Cytokines and immunoregulatory molecules in malignant glial neoplasms

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Cytokines are important regulatory proteins controlling growth and differentiation of normal and malignant glial cells. Astrocytes and microglial cells produce and respond to many of the same cytokines employed by cells of the immune system. The authors have analyzed 15 histologically confirmed malignant glial neoplasms for the presence of infiltrating lymphocytes, macrophages, cytokines, and other immunoregulatory molecules using a panel of specific monoclonal and polyclonal antibodies on frozen-tissue sections. All neoplasms showed focal T-cell infiltration with CD8 cells predominating. Infiltration of activated macrophages (positive for CD8c, class II, and interleukin-2 receptor) was marked in all tumors. Within the neoplasm, tumor necrosis factor-α (TNF-α)- and interleukin (IL)-6-positive macrophages were prominent in five cases, while the tumor cells themselves were only weakly positive. In the other 10 cases, the numerous infiltrating macrophages were only rarely immunoreactive for TNF-α or IL-6. Transforming growth factor-β (TGF-β) immunoreactivity was most prominent in those tumors with little TNF-α-positive macrophage infiltration, although intratumoral variability was present. This study suggests that, in malignant gliomas, the cytokines TNF-α and IL-6, although weakly present in neoplastic cells, are most prominent in infiltrating macrophages and in those regions of the tumors that show little immunoreactivity for TGF-β. The important interactions among neoplastic, reactive glial, and inflammatory cells, which regulate tumor growth, are likely to be in part mediated through these molecules.

KEY WORDS • glioblastoma multiforme • anaplastic astrocytoma • interleukin-6 • tumor necrosis factor-α • transforming growth factor-β

MALIGNANT glial neoplasms and, in particular, glioblastoma multiforme are rapidly growing tumors for which limited therapy is currently available. Little is known about the factors regulating cellular proliferation and differentiation within these tumors, although tumor suppressor genes, growth factors, and signal transduction mechanisms may be involved. The role of the immune response in control of malignant glial tumor growth has been studied extensively and recently has been the basis for immunotherapeutic approaches. Infiltrating mononuclear cells are common in malignant glial neoplasms, suggesting a possible immunological response by the host; however, attempts to correlate this response with survival are inconclusive. Numerous studies have examined the phenotype of infiltrating leukocytes in glial tumors and have found, in general, a predominance of cytotoxic lymphoid cells. Other authors have stressed the importance of macrophage infiltration in these tumors and suggested the possible release of cytotoxic compounds by macrophages as a mechanism for the necrosis characteristic of glioblastoma multiforme.

Cytokines are important regulatory proteins controlling growth and differentiation, often in an autocrine or paracrine manner. Originally thought to be derived solely from lymphocytes and macrophages, they have more recently been discovered to be produced by cells not traditionally part of the immune system, including astrocytes and microglial cells. Cytokines have been identified and are thought to play a role in a variety of neurological diseases including infectious diseases of the central nervous system (CNS) and multiple sclerosis. Glioblastoma multiforme cell lines have also been shown to produce and respond to several cytokines including interleukin (IL)-1, tumor necrosis factor (TNF)-α, and IL-6. Recently, primary glioblastomas multiforme have been shown to have IL-
FIG. 1. Photomicrographs showing histological features of three glioblastomas multiforme studied. Paraffin tissue sections. H & E. A to C: Sections from the neoplasm in Case 1, showing a hypercellular tumor with necrosis (n) (A, × 63), prominent vascular proliferation (B, × 63), and occasional mitotic (arrow) figures (C, × 250). All of the other tumors showed similar features, however the vascular proliferation was most prominent in Case 1. D: Representative sections of the tumor in Case 2. × 250. E: Section from the tumor in Case 3 showing mitotic figures (arrows). × 250.

6 messenger ribonucleic acid (mRNA) by Northern blot analysis and IL-6 protein by the diffuse reactivity of tumor cells with an anti-IL-6 monoclonal antibody. In this report, we characterize immunohistochemically the infiltrating lymphocytes and macrophages in 15 malignant glial tumors, determine their distribution, and identify cytokines and other immunoregulatory molecules in order to understand more completely the tumors' local immune environment.

