Human Glioblastoma Cells Release Interleukin 6 in Vivo and in Vitro

Erwin Van Meir, Yutaka Sawamura, Annie-Claire Diserens, Marie-France Hamou, and Nicolas de Tribolet

Neurosurgical Service, University Hospital (CHUV), 1011 Lausanne, Switzerland

ABSTRACT

This study demonstrates interleukin 6 (IL-6) production and release by human glioblastomas. Twenty glioblastoma cell lines were tested for IL-6 bioactivity using an IL-6-dependent cell line (7TD1). All of the lines tested with one exception (LN-229) constitutively released IL-6. A significant induction of IL-6 production and secretion was observed when LN-229 cells were treated with interleukin 1β (IL-1β) or tumor necrosis factor α. Various amounts of IL-6 mRNA were found in five of six cell lines tested. IL-6 mRNA was detected in one line LN-229 only when the cells were treated with IL-1β or tumor necrosis factor α, confirming the bioassay data. Glioblastoma cells also produce IL-6 in vivo. (a) IL-6 activity was detected in 11 of 13 cerebrospinal fluids and five of five tumor cyst fluids. (b) IL-6 mRNA was found in four of four tumors. (c) Immunohistochemical analysis showed IL-6 within the tumor cells in 15 of 20 glioblastoma sections. In conclusion, biologically active IL-6 is released by almost all glioblastomas both in vitro and in vivo. The elevated levels of serum acute phase proteins and immune complexes found in glioblastoma patients may be the result of this secretion.

INTRODUCTION

Little is known about the way brain tumors can modulate the host immune system. Glioma cells have been shown to be capable of releasing various cytokines, such as an IL-1-like factor (1), an IL-3-like factor (2), an IFN-β-like activity (3), and TGF-β2 (reviewed in Ref. 4). Although it has been recently reported that an astrocytoma line (U373) and a glioblastoma cell line (SK-MG-4) expressed IL-6 mRNA when stimulated with IL-1β (5), until now IL-6 production by human glioblastomas was still unknown.

IL-6 is a lymphokine produced by a variety of cells with pleiotropic functions and is one of the major immunomodulators (reviewed in Ref. 6). Three functional properties of IL-6 are of particular interest for brain tumor biology. (a) IL-6 controls the final maturation of B-cells into antibody-producing cells and stimulates immunoglobulin synthesis by plasmocytes. Autologous humoral immune responses associated with gliomas have been described (7), and increased amounts of immune complexes have been found in patients’ sera which correlated with poor prognosis (8). (b) IL-6 can stimulate the activation of NK cells (9) and the generation of cytotoxic T-lymphocytes (10, 11). Therefore endogenous production of IL-6 by glioblastomas should favor a cell-mediated antitumor response. (c) IL-6 can act as a hepatocyte-stimulating factor which promotes hepatic plasma protein synthesis known as acute phase proteins. Elevated serum levels of these proteins have been found in glioblastoma patients and correlated with tumor extent (12).

Received 11/18/89; accepted 7/18/90.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work was supported by Grant 3.314-0.82 of the Swiss National Research Foundation. To whom requests for reprints should be addressed.

The abbreviations used are: IL-1, interleukin 1 (IL-2, IL-3, IL-4, and IL-6 defined similarly); rIL-6, recombinant interleukin 6; CSF, cerebrospinal fluid; CNS, central nervous system; FCS, fetal calf serum; GFAP, glial fibrillary acidic protein; TGF-β2, transforming growth factor β2; TNF-α, tumor necrosis factor α; rTNF-α, recombinant tumor necrosis factor α; IFN-γ, γ-interferon (IFN-β defined similarly); PGE2, prostaglandin E2; cDNA, complementary DNA; PBS, phosphate-buffered saline; AEC, aminoethylcarbazol.

IL-6 has been extensively studied in the acute phase response to infections, inflammation, and tissue injuries; however, the role of IL-6 production by human cancers, especially solid tumors, is still obscure. IL-6 was described in cells derived from several tumor types or in cell lines: 6 cardiac myxomas; one uterine carcinoma (13); 6 chronic lymphocytic leukemias (14); 3 bladder carcinomas (15); and 9 myelomas (16). In certain cases such as cardiac myxomas, IL-6 overproduction was supposed to be responsible for the generation of autoantibodies due to B-cell hyperactivation (13). Furthermore, IL-6 may be an autocrine (16) or paracrine (17) growth factor for myelomas. Alternatively, IL-6 is able to inhibit the growth of human breast and leukemia/lymphoma cell lines (18).

