macrophages were observed in areas of pseudopalisading necrosis (data not shown). Some neoplastic cells also expressed MHC class II antigens (Fig. 1C), as observed previously [46].

Cytokine and cytokine receptor mRNA expression

Snap-frozen samples from the 12 cases of glioblastoma and 6 well-established malignant glioma cell lines were tested by RPA, which detected two patterns of mRNA expression. As seen in Fig. 2A, some transcripts, such as IFN-γ receptors (IFN-γRα and IFN-γRβ), were detected in both tumor specimens and tumor cell lines, whereas other receptor transcripts, such as IL-10R, IL-3Rα, and IL-12Rβ1, were expressed only in tumor specimens. The expression level of each transcript was relatively consistent through all the tumor specimens, as evidenced by the labeling of two housekeeping genes (ribosomal RNA L32 and GAPDH), used as RNA loading controls (Fig. 2A).

All the tumor samples and cell lines were then tested for their expression of Th1, Th2 and Th3 cytokines and cytokine receptor transcripts (Fig. 2B). Relative cytokine and receptor mRNA expression levels were calculated and are summarized in Table 2. Three Th1 cytokine receptor transcripts (IFN-γRα, IFN-γRβ, TNFRI) were highly expressed in both cell lines and tumors, but the corresponding cytokine transcripts (IFN-γ, TNF-α, LT-α) were not. All cell lines and tumor samples expressed very limited, if any, transcripts for the following: IL-2 family cytokines (IL-2, IL-7, IL-9, IL-15) and receptors (IL-2Rα, IL-2Rβ, γc, IL-7Rα, IL-9Rα, IL-15Rα), IL-12 cytokines (IL-12p35, IL-12p40) and receptors (IL-12Rβ1 and IL-12β2).

In contrast, both Th2 cytokines [IL-6 and, to a lesser extent, leukemia inhibitory factor (LIF) and oncostatin M (OSM)] and Th2 cytokine receptors (IL-4Rα, IL-6Rα, IL-10R, IL-13Rα, gp130, LIFRα) were expressed in cell lines and tumors. Similarly, both Th3 cytokines (TGFβ1, TGFβ2, TGFβ3) and their corresponding receptors (TGFβRI, TGFβRII) were highly expressed in cell lines and tumors. Transcripts for IL-1 family cytokines [ILl-1α, IL-1β and IL-1 receptor antagonist (IL-1Ra)] and receptors (IL-1RI, IL1RII) were detected in some cell lines and tumors. Of the CSF family of cytokines, only M-CSF was detected in the cell lines and tumors. In contrast, receptor transcripts of these cytokines [granulocyte (G)-CSFRα, granulocyte/macrophage (GM)-CSFRα, IL-3Rα, IL-5Rα, β chain (βc)] were evident only in the tumor specimens (Fig. 1B).

Discussion

A comprehensive analysis of cytokine and cytokine receptor mRNA expression in human glioblastoma tumors and cell lines has been performed. Although studies of cytokines and their receptors in the tumor and cell lines have