Materials and Methods

Tissue Samples

Tissue was obtained at surgery from 15 patients with supratentorial malignant glial tumors, of which 12 were glioblastoma multiforme (Cases 1 to 12) and three were high-grade anaplastic astrocytomas (Cases 13 to 15). The tissues were all from diagnostic stereotactic or open biopsy specimens obtained from untreated patients. The patients (eight women and seven men) ranged in age from 37 to 72 years. Imaging studies revealed that nine of the lesions were multicystic or had a necrotic center (Cases 1, 2, 4, 6, 7, 9, 10, 11, and 12) and six were solid enhancing lesions (Cases 3, 5, 8, 13, 14, and 15). All tumors demonstrated mass effect on surrounding brain at the time of diagnosis. No lesion had radiographic evidence of recent hemorrhage. The samples were snap-frozen within minutes of biopsy in liquid-nitrogen-cooled isopentane and stored at −80°C. The histological diagnosis was made on adjacent tissue samples fixed in formalin and embedded in paraffin (Fig. 1) using histological criteria reported by Burger, et al.5 Only one of the frozen biopsy samples examined contained areas of frank necrosis (Case 12), although necrosis was present in other areas of the tumors. Control normal brain tissues were obtained from Dr. Carol Miller at our institution; tissues included cerebral cortex and white matter obtained at autopsy, with a postmortem interval of less than 12 hours, from patients who had died from non-neurological causes.

Immunoperoxidase Method

Frozen-tissue sections were fixed in reagent grade acetone for 10 minutes, then rehydrated in phosphate-buffered saline (PBS, pH 7.4) for 5 minutes, followed by incubation with the primary antibody for 30 minutes at 25°C in a humidified chamber. The slides were
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then washed in PBS for 10 minutes. The biotin-labeled secondary antibody used was either horse anti-mouse immunoglobulin G (IgG) or goat anti-rabbit IgG depending on the nature of the primary antibody. This secondary reagent was applied to the section for 30 minutes, followed by a 10-minute wash in PBS. The tissue sections were then incubated in a complex of avidin and biotinylated horseradish peroxidase for 30 minutes, and given another 10-minute wash in PBS. After this wash, the colored substrate amino-ethyl carbazole was applied to the tissue and the preparation was incubated for 10 minutes. The slides were then rinsed in tap water for 5 minutes, stained with Mayer's hematoxylin for 10 minutes, and mounted with glycerin-gelatin mounting medium. Control preparations included the use of appropriately diluted mouse or rabbit immunoglobulins in place of the primary antibody; negligible background staining was observed. In a second control, the primary antibody was omitted to determine whether granulocytes, macrophages, or other cells with endogenous peroxidase activity were present; very few positive cells were observed in the brain tissues examined.

Results

Histology of Tumors Studied

All of the tumors showed histological features diagnostic of high-grade anaplastic astrocytic tumors including hypercellularity, prominent nuclear pleomorphism, occasional mitotic figures, and varying amounts of vascular proliferation. In addition, the glioblastomas multiforme showed zones of characteristic necrosis (Fig. 1). The tumor in Case 1 was notable for the extent of vascular proliferation present. The tumor from Case 10 contained numerous pleomorphic giant cells. We cannot rule out that the tumors designated as high-grade anaplastic astrocytoma were not in fact glioblastoma multiforme in which necrosis was not identified due to sampling errors. Immunoperoxidase staining of paraffin-embedded tissue sections showed focal to strong positivity of tumor cells for glial fibrillary acidic protein in all cases (results not shown).

Analysis of Infiltrating Lymphocytes

The presence of infiltrating lymphocytes was determined by immunoperoxidase staining using the monoclonal antibodies CD4 (T helper cells, Leu-2) and CD8 (cytotoxic T cells, Leu-3).* In six of the specimens, equal proportions of CD4- and CD8-positive lymphocytes were present predominantly in a perivascular location with occasional cells within the tumor parenchyma. The other nine specimens showed more frequent infiltration of lymphocytes with a higher proportion positive for CD8 (Table 1 and Fig. 2A). In all cases, only rare CD22-positive B cells were present. In control brain tissues, only rare intravascular T cells were identified.

* Mouse monoclonal antibodies obtained from Becton-Dickinson, San Jose, California.

Fig. 2. Photomicrographs showing interleukin-2 (IL-2) and IL-2-receptor localization in representative glioblastomas multiforme. All tumors showed occasional infiltrating lymphocytes with an overall predominance of CD8 cells. H & E. A: Only occasional CD8 cells infiltrated the tumor parenchyma (arrowheads). × 250. B: Very rare perivascular lymphocytes showed immunoreactivity for IL-2 (arrows). × 500. C and D: The immunoreactivity of IL-2 receptor was prominent on a subset of morphologically identified macrophages. All cases were positive for this feature, but representative cells from Cases 1 (C) and 3 (D) are shown. × 500.