The present study focuses on the release of IL-6 by glioblastomas and discusses its possible role in glioblastoma patients. The presence of IL-6 was analyzed in supernatants of glioblastoma cell lines, in CSFs, in cyst fluids, and in tumoral tissues of glioblastoma patients. The conditioned media of cultured cells were found to contain significant amounts of IL-6. IL-6 mRNA was detected in the cell lines and was shown to be inducible by IL-1β in vitro. IL-6 activity was also found in the body fluids of patients, and IL-6 mRNA was present in solid frozen tumors. Furthermore, immunohistochemical analysis revealed that IL-6 is produced by GFAP-positive cells in glioblastoma. This is the first extensive study demonstrating that release of biologically active IL-6 is a general property of human glioblastomas.

MATERIALS AND METHODS

Preparation of Supernatants from Glioma Cell Lines. The 20 human glioblastoma cell lines used in this study were established and extensively characterized in our laboratory as described elsewhere (19). All cell lines studied were maintained in monolayer cultures in RPMI 1640 medium with penicillin (100 units/ml), streptomycin (0.1 mg/ml), 2 mm L-glutamine, and 5 to 10% FCS (complete medium, Seromed). For production of supernatants, the glioblastoma cells were plated in 25-cm² flasks. After 24 h, the subconfluent cultures were washed 3 times and replaced with 5 ml of either HB-102 serum-free medium or with RPMI 1640 medium plus 5% FCS. The supernatants were collected from Day 0 to Day 4 and stored at −70°C. In some experiments, human IL-1β (1 to 20 units/ml; Genzyme, Cambridge, MA), IFN-γ (10² to 10⁴ units/ml; Glaxo, Geneva, Switzerland), TNF-α (10 to 10⁴ units/ml; Glaxo), IL-2 (10⁴ units/ml; Glaxo), or IL-4 (10³ units/ml; Genzyme) was added at the initiation of a 24-h culture. A fibroblast culture obtained from a patient’s scalp, primary cultures of 2 benign astrocytomas, and one cerebral neurocytoma were also used to analyze IL-6 activity in their supernatants. In addition, supernatants of 2 primary cultures of reactive astrocytes isolated from metastatic brain tumors (one lymphoma and one undifferentiated carcinoma of unknown origin) were also studied to test IL-6 production by nonneoplastic brain cells.

Harvesting Cerebrospinal Fluid and Glioma Cyst Fluid. CSF samples were tested for IL-6 activity from 13 patients with supratentorial glioblastoma, from 8 patients with meningioma, and from 16 patients with unrelated diseases such as herniated lumbar disc. In the latter patients, inflammatory or tumoral diseases of the CNS were excluded. CSF was aspirated by a lumbar puncture before operation at myelography. Glioma cyst fluids were obtained preoperatively from 5 patients. Some glioblastoma patients received p.o. or parenteral corticosteroid therapy before the operation. The CSF and cyst fluid samples were...
centrifuged, filtered, pooled, and stored until the assay for a maximum of 6 mo at -70°C.