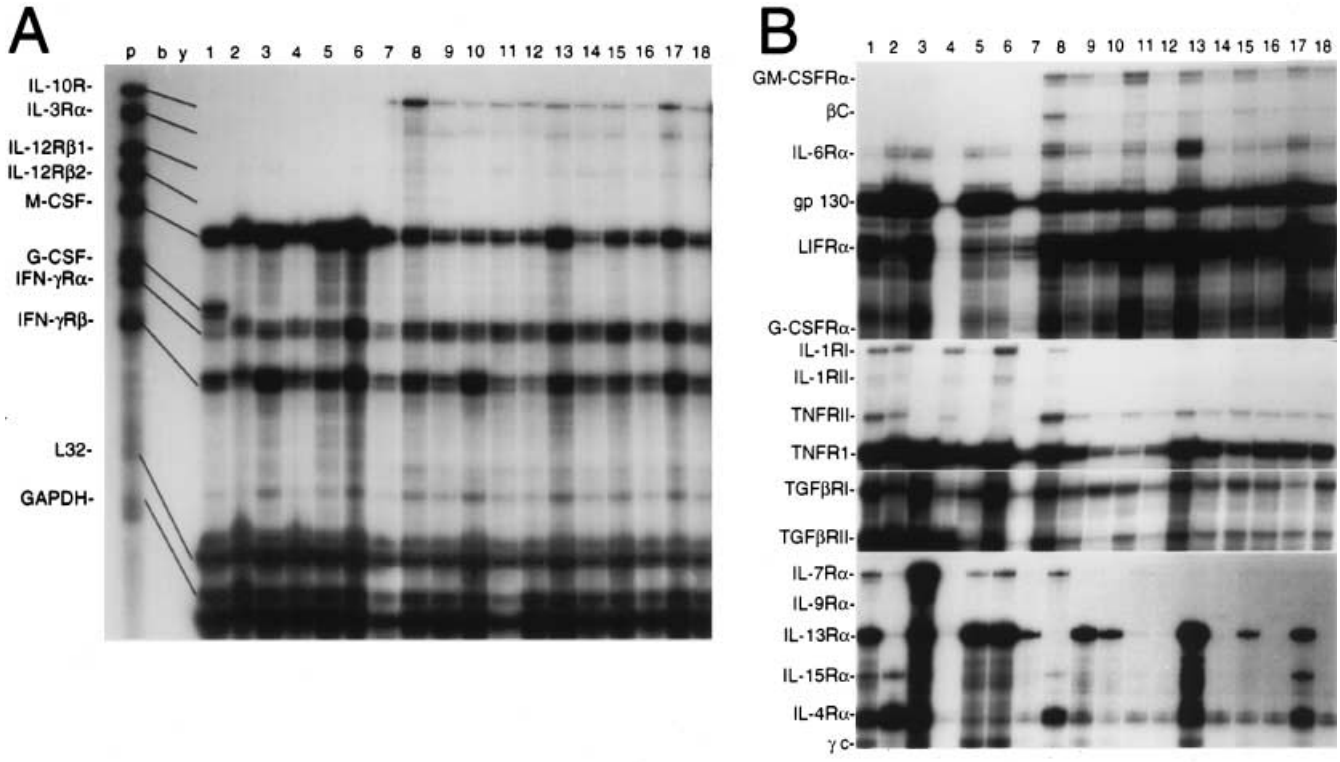

Fig. 2A, B RPA analysis for cytokine and cytokine receptor mRNA expression. Total RNA was isolated from 6 glioma cell lines (*lanes 1–6* U87, U118, D54, U138, U373, U251) and 12 human glioblastomas (*lanes 7–18* BTTB 197, 335, 353, 356, 389, 564, 256, 270, 337, 437, 439, 450). **A** RPA autoradiograph shows blank (*b*) and yeast total RNA (*y*) as negative controls and L32 and GAPDH as total RNA loading controls. The protected transcripts of cytokines and receptors were identified by the length of their respective fragments in relationship to unprotected probes (*p*). **B** Representative RPA autoradiographs show the cytokine receptor expression in glioblastoma tumors and cell lines (*RPA* RNase protection assay)

been published, simultaneous quantification of a large number of these agents is new and provides direct evidence of Th1 and Th2/Th3 cytokine dysregulation in glioblastomas.

Th1 cytokines

The IFN-γ response is mediated by a specific receptor with two functionally distinct subunits, one binding IFN-γ (IFN-γRα subunit) and the other mediating IFN-γ signal-transduction (IFN-γRβ subunit) [41]. IFN-γRα mRNA has been demonstrated previously in gliomas, based on the reverse transcriptase polymerase chain reaction (RT-PCR) [43]. Our observations using RPA confirm this finding and additionally demonstrate IFN-γRβ transcripts in all glioblastoma tumors and cell lines, indicating the synthesis of a functional IFN-γ receptor. IFN-γ affects glioblastoma cells in vitro by directly stimulating MHC class I and II expression by the tumor cells and inhibiting tumor growth [24, 37]. In contrast, although contradicting findings in an earlier study in which RT-PCR demonstrated IFN-γ mRNA in two glioma cell lines [32], our failure to detect mRNA for this cytokine in glioblastomas and glioma cell lines is consistent with the biological principal that IFN-γ secretion is restricted to Th1 lymphocytes [29].