Analysis of Infiltrating Macrophages

The presence of infiltrating macrophages was identified by immunohistochemical staining for the macrophage-specific marker CD11c (Leu-M5). All 15 specimens showed significant macrophage infiltration both
Fig. 3. Photomicrographs demonstrating reactivity of glioblastoma multiforme tissue to cytokines and immunoregulatory molecules. Immunoperoxidase staining of sections from Case 1 (columns 1 and 2), Case 2 (column 3), and Case 3 (column 4) with antibodies reactive with CD11c (row 1), HLA-DR (row 2), tumor necrosis factor (TNF)-α (row 3), interleukin-6 (IL-6) (row 4), and transforming growth factor (TGF)-β (row 5). All cases showed prominent macrophage (CD11c-positive) infiltration which was both perivascular (arrow) (A, × 50) and intraparenchymal (D, × 200). Morphology of the macrophages at higher magnification is shown (B and C, × 500). The macrophages were strongly positive for class II antigens (HLA-DR), while the tumor cells were weakly positive. In Case 1, the blood vessel (v) is surrounded by numerous positive macrophages (E, × 50; F, × 200). In Case 2 (G, × 500), a single strong positive macrophage is identified with only weakly or negatively stained endothelial cells. In Case 3...

Fig. 3 (continued →)
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TABLE 1
Infiltrating leukocytes, cytokines, and other immunoregulatory molecules in 15 malignant glial neoplasms*

| Factor | Case No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|--------|----------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|
| CD8    |          | ++| +| +/| +/| +/| +/| +/| +/| +/| +  | +/ | +/ | +/ | +/ | +/ | +/ |
| CD4    |          | +/| +| +/| +/| +/| +/| +/| +/| +/| +  | +/ | +/ | +/ | +/ | +/ | +/ |
| CD22   |          | +/| +| +/| +/| +/| +/| +/| +/| +/| +/ | +/ | +/ | +/ | +/ | +/ | +/ |
| CD11c  |          | +++| +++| +++| +++| +++| +++| +++| +++| +++| +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| IL-2 receptor |          | +++| +++| +++| +++| +++| +++| +++| +++| +++| +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| IL-6   |          | +T| +T| +T| +/T| +T| +T| +T| +T| +T| +T  | +T | +/ | +/ | +/ | +/ | +/ |
| MNF-a  |          | ++/| T| +/M| +/M| +/M| +/M| +/M| +/M| +/M| +/M| +/M | +/M | +/M | +/M | +/M | +/M |
| TGF-ß  |          | ++/| T| +/M| +/M| +/M| +/M| +/M| +/M| +/M| +/M| +/M | +/M | +/M | +/M | +/M | +/M |

* Frozen sections of 15 malignant glial tumors (Cases 1 to 12 are glioblastomas multiforme, while Cases 13 to 15 are high-grade anaplastic astrocytomas) were stained immunocytochemically for the presence of leukocyte subpopulations, cytokines, and other immunoregulatory molecules as described in the Materials and Methods section. Leukocytes were identified as follows: CD4 (T helper cells, Leu-3), CD8 (cytotoxic T cells, Leu-2), CD22 (B cells, Leu-14), and CD11c (macrophages, Leu-M5). Leukocytes were counted/sq mm and were graded as follows: < 1 cell (−); 1 to 5 cells (+/+); 5 to 20 cells (+); 21 to 100 cells (++); and > 100 cells (+++). Tumor necrosis factor-α (TNF-α), and transforming growth factor-ß (TGF-ß). HLA = human leukocyte antigen.

† The reactivity for TGF-ß in Case 10 was very heterogeneous; while most of the tumor showed small numbers of immunoreactive cells, focal areas of ++++ reactivity were present.

in a perivascular and in a parenchymal location within the tumor (Fig. 3A to D). The numbers of macrophages/sq mm ranged from 80 (Case 4) to 278 (Case 5) (Table 1). The control brain tissues showed weakly stained microglial cells in the gray and white matter.

