Journal of Neuropathology and Experimental Neurology

Vol. 57, No. 7
July, 1998
pp. 653–663

Expression of Interleukin-1β mRNA and Protein in Human Gliomas Assessed by RT-PCR and Immunohistochemistry

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Abstract. To characterize the expression and localization of interleukin (IL)-1β in human gliomas, both reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry were used on surgically excised human gliomas, human malignant glioma xenografts, and human glioblastoma cell lines. The RT-PCR products for IL-1β mRNA were quantified by computerized image analysis. IL-1β mRNA was detectable in 30 out of 35 (86%) surgically resected gliomas. An abundant expression of IL-1β mRNA was often found in the glioblastomas, anaplastic astrocytomas, and pilocytic astrocytomas, but not in other types of gliomas. Quantitatively, in both the grade 2 astrocytomas and the oligodendrogliomas, the IL-1β mRNA levels were significantly ($p < 0.05$) lower than those of the grade 3/4 astrocytomas. Immunohistochemically, IL-1β was localized in the pleomorphic tumor cells of the astrocytic tumors and in macrophages. In contrast to the astrocytic tumors, low and high grade oligodendrogliomas showed no or little expression of IL-1β antigen. IL-1β was present less frequently than IL-1α and IL-1 receptor type 1 in 4 malignant gliomas transplanted into nude mice by RT-PCR. All 2 cell lines showed IL-1β expression at both the mRNA and protein levels. It is concluded that in human gliomas, both high-grade astrocytomas and pilocytic astrocytomas often express high IL-1β production, and that IL-1β is mainly localized in astrocytic tumor cells and macrophages.

Key Words: Cytokine; Glioma; Interleukin-1; Macrophage; Microglia; Nude mice; Polymerase chain reaction.

INTRODUCTION

Interleukin-1 (IL-1) plays a key role in inflammatory and immune responses, and has a number of biologic activities, including prominent effects on the central nervous system (CNS) (1–4). Two forms of IL-1 have been isolated: IL-1α and IL-1β. IL-1β is the predominant form of IL-1 released by human monocytes/macrophages (5). Varying numbers of macrophages (which are derived from blood monocytes or intrinsic microglia) are observed in glioma (particularly glioblastoma) tissues (6–10). Since the morphological distinction between macrophages and activated microglia is not clear in a tumor environment, macrophages were used as a generic form for cells showing a macrophage-specific marker in the tumor tissue in the present study, and microglia were used as a generic form for cells with rod nuclei and dendritic cell processes within non-neoplastic brain tissue.

IL-1β is synthesized as an inactive 31 kDa precursor (pro-IL-1β), and the 17 kDa active IL-1β results from cleavage of the pro-IL-1β by IL-1β-converting enzyme (ICE), which plays a pivotal role in programmed cell death (11–13). Recently, ICE-like immunoreactivity was localized selectively in microglia in the hippocampus after ischemia (14). Immunoreactivity for IL-1β has been demonstrated in microglia and astrocytes in the CNS (15, 16). It remains to be determined which cells initially produce IL-1β in glioma tissues.

Several cytokines are reported to be expressed during CNS neoplasia (17). IL-1 is likely to be one of the most important cytokines in gliomas, since IL-1 can directly stimulate tumor cell proliferation (18, 19) and induce the expression of many other cytokines (19–21). IL-1β mRNA and protein expression were detected in gliomas in vitro and in vivo (18, 22–25). However, the number of in vivo studies of gliomas other than high-grade astrocytomas is limited. The type of glioma that initially produces IL-1β is not known, nor is the mechanism underlying the involvement of IL-1β in gliomas.

In this study, to clarify the correlation between IL-1β expression and histological types of gliomas, and to determine the main cell types that express IL-1β, surgically resected gliomas of various histologic types, malignant gliomas transplanted into athymic nude mice, and 2 human glioblastoma cell lines were investigated using reverse-transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry. IL-1β mRNA was also quantified by RT-PCR and computerized image analysis.

MATERIALS AND METHODS

Glioma Cell Line

The two human glioblastoma cell lines (TATE-87 and CGNH-89) used in this study were maintained in Eagle's minimum essential medium (Nissui, Tokyo) supplemented with 10% fetal calf serum (Gibco, Grand Island, NY) and 5% L-glutamine. TATE-87 and CGNH-89 were originally derived from explants of human glioblastomas of a 51-year-old male patient and a 56-year-old female patient, respectively. Both glioblastoma cell lines were positive for glial fibrillary acidic protein (GFAP), vimentin, A2B5, O4, and myelin basic protein.