Bioassay of IL-6. IL-6 bioactivity was measured on BSF-2-dependent B-cell hybridoma 7TD1 (mouse-mouse cells) (Dr. J. Van Snick, Brussels, Belgium). 7TD1 cells do not grow in response to the following recombinant factors: human IL-1α; IL-1β; TNF-α; IFN-γ; poly rI:rC (20); granulocyte-CSF (3 to 400 units/ml); macrophage-CSF (40 to 50,000 units/ml); TGF-β1 (0.1 to 5 ng/ml); TGF-β2 (0.1 to 5 ng/ml); or murine granulocyte-macrophage CSF (1 to 1,000 units/ml).* Briefly, 7TD1 cells were seeded at a density of 2,000 cells/well in 96-well flat-bottomed microtiter plates in Iscove's modified Dulbecco's medium supplemented with 5% FCS, 50 µg 2-mercaptoethanol, 0.1 mM hypoxanthine, streptomycin (0.1 mg/ml), and penicillin (100 units/ml) (21). The cells were cultured in the presence of serial dilutions of samples or human rIL-6 as a standard (specific activity, 107 units/mg of IL-6; Genzyme). In addition in some assays TGF-β2 (4 to 40 ng/ml); Dr. S. Bodmer, Zürich, Switzerland) or PGE2 (30 ng/ml; Sigma, St. Louis, MO) was added with rIL-6. For the final 16 h of a 72-h culture, the cells were pulsed with 0.5 µCi of [3H]thymidine (5 Ci/mmol; Amer- sham, Buckinghamshire, United Kingdom). One unit of IL-6 activity was defined as the amount of a sample that resulted in half-maximal thymidine incorporation in the assay. When testing human rIL-6, the detection limit of the IL-6 bioassay was found to be 0.1 unit/ml. When testing supernatants, CSF, or cyst fluids, the detection limit was between 4 units/ml and 20 units/ml due to inhibitory effects of certain samples tested at low dilutions. To characterize the IL-6-like activity in samples, a neutralizing goat anti-IL-6 serum was used (Dr. T. Hirano and Dr. T. Kishimoto, Osaka, Japan). After a 3-h incubation of the samples with the anti-IL-6 serum (final dilution, 1:2,000) at 37°C, the residual activity was determined in the assay. The anti-IL-6 serum neutralized 1,000 units of IL-6 activity at a 100-fold dilution.

RNA Isolation and Hybridization. Human glioblastoma cells were seeded at a concentration of 6 x 104 cells/ml in 75-cm2 flask and incubated in medium for 4 or 24 h. IL-6 (5 units/ml) or rTNF-α (100 units/ml) was added at the beginning of some cultures as indicated. The adherent cells were harvested with a rubber scraper into 5% citric acid. Frozen tumor samples were ground in liquid nitrogen and then homogenized in 5% citric acid. Total RNA was then isolated according to the citric acid procedure (22). Northern blot analysis was performed on 10 µg of total cellular RNA, as described previously (23), with random labeled probes (24). The 298-base pair Saff/XbaI fragment of the IL-6 coding region was used as a probe for IL-6 (25). The mouse β-actin cDNA used was a PstI 1100-base pair fragment of plasmid pAL41 (26). The filters were washed in 0.1% sodium dodecyl sulfate: 18 mM NaCl:1 mM sodium phosphate (pH 7.7):0.2 mM EDTA at 65°C (high stringency) for the IL-6 probe and at 25°C for the mouse β-actin probe. Autoradiography was performed at 70°C with an intensifying screen.

Immunohistochemical Staining. Biopsy samples obtained from 20 glioblastomas, 5 low-grade gliomas, 7 meningiomas, one neurinoma, 2 metastatic brain tumors, one fetal brain, and 3 normal adult brains (autopsy or lobectomy) were stored at -70°C. Several serial sections of 7- to 8-μm thickness were made from each sample, air-dried overnight and immersed in fresh acetone at —20°C for 5 min. The slides were thawed, washed in PBS (pH 7.4), and incubated for 60 min with a 1/100 dilution of monoclonal anti-IL-6 antibody BSF-166 (Dr. T. Hirano and Dr. T. Kishimoto, Osaka, Japan), with a 1/800 dilution of monoclonal anti-IFN-γ antibody N358 (Amerham), or with the supernatant of P3X63Ag8, a γ-1 producing myeloma cell line as a control (Dr. S. Carrel, Lausanne, Switzerland). Slides were incubated with a secondary antibody, biotinylated horse anti-mouse or goat anti-rabbit immunoglobulin at 50 µg/ml in PBS, for 20 min and then with avidin-DH-biotinylated horseradish peroxidase at 90 µg/ml in PBS for 20 min (Vector Labs., Inc., Burlingame, CA). The peroxidase was visualized with AEC (10 mg of AEC in 2.5 ml of dimethylformamide diluted in 50 ml of 0.05 M acetate buffer, pH 4.9) and 0.01% H2O2 for 5 min. Positive cells stained brown-red. The slides were then counterstained with Gills hematoxylin for 1 min. For double staining, the counterstaining step was omitted. The sections were further washed for 10 min in PBS and incubated with rabbit anti-GFAP antiserum (1/100) for 45 min and then with the secondary goat anti-rabbit antibody conjugated with peroxidase (1/30) (Nordic, The Netherlands). Positive cells (dark gray) were revealed in 5 min with chloro-naphthol (25 mg of chloro-1-naphthol in 200 µl of dimethylformamide diluted in 50 ml of 0.05 M Tris-HCl, pH 7.6) and 0.01% H2O2.