Expression of HLA-DR

Morphologically identified macrophages in all 15 tumors showed prominent expression of human leukocyte antigen (HLA)-DR (major histocompatibility complex class II) by immunoperoxidase staining (Fig. 3E to H).† No definitive HLA-DR expression was found on endothelial cells either in the tumor or the adjacent reactive brain, resulting in a striking contrast between the negative endothelium and the intense reactivity of the adjacent perivascular macrophages (Fig. 3E to H). Occasional microglia in the adjacent brain were also positive. The tumor cells also stained diffusely positive for HLA-DR, but this was much less intense than in the macrophages. Microglial cells in the control brain tissues did not express HLA-DR.

† HLA-DR monoclonal antibody obtained from Becton-Dickinson, San Jose, California.

Interleukin-2 Receptor and Localization

The IL-2 receptor was immunocytochemically identified as punctate membrane staining of a subpopulation of morphologically identified macrophages and lymphocytes in all cases (Fig. 2C and D).‡ Staining was most frequent in those cases with the highest number of macrophages. Control brain tissues were nonreactive for IL-2 receptor. Interleukin-2 was identified in association with only very rare perivascular lymphocytes (Fig. 2B).

Cytokine Localization

Intense reactivity for TNF-α was found in morphologically identified tumor infiltrating macrophages of Cases 1, 2, 10, 13, and 14 (Fig. 3I to K).§ Seven other cases showed occasional macrophages positive for TNF (Cases 5, 6, 8, 9, 11, 12, and 15), while rare immunoreactive astrocyte processes were found in Case 3 (re-...
results not shown). In 10 cases, very weak diffuse reactivity of tumor cells was present (Fig. 3L).

Intense IL-6 immunoreactivity was present in a subset of morphologically identified macrophages (Fig. 3M to O), infiltrating seven of the tumors (Table 1). In all 15 cases, the tumor cells showed weak diffuse staining for IL-6 (Fig. 3P).

In 11 cases prominent diffuse cytoplasmic reactivity of the tumor cells, and in many cases macrophages, for transforming growth factor (TGF-β) was present (Fig. 3T). Intratumoral variability in intensity and degree of immunoreactivity was common. In contrast, in Cases 1 and 2, only weak, diffuse, predominantly perinuclear staining was identified (Fig. 3Q to S). In Case 10, a specimen from a large open biopsy, immunoreactivity for TGF-β varied from region to region; it was most prominent in those areas of the tumor in which TNF-α and IL-6 reactivity was minimal.*

The control brain tissues were nonreactive for IL-6 and TNF-α. No definite positive staining was identified for IL-1, IL-4, or interferon-γ (IFN-γ) either within the tumors or in the normal control brain samples (results not shown).† Reactivity to TGF-β was limited to weak, focal perinuclear staining.

Discussion

Leukocyte Infiltration

We have shown that there is an active immune environment within primary malignant glial tumors. These tumors are infiltrated by T lymphocytes and activated macrophages, and cytokines are present at the site of the infiltrating macrophages and associated with the tumor cells themselves. We did not find significant differences, with respect to these features, between high-grade anaplastic astrocytomas and glioblastoma multiforme specimens. Leukocyte infiltration into malignant gliomas has been extensively studied over the last 20 years. Immunohistochemical studies showed that many of the infiltrating cells are T cells with a predominance of CD8-positive T-suppressor/cytotoxic cells. In the present study, all 15 cases showed some degree of leukocyte infiltration. Although the degree of infiltration was small, the predominant phenotype of the lymphocytes was CD8-positive. As expected, only rare B cells were identified. The effect of this infiltration on tumor cell growth is unknown. Although CD4 cells were present in all of the tumors, their cytokine products (IL-2, IL-4, and IFN-γ) were either absent or only rarely identified. This suggests either that cytokines were produced only very early in the process of infiltration or that the secretion of these factors is down-regulated possibly by immunoregulatory cells, CD8, or other cytokines such as TGF-β.

Functional studies have shown that malignant gliomas contain a population of lymphocytes with potential cytolytic activity against the tumor; however, this response is weaker than that obtained from peripheral blood lymphocytes from the same patients. The CD8 cells in the tumors of the present study were not spatially associated with areas of necrosis, indicating a lack of obvious cytotoxicity. These features suggest that in glioblastomas multiforme, as well as in malignant glial tumors, there is an immune-suppressed environment.