Lymphotoxin (LT or TNFβ) and TNF-α are closely related cytokines that bind with nearly identical affinities to the same pair of cell surface receptors, TNFRI (p55, CD120a) and TNFRII (p75, CD120b) [40]. Our RPA reported here and an RT-PCR analysis described by others [43] show TNFRI in the glioblastoma tumors and cell lines, but no evidence or limited expression of TNFRII and LT or TNF-α cytokine transcripts. TNFRI appears to be involved in inducing apoptosis [27], but glioma cell lines are resistant to TNF-α-induced cytotoxicity [16], possibly related to the expression in glioma cell lines of the silencer of death domain [3], a potential inhibitor of TNFRI [23].

The IL-2R is made up of at least three subunits: IL-2Rα shares homology with the α chain of the IL-15Rα, whereas IL-2Rβ is shared with IL-15R [45]. The IL-2Rγ chain (γc) participates in the receptor formation of five cytokines (IL-2, IL-4, IL-7, IL-9 and IL-15). Except for weak IL-15 mRNA expression in five of six cell lines, neither the glioblastomas nor the cell lines contained transcripts for IL-2, IL-7, IL-9, or IL-15. Although sparse mRNA was detected for some receptor subunits in a few cell lines and tumors, transcripts for cytokine specific α chains (IL-2Rα, IL-9Rα, IL-15Rα) and common receptor chains (IL-2Rβ and γc) were essentially absent. These cytokines are, therefore, unlikely to exert any direct effect on glioblastoma cells.

Bioactive IL-12 is composed of two subunits: p35 and p40 [47]. The presence of p35 mRNA, but the absence of p40 mRNA suggests that glioblastoma cells do not secrete

Table 2 RNase protection assay quantification of mRNA expression of 53 cytokines and receptors in glioblastoma tumors and cell lines Values represent: – 0–0.9%, + 1–5%, ++ 6–10%, +++ 11–20%, ++++ >20% (a specific cytokine or receptor band / internal control L32 band)