RESULTS

Fourteen permanent and 6 short-term cultured (within 3 passages) glioblastoma cell lines were tested for IL-6-like bioactivity measured as hybridoma growth factor on the IL-6-dependent B-cell hybridoma 7TD1 (Fig. 1A). These cells were cultured in serum-free medium in order to avoid any nonspecific serum effect of FCS. Short-term cultures of human glioblastoma cells may contain a few normal astrocytes and fibroblasts, but permanent cultures do not. All of them except LN-229 showed variable constitutive IL-6-like activity ranging from 12 to 14,000 units/ml. Since cell numbers and culture periods were different in each line, no precise quantitative comparison is possible in this instance. When defined numbers of cells (5 x 104) were cultured under identical growth conditions in medium containing 5% FCS, a time-dependent accumulation of IL-6 (per 1,000 cells) was observed in 5 stable permanent glioblastoma cell lines, LN-382, LN-428, LN-443, LN-444, and LN-992 (Table 1). The conditioned media obtained from a fibroblast cell culture (Fbl 445), 2 cultures of nonneoplastic astrocytes obtained from metastasized brain (Patients 512 and 534), and 2 benign astrocytoma primary cultures (Patients 501 and 496) also exhibited IL-6 activity (210, 130, 420, 12, and 58 units/ ml, respectively). A primary culture of a nerve cell tumor (neurocytoma; Patient 500) was negative. A neutralizing goat anti-human IL-6 serum was able to abolish IL-6 activity present in the supernatants of cell lines LN-18 and LN-428, but only partial neutralization was observed for LN-319 (data not shown). We are currently investigating the nature of the remaining activity.

Six glioblastoma cell lines were analyzed by Northern blotting techniques for the presence of IL-6 mRNA using an IL-6 cDNA probe (Fig. 1B). The total RNA of a fibroblast cell line (Fbl 445) was used as a positive control as fibroblasts are known to express IL-6 mRNA (20). Variable amounts of IL-6 mRNA were found in five of the cell lines tested. No mRNA was detected in line LN-229, confirming the negative bioassay data.

To investigate the in vivo existence of IL-6 released from glioblastoma cells, cryopreserved CSF of 13 glioblastoma patients and the tumor cyst fluid of 5 patients were tested (Fig. 2A). Eleven CSFs of glioblastoma patients scored positive in the bioassay (>10 units/ml). In contrast, only 3 of 16 control CSFs obtained from patients with herniated discs without any CNS inflammation had low detectable IL-6 activity (<10 units/ml). Six of 8 meningioma CSFs were also weakly positive (10 to 30 units/ml) (data not shown). Of particular interest are patients LN-443, LN-444, and LN-566, who showed IL-6 activity.

Table 1 IL-6-like activity released by 106 cells of 5 stable glioblastoma cell lines in the presence of 5% FCS

<table>
<thead>
<tr>
<th>Days of culture</th>
<th>LN-382</th>
<th>LN-428</th>
<th>LN-443</th>
<th>LN-444</th>
<th>LN-992</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.6</td>
<td>8.4</td>
<td>7.2</td>
<td>5.6</td>
<td>7.8</td>
</tr>
<tr>
<td>2</td>
<td>6.4</td>
<td>12.0</td>
<td>10.8</td>
<td>8.4</td>
<td>10.4</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>36</td>
<td>22</td>
<td>11</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>104</td>
<td>52</td>
<td>40</td>
<td>116</td>
</tr>
</tbody>
</table>

* Five permanent cell lines were cultured for 4 days in the presence of 5% FCS.
* IL-6-like activity in the culture supernatants was assessed on the IL-6-dependent 7TD1 cell line.