Macrophage infiltration into malignant gliomas has previously been studied immunocytochemically; however, little attention has been paid to the significance of this infiltration. Shionaga, et al. studied a variety of brain tumors and found that glioblastoma multiforme showed moderate or marked macrophage infiltration. In a semiquantitative study by Hitchcock and Morris, 38.3% of the infiltrating cells in malignant gliomas were found to be macrophages. We have shown that there was heavy macrophage infiltration in 11 of 15 tumors; in all cases, macrophages made up at least 80% of the infiltrating leukocyte population. Many of these macrophages are activated, as shown by the presence of HLA-DR and IL-2 receptor, and in seven of the tumors, they are a major site of TNF-α and/or IL-6 immunoreactivity.

Class II Antigen Expression

Class II antigen expression, usually by macrophages, is necessary for the presentation of processed antigen to the T lymphocyte. In normal brain, there is minimal production of major histocompatibility complex cell-surface antigens, which is usually limited solely to perivascular macrophages/microglia. Primary astrocyte cultures may also express class II antigens when exposed to cytokines, virus, or bacterial preparations. Glial cell lines can also express class II antigens, and this expression can be augmented by IFN-γ. Although astrocytes, endothelial cells, and microglial cells can all express class II antigens in vitro, their functional role in vivo antigen presentation is questionable. In this study, intense class II antigen positivity of macrophages was found in all cases. Many of the positive macrophages were in a perivascular location and contrasted strikingly with the negative endothelium, suggesting that in these tumors the macrophage may be the predominant site of antigen presentation. The tumor cells also showed diffuse reactivity for HLA-DR; although this was much less intense than that found in macrophages, it suggests that the tumor cell could have a functional role in T cell recognition.

Previous Reports of Cytokines in CNS Tumors

Little is known about the presence and localization of cytokines in primary human tumors of the CNS;
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most of what is known has been gleaned from studies of cell lines. Glioma cell lines have been shown to release several cytokines including an IL-1-like factor.\(^2\) All 20 glioblastoma multiforme cell lines studied by Van Meir, et al.,\(^4\) were shown to secrete IL-6 either constitutively or upon stimulation; five of six cell lines tested also expressed IL-6 mRNA. Tumor necrosis factor-\(\alpha\) was also secreted by a malignant glioma cell line (D54-MG) after stimulation.\(^4\) This cell line possesses in addition both high- and low-affinity TNF receptors which can be modulated with IFN-\(\gamma\) treatment.\(^4\)

The related literature to cell lines suggests that cytokines are likely to be found in primary glioblastoma multiforme. Van Meir, et al.,\(^4\) found that IL-6 activity was detectable in cerebrospinal fluid and tumor cyst fluid of patients with glioblastoma multiforme. Their immunohistochemical studies revealed diffuse IL-6 positivity in GFAP tumor cells in 15 of 20 sections. Tumor necrosis factor-\(\alpha\) has not previously been identified in primary glioblastoma multiforme.

**Localization of IL-6 and TNF-\(\alpha\)**

In this study, we found diffuse, weak IL-6 and TNF-\(\alpha\) staining of tumor cells in 10 of 15 cases, with the remaining five cases being positive for IL-6 only. In contrast to the study of Van Meir, et al.,\(^4\) we found that by far the most intense positivity for these cytokines was in infiltrating macrophages. It is of interest that thymomas were recently reported to contain TNF-\(\alpha\)-positive macrophages;\(^3\) the tumor cells themselves were negative. The localization of these cytokines in macrophages is not unexpected, since macrophages are numerous in these tumors, are activated, and are known to be major producers of these cytokines in the brain's immune response.\(^1\) Recently, Barna, et al.,\(^2\) found that monocytes harvested from the blood of patients with malignant gliomas retain tumoricidal activity and can secrete TNF-\(\alpha\).

The specific role of these cytokines in the in vivo environment is yet to be determined. Interleukin-6 acts to stimulate the hepatic acute-phase response, enhances natural killer-cell and cytotoxic T-cell activity, and induces the growth and differentiation of B cells; however, B cells were exceedingly rare in these tumors, suggesting that the latter mechanism is unlikely. The presence of weak IL-6 immunoreactivity in the tumor cells suggests that this cytokine may have another function, which may be tumor-promoting at some stage as has been described for renal-cell carcinoma.\(^1,2\) The possible conflicting response to IL-6 production by both infiltrating macrophages and tumor cells is yet to be resolved.