Samples	Glioma cell lines						Glioblastoma tumors (BTTB)											
	U87	U118	D54	U138	U373	U251	197	335	353	356	398	564	256	270	337	437	439	450
IFN-γ	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IFN-γRα	++	++	++	++	++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++
IFN-γRβ	+++	+++	++++	+++	+++	+++	++++	++++	++++	++++	++++	+++	++++	++++	+++	++++	++++	++++
TNF-α	–	–	–	–	–	–	–	+	+	–	+	+	–	+	+	–	–	–
LT-α	–	–	–	–	–	–	–	–	+	–	+	+	–	+	–	–	–	–
TNFRI	++++	++++	++++	++++	++++	++++	++++	++++	++	+	++++	++++	++++	++++	++++	++++	++++	++++
TNFRII	+	+	–	+	–	–	+	+	+	+	++	+	+	+	+	+	+	+
IL-2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-2Rα	+	–	–	–	+	+	–	–	–	–	–	–	+	–	–	–	–	–
IL-2Rβ	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
γc	+	–	–	–	+	+	–	–	+	–	–	–	+	–	–	–	–	–
IL-7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-7Rα	+	+	++	+	+	+	–	+	–	–	+	–	–	–	+	+	+	+
IL-9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-9Rα	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-15	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
IL-15Rα	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–
IL-12p35	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
IL-12p40	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-12Rβ1	–	–	–	–	–	–	–	+	–	–	+	+	+	+	+	+	+	–
IL-12Rβ2	–	–	–	–	–	–	–	+	+	–	+	+	+	+	+	+	+	–
IL-1α	+	–	–	–	–	–	–	+	+	–	–	–	+	–	–	–	–	–
IL-1β	+	–	–	–	–	+	+	+	+	–	+	+	+	+	+	+	+	+
IL-1Ra	+	+	–	–	–	+	–	+	+	–	+	+	+	+	+	+	+	+
IL-1RI	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+
IL-1RII	+	+	–	+	+	–	+	–	–	–	–	+	–	–	+	–	+	+
TGFβ1	+++	++	++++	+	++	+	+++	+++	+++	++	+++	+++	++++	+++	++	+++	++	+++
TGFβ2	++	++	++++	+++	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++++	++++	++++
TGFβ3	+	+	+	+	+	+	++	+	++	+	+	+	+++	+++	++	++	+++	+++
TGFβRI	+++	++++	+++	+++	++++	++++	+++	+++	+++	+++	++++	++++	++++	+++	+++	++++	++++	++++
TGFβRII	++	++++	+++	+++	+++	+++	+++	++++	+	+++	++++	+++	+++	+++	++++	+++	++++	++++
IL-6	++	+	–	++	++	+	+	++	–	–	+	+	+	+	+	+	+	+
IL-6Rα	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
gp130	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIF	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	+	+	+
LIFRα	+++	+++	+++	++	+++	++	+++	++++	+++	++	++++	+++	+++	+++	++	+++	++++	+++
OSM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2 (continued)

Samples	Glioma cell lines						Glioblastoma tumors (BTTB)									
	U87	U118	D54	U138	U373	U251	197	335	353	356	398	564	256	270	337	437	439	450
IL-4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-4Rα	+++	++++	++	+	+	+	+	++	+	+	+	+	++	+	+	+	++	+
IL-13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-13Rα	+++	+	++++	+	++++	++++	+	–	++	+	+	+	+++	+	+	–	+	–
IL-10	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–
IL-10R	–	–	–	–	–	–	+	++	+	+	+	+	+	+	+	+	+	+
IL-3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-3Rα	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
βc	–	–	–	–	–	–	+	+	+	+/–	+	+	+	+	+	+	+	+
IL-5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-5Rα	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
G-CSF	++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
G-CSFRα	–	–	–	–	–	–	++	++	++	+	+++	+++	++	+++	++	++	+++	++
GM-CSF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GM-CSFRα	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
M-CSF	+++	++++	++++	++++	++++	++++	+++	++++	++	++	+++	+++	++++	+++	+++	++++	++++	+++

functional IL-12. Moreover, transcripts for the IL-12 receptor, which is composed of two subunits (IL-12Rβ1 and IL-12Rβ2) [10], were not detected in any of the cell lines nor in most of the tumor samples, suggesting that IL-12 does not play a role in the modulation of glioblastoma growth.

Th2/Th3 cytokines

Expression of all three TGF-β isoform transcripts and secretion of TGF-β1 and β2 (but not TGF-β3) by glioblastomas has been reported previously [5, 34] and is confirmed here. In addition, our quantitative RPA analysis shows that the expression of TGF-β mRNA is the highest of all the cytokines examined. The effects of TGF-β have been implicated in the immunosuppressive status of glioblastoma patients [28] and animal models [8]. TGF-β signals are normally mediated through heteromeric complexes of type I (TGFRI) and II receptors (TGFRII) [52]. Activating these receptors induces G1 cell cycle arrest by inhibiting G1 cyclins and cyclin-dependent kinases [11]. TGFRI and TGFRII have been detected at the mRNA level by RPA in this study, and elsewhere by Northern blot and a functional affinity-labeling assay [22]. Most glioblastoma cells, however, are resistant to TGFβ-mediated growth inhibition and can proliferate in vitro despite exposure to TGFβ cytokines [22], possibly reflecting G1/S dysregulation in gliomas due to p16, cyclin D_{1-3} and pRB loss [19].