K. Frei, Zürich, Switzerland, personal communication.
activity in both the CSF (670, 540, and 220 units/ml, respectively) and the corresponding established cell lines (Fig. 1). All 5 cyst fluids of glioblastoma patients were positive (Fig. 2A). Patients LN-467 and LN-469 were positive for IL-6 in the cyst fluid (780 and 2100 units/ml, respectively) and the tumor-derived cell lines (Fig. 1). In Patient G-549, where CSF and cyst fluid were available, both scored positive for IL-6 (12 and 450 units/ml, respectively). These data suggest that the IL-6 present in the CSF originates from the tumor tissue.

To further confirm that the IL-6 activity detected in these body fluids is IL-6 and that it originates from the tumor, the IL-6 mRNAs extracted from 4 frozen tissues of glioblastoma and one meningioma were analyzed (Fig. 2B). Specific IL-6 mRNA was detected in all 4 glioblastomas and had the same molecular size (1.3 kilobases) as in fibroblasts. No IL-6 mRNA was detected in the only meningioma analyzed. In Patient G-403, the IL-6 mRNA was present in the tumor, and IL-6 activity was detected in both CSF (670 units/ml) and the tumor-derived cell line LN-443 (Table 1).

The presence of IL-6 bioactivity or mRNA in both cell lines in vitro and tumor specimens in vivo strongly suggests that cells within the glioblastoma tissue, such as tumor cells, reactive astrocytes, or fibroblasts, release IL-6 in vivo in CSF and cyst fluid. To investigate which cell types are responsible for this IL-6 release, immunohistochemical analysis on frozen tissue sections was used. Adjacent serial sections were studied by utilizing monoclonal anti-IL-6 antibody BSF2-166 (27), anti-GFAP serum, and supernatant of P3X63Ag8 as a control. In 15 of 20 glioblastomas studied, numerous neoplastic cells were found to be IL-6 positive (Fig. 3A). In some cases, up to 50% of the cells stained weakly positive, and adjacent sections showed more than 95% GFAP-positive cells, but none with the P3X63Ag8 supernatant. In other cases, densely staining regions were seen beside negative area showing heterogeneity of the IL-6 expression even in a given tumor. The existence of both IL-6 and GFAP-positive cells was confirmed by double staining with both antibodies (Fig. 3B). In 3 of 5 benign astrocytomas, only a few isolated positive cells were observed, which were also
HUMAN GLIOBLASTOMA CELLS RELEASE IL-6 IN VIVO AND IN VITRO

Fig. 3. A. high magnification of a glioblastoma section from Patient G-403 showing multiple IL-6-positive cells. B. high magnification (× 400) of positively double-stained glioblastoma cells (Patient G-403). Top, two cells showing cytoplasmic IL-6 staining with nuclei remaining unstained (arrows). GFAP dark gray filaments stain positive on the same cells.

GFAP positive and homogenously distributed throughout the benign astrocytoma tissue. The anti-IL-6 monoclonal antibody could not stain any cells in either one normal fetal brain, 3 normal adult brains, or in one neurinoma (not shown). In addition, neither normal nor abnormal vascular endothelial cells within the glioblastoma stained positively. In 2 of 7 meningiomas, positive cells were scarcely present (less than 1 cell in 10,000 cells) which could not be distinguished from infiltrating inflammatory cells. In the 2 CNS metastatic cases studied (Patients 553 and 570), gliosis surrounding the negatively staining tumor cells included groups of both IL-6- and GFAP-positive staining cells.

Four glioblastoma cell lines were treated with IL-1β and TNF for 4 to 24 h. Both cytokines enhanced IL-6 production (Fig. 4; Table 2). As a control, IL-1β or TNF was added to the bioassay in the presence of various concentrations of IL-6 and found to have no direct effects on the growth of the hybridoma cells. These two cytokines were able to induce or to enhance IL-6 production by glioblastoma cell lines (Table 2). Other cytokines tested such as IL-2, IL-4, or IFN-γ had no stimulatory effect on IL-6 production by cultured cell lines. In addition, 0.004 to 40 ng/ml of TGF-β2 (4) and 10⁻⁹ to 10⁻⁵ M PGE2 (28) showed no inhibitory effect (data not shown).