Tumor necrosis factor-\(\alpha\) acts in numerous ways, including stimulation of major histocompatibility complex class I antigens\(^4\) on endothelium, fibroblasts, and astrocytes, induction of IL-1 secretion by mononuclear cells, and tumor cytotoxicity. The effects of TNF-\(\alpha\) on glioma cell lines have been variable; some lines show a proliferative effect while others are inhibited. Glioma cell lines have been shown to have both high- and low-affinity receptors for TNF-\(\alpha\), which can be modulated by IFN-\(\gamma\).\(^4\) Despite the finding that all tumors had activated macrophages, only a subset had TNF-\(\alpha\) positive macrophages, suggesting the possibility that this heterogeneity may be related to the individual tumor's susceptibility to TNF-\(\alpha\) action. Studies correlating the presence of TNF-\(\alpha\)-positive macrophages in situ with the proliferative/inhibitory response of primary cultures to TNF-\(\alpha\) are under way to answer this question. Tumor necrosis factor-\(\alpha\) may also act on endothelial cells in vivo to promote angiogenesis\(^2\) and thus may help to explain the prominent endothelial proliferation found in these tumors. It is of interest that the tumor in Case 1 showed marked endothelial proliferation (Fig. 1) and also was infiltrated by numerous TNF-\(\alpha\)-positive macrophages.

**Transforming Growth Factor-\(\beta\)**

Transforming growth factor-\(\beta\) was originally derived from glioblastoma multiforme cells. Both TGF-\(\beta\) and TGF-\(\beta\) mRNA have been identified in glioblastoma multiforme; however, studies of glioblastoma multiforme cell lines have shown that only TGF-\(\beta\) is secreted by these cells.\(^3\) Our immunocytochemical studies showed that TGF-\(\beta\) was present to some extent in the tumor cells of 14 of 15 cases; however, reactivity was prominent in 12 tumors. Regional differences in immunoreactivity were quite prominent in some tumors, suggesting local microenvironmental regulation. The tumors that are most positive for TGF-\(\beta\) appear to be the ones with the fewest TNF-\(\alpha\)-infiltrating macrophages, thus suggesting the possibility that TGF-\(\beta\) may have an inhibitory or down-regulatory effect on this cytokine, either directly or indirectly. In vitro studies are necessary to determine whether this is true; however, recent reports substantiate the immunosuppressive activities of TGF-\(\beta\), including inhibition of proliferation in both major histocompatibility complex restricted T cells and IL-2-dependent T-cell lines, and down-regulation of HLA-DR expression in malignant glioma cell lines.\(^2,4\) Large biopsy samples (Case 8, 9, and 12) showed heterogeneity in the expression of TNF-\(\alpha\) and TGF-\(\beta\). Foci with prominent tumor-cell TGF-\(\beta\) expression exhibited rare TNF-\(\alpha\)/IL-6 macrophages, while areas with prominent TNF-\(\alpha\)/IL-6 macrophages showed weak reactivity for TGF-\(\beta\). Transforming growth factor-\(\beta\) may also have proliferative activities both on glioma cells in culture and tumor endothelial cells, possibly resulting in tumor cell growth and the endothelial proliferation characteristic of this tumor.\(^3\) The finding of increased TGF-\(\beta\) immunoreactivity in those tumors with absent or few TNF-\(\alpha\) and IL-6-infiltrating macrophages is intriguing, since TGF-\(\beta\) can inhibit the induction of IL-6 by either IL-1 or TNF-\(\alpha\).

**Cytokine Interactions**

Cytokine interactions within malignant glial tumors are complex and heterogeneous. Cytokines may have
both stimulatory or inhibitory effects on the tumor and may be produced primarily by either the tumor cells, infiltrating macrophages, or adjacent reactive glial cells; in our study, macrophages appeared to be the predominant source of IL-6 and TNF-α. The lack of immunoreactivity for IL-4 and IFN-γ and only very occasional reactivity for IL-2 suggested that CD4 cells were not producing cytokines at this time, perhaps due to CD8 inhibition or the immunosuppressive action of IL-6 or TGF-β.

The apparent reciprocal relationship between the presence of IL-6 and TNF-α in infiltrating macrophages and TGF-β in tumor cells needs to be further explored in order to understand more completely local immune response within this apparently heterogeneous group of tumors. This knowledge may allow for more specific and individualized immunotherapy for patients with malignant glial neoplasms.22,27

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