The IL-6 family of cytokines includes IL-6, IL-11, ciliary neurotropic factor (CNTF), cardiotrophin (CT)-1, LIF and OSM [44]. This family acts via receptor complexes composed of at least one subunit of the signal transducing protein gp130 and a specific receptor subunit (IL-6Rα, IL-11Rα, CNTFRα or LIFRα) [44]. The only exception to this is OSM, which binds to gp130 and LIFRα. The expression of all IL-6-type cytokines and their receptors has been reported at both mRNA and protein levels in glioma cell lines [13, 14, 15, 30] and is further confirmed in this study and demonstrated in vivo in solid glioblastomas. Functional studies have indicated that IL-6 may form an autocrine growth loop in glioblastomas [13], although the roles of other IL-6 family cytokines remain to be investigated.

IL-4 and IL-13 have similar biological activities, possibly reflecting the use of both cytokine receptors of the IL-4Rα chain for signal transduction [33]. Although neither IL-4 nor IL-13 mRNA was detected in the glioblastoma tumors and the cell lines, their receptor transcripts (IL-4Rα and IL-13Rα) were found. These receptors have been shown to be functional in affinity-binding assays and by being specifically targeted by *Pseudomonas* exotoxin-based IL-4 or IL-13 chimeric anti-tumor proteins [6, 38]. Although IL-4 receptors share the γc chain with IL-2R in normal cells [45], the absence of γc transcripts in majority of the cell lines and tumors suggests γc may not contribute to the IL-4 receptor function in these cells.

IL-10 mRNA was reported in glioblastomas tumors using RT-PCR [20]. However, recent in situ studies indicate that microglia/macrophages, rather than tumor cells, are the source of such IL-10 expression [21, 49]. This is con-

sistent with the observations in our study of weak IL-10 mRNA expression in 2 of 12 tumors but not in any of the cell lines. The finding of IL-10R transcripts in all 12 tumors but not in the cell lines is interpretable as an influence of trapped inflammatory cell infiltrates such as microglia, lymphocytes and/or endothelial cells.

CSF family

G-CSF and GM-CSF expression by malignant cell lines was reported using RT-PCR [31]. However, subsequent studies using RT-PCR and immunohistochemistry [9, 42] failed to detect GM-CSF or G-CSF transcripts and proteins in malignant glioma tumors. This is in keeping with our study, which failed to find transcripts for GM-CSF, G-CSF, IL-3 and IL-5 in the tumors and all but one (U87MG) of the cell lines in G-CSF expression. In contrast, transcripts for the ligand-specific/low-affinity binding α-chain receptor subunits (GM-CSFRα, G-CSFRα, IL-3Rα and IL-5Rα) and the common high-affinity binding/signal transduction βc shared by these four cytokines [51] were detected in all glioblastoma tumors at a low level, but not in the cell lines. Microglia and macrophages normally express CSF receptors when exposed to CSFs [25] and may therefore be responsible for this finding.

Of the CSF family of cytokines, only M-CSF was highly expressed in both the tumors and cell lines. M-CSF receptor mRNA (encoded by c-*fms* proto-oncogene) has been reported in glioblastoma cell lines and tumors using RT-PCR [1] and it has been shown that astroglia produce M-CSF [17]. Taken together, these observations suggest a paracrine loop that may promote the intratumoral proliferation of microglia as shown here and in other studies [39, 49].

In summary, our study has shown an unbalanced expression Th1, Th2/Th3 cytokines and their receptors in human glioblastomas. The dominant expression of Th2/Th3 is associated with a paucity of lymphocytes in glioblastomas and could account for the 'immunosuppressive status' of these tumors. Further study of the complex interaction of cytokines and their receptors between the neoplastic and infiltrating cells in glioblastomas may provide useful information toward the search for effective immunotherapies for this devastating disease.

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