It was of interest to see whether the increased amount of IL-6 biological activity obtained in response to IL-1β or TNF could correspond to an increased steady-state mRNA level in the glioblastoma cell lines LN-229, LN-18 (Fig. 4), LN-319, and LN-428 (not shown). A significant induction of IL-6-specific mRNA was detected 4 h after incubation in medium containing IL-1β (Fig. 4) or TNF (data not shown) increasing up to 24 h. The relative levels of IL-6 mRNA in line LN-229 showed a 6-fold increase between 4 and 24 h as estimated by scanning of the autoradiograph and standardized to the β-actin mRNA levels.

DISCUSSION

The results presented here indicate that glioblastoma cells produce variable levels of IL-6 in vivo and in vitro.
Recently Yasukawa et al. (5) showed that a glioblastoma cell line expressed IL-6 only when induced by IL-1β. However, in our study, 19 of 20 cell lines without any stimulation released IL-6-like activity, and 5 of 6 were shown to constitutively express IL-6 mRNA. In 5 cell lines, the IL-6-like activity was quantified and ranged from 36 to 116 units with a mean value of 70 units per 1000 cells. IL-1β and TNF were able to enhance IL-6 mRNA steady-state levels and IL-6 secretion by different glioblastoma cell lines. They also induced IL-6 production and secretion by line LN-229, which was the only cell line not able to secrete IL-6 spontaneously. These results are in agreement with previous studies; IL-6 secretion by various cell types can be regulated by inflammatory cytokines (20, 29–32). The control mechanism for induction or enhancement of IL-6 mRNA in stimulated glioblastomas may be due to increased transcription or mRNA stabilization.

IL-6-like activity was also observed in 11 of 13 CSF and 5 of 5 tumor cyst fluids obtained from glioblastoma patients. This shows that IL-6 activity in the CSF is probably related to the presence of the glioblastoma. IL-6 has been previously found in the CSFs of the patients with infectious CNS diseases, such as aseptic meningocencephalitis due to HSV-1 or HIV-1 infection (33, 34), and acute bacterial infection (35).

These data suggested that the tumor cells may produce IL-6 in vivo. The activity could, however, also originate from the nonneoplastic cells. T-cells, monocytes and macrophages, fibroblasts, microglial cells, astrocytes, and endothelial cells, which all commonly exist in the glioma tissue, are known to release IL-6 (6, 29–31, 33, 34). Immunohistochemical analysis showed that, in some glioblastoma, up to 50% of the cells produce IL-6. Adjacent sections of the same specimens showed more than 95% positive GFAP cells. Double staining studies allowed us to confirm that at least part of the cells producing IL-6 in the tumor is GFAP positive and, therefore, glioblastoma cells. In addition, benign astrocytomas showed dispersed positive cells, and IL-6 biological activity was found in their primary cultures.

During the preparation of this paper, the CSFs of 3 astrocytomas, one of 3 glioblastomas, 3 meningiomas, and 7 CNS metastases were shown to contain IL-6-like bioactivity (36). On this basis, the authors proposed that IL-6 is secreted by primary CNS tumor cells or metastatic tumor cells. We have now established that this is the case for at least human glioblastomas. In the meningiomas, we found weak IL-6 activity in 6 of 8 CSFs, but in only 2 of 7 cases could we observe isolated IL-6 positive staining cells which might be infiltrating macrophages. Since most of the meningiomas are intracranial extraaxial tumors surrounded by a fibrous capsule, there is no direct contact between the meningioma cells and the CSF circulation (37). Therefore the presence of IL-6 in CSFs of meningioma patients could originate from IL-6-secreting reactive astrocytes present in the adjacent normal brain in response to compression by meningioma. In the 4 cases of metastatic brain tumors we studied so far, only astrocytes adjacent to the tumor but not the tumor cells were stained by anti-IL-6 antibody. Furthermore cultured astrocytes isolated from metastatic brain tumors could release IL-6 activity in vitro. We would like to suggest that, in these cases, the IL-6 activity detected in the CSF may originate at least in part from the gliosis surrounding the metastatic tumor.

What is the consequence of the presence of IL-6 in body fluids of glioblastoma patients? IL-6 has recently been shown to be a hepatocyte-stimulating factor (38), the major factor regulating, essentially at the transcriptional level, the plasma concentration of the acute phase proteins released by the liver (39). The elevated plasma levels of these proteins are useful as nonspecific indicators of tumor presence, and they increase with the tumor size (40, 41). They were also shown to be related to tumor extent in patients with gliomas (12). This leads us to suggest that the IL-6 produced by the glioblastoma cells may be released into the blood and elicit an acute phase response in the liver. Other factors secreted by glioblastoma cells (42), like IL-1, may play a role in a number of the biological activities mentioned for IL-6. For instance IL-6 has been shown to be the major inducer of the C-reactive protein gene expression, although it acts cooperatively with IL-1 (43).

On the other hand, does IL-6 play a role in the immune response against brain tumors in situ? First, IL-6 may be involved in a cellular antitumor response as IL-6 enhances the activity of natural killer cells (9) and cytotoxic T-lymphocytes (10, 11). Second, IL-6 may stimulate the growth and maturation of tumor infiltrating B-lymphocytes and activate them in situ to elicit antibody secretion. Although B-lymphocyte infiltration in human glioblastomas is usually scarce, antibodies against glioma-associated antigens have been described (44). Some inhibitory factors released by the tumor, such as PGE2 (28) or TGF-β2 (4), could interfere with IL-6 effects on both T- and B-cells, although we could find no effect of these two factors on IL-6 in vitro. Serum immunoglobulins are not significantly increased in patients with glioma (12), but elevated immunoglobulin G levels have been reported in the CSF of 2 glioblastoma patients without blood brain barrier disruption (45). This leads us to speculate that apart from an intratumoral local influence IL-6 may also be responsible for a general stimulation of B-cell maturation and lead to nonspecific antibody release. Nonspecific cytotoxic activity has been reported in glioblastoma patients' sera using an antibody-dependent cell-mediated cytotoxicity assay (46). It has also been previously shown that 28% of 96 high grade glioma patients had immune complexes as compared with 14% of 28 low-grade glioma patients. The presence of the immune complexes correlated with reduced survival (8).

In conclusion, the present study demonstrates for the first time the in vivo production of IL-6 by human glioma cells. The clinical consequences of this cytokine being released in the cyst fluid and CSF of the patients is, at the present time, not clear. We suggest that it might be responsible for the hepatic acute phase response and the elevated immune complexes previously observed in glioblastoma patients. It is also at the moment not obvious how IL-6 may modulate cellular immune responses and B-cell growth and maturation in situ or if its action could be antagonized by tumor-released suppressor factors. Adversely, IL-6 could also lead to the establishment and progression of neoplastic transformation as has been proposed in an autocrine way for myelomas (16) and renal cell carcinomas (47). Clearly further studies are necessary to see whether this also holds true for glioblastomas.

ACKNOWLEDGMENTS

We wish to thank Dr. S. Bodmer, Dr. J. Brakenhoff, Dr. M. Buckingham, Dr. S. Carrel, Dr. K. Frei, Dr. T. Hirano, Dr. T. Kishimoto, and Dr. J. Van Snick for the generous gifts of cytokines, cDNA probes, hybridomas, antibodies, and unpublished data. We are grateful to Dr. S. Bodmer, Dr. A. Fontana, and Dr. M. Kupner for critically reading the manuscript.

REFERENCES

1. Fontana, A., Hengartner, H., de Tribollet, N., and Weber, E. Glioblastoma cells release interleukin 1 and factors inhibiting interleukin 2-mediated...
24. Feinberg, A. P., and Vogelstein, B. A technique for radiolabeling DNA
17. Klein, B., Zhang, X-G., Jourdan, M., Content, J., Houssiau, F., Aarden, L.,
13. Mirano, T., Taga, T., Yasukawa, K., Nakajima, K., Nakano, N., Takatsuki, S.
Human Glioblastoma Cells Release Interleukin 6 in Vivo and in Vitro


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/20/6683

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.