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TABLE 1
Sequence and Characterization of the Oligonucleotide Primers used in the RT-PCR

Primer	Sequence (5′ - 3′)	Length	Expected PCR product
IL-1α			491 bp
sense	CAAGGAGAGCATGGTGGTAGTAGCAACCAACG	32	
antisense	TAGTGCCGTGAGTTTCCCAGAAGAAGAGGAGG	32	
IL-1β			802 bp
sense	ATGGCAGAAGTACCTAAGCTCGC	23	
antisense	ACACAAATTGCATGGTGAAGTCAGTT	26	
IL-1R			300 bp
sense	ACACATGGTATAGATGCAGC	20	
antisense	TTCCAAGACCTCAGGCAAGA	20	
GAPDH			452 bp
sense	ACCACAGTCCATGCCATCAC	20	
antisense	TCCACCACCCTGTTGCTGTA	20	

Transplanted Gliomas

Surgical biopsies from 4 human brain tumors (3 glioblastomas and 1 ependymoblastoma) were transplanted into athymic nude mice subcutaneously. The transplanted tumors developed into long-term serial lines, designated as KYG, TYG, and YAG for the glioblastomas, and NNE for the ependymoblastoma (26, 27). All of the tumors serially transplanted into mice showed unchanged histologic characteristics of the original tumors, and were shown immunohistochemically to contain various numbers of GFAP-positive tumor cells. In this study, tumors at passages 31–46 were used.

Primary Gliomas

For RT-PCR, fresh tumor tissues were obtained from 35 patients undergoing craniotomy for gliomas (cases 1–35). Twenty-nine of the tumor tissues were embedded in OCT compound and immediately frozen in liquid nitrogen. After the cryostat sections were cut for histological diagnosis, the frozen fragments were stored at −70°C until use. In 6 patients, part of the tumor tissues was immediately frozen in liquid nitrogen and stored in an airtight plastic tube at −70°C until use. For immunohistochemistry, we investigated 55 surgical biopsy specimens from gliomas (cases 1–55) and 5 autopsy samples from the brains of patients with gliomas (cases 56–60). The tissues were fixed in buffered formalin and embedded in paraffin. The neoplasms were classified according to the World Health Organization (WHO) classification.

RT-PCR

Total cellular RNA was obtained from OCT-embedded, frozen tissues (10–15 cryostat sections, 10 microns thick each), fresh frozen tissues (0.1 to 0.5 gm) and cultured cells (1×10^7 or 5×10^6 cells). We used a guanidinium thiocyanate-phenol-chloroform method using an RNA isolation kit (Nippon Gene, Tokyo) according to the manufacturer's instructions. The extracted RNA was finally resuspended in DEPC-treated (Sigma Chemicals, St. Louis, Mo) water, and quantified spectrophotometrically (OD_{260}/OD_{280}).

The RT and PCR were carried out in a Perkin-Elmer Cetus Gene Amp PCR System 2400 using the GeneAmp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, Conn). The RT was performed in a 20 μl reaction containing total RNA (0.5 or 1 μg of RNA), 5 mM $MgCl_2$, 1 X PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 1 mM each dNTP, 20 units of Rnase inhibitor, 2.5 μM random hexamers, and 50 units of reverse transcriptase. The RT mixture was incubated at room temperature for 10 min, 42°C for 15 min, 99°C for 5 min, and 5°C for 5 min. The resulting cDNA solution was amplified with one of the cytokine-specific primer pairs IL-1α, IL-1β, and IL-1 type 1 receptor (IL-1R) and GAPDH (Clontech Laboratories, Palo Alto, Calif). The oligonucleotide primers are depicted in Table 1. The cDNA solutions were mixed in a final volume of 100 μl to obtain the final concentration of 2 mM $MgCl_2$, 1 X PCR buffer, 200 μM each dNTP, 2.5 units of AmpliTaq DNA polymerase, and 0.2 μM Clontech primers. The PCR reaction was performed under the following conditions: initial melting at 95°C for 105 seconds, 30 or 35 cycles (95°C for 30 seconds; 60°C for 45 seconds; 72°C for 2 min), final elongation at 72°C for 7 min, and cooling to 4°C. The positive DNA control for each cytokine and GAPDH was also amplified and run in parallel in the agarose gel.

The reaction products (10 μl) were applied to a 2% agarose gel with a DNA ladder marker and stained with ethidium bromide (EtBr, 1 μg/ml). Bands were visualized under ultraviolet illumination, and the fluorescence intensity was captured with a camera using Polaroid type 667 coated black and white instant films. The intensity of the ethidium-bromide fluorescence of each band for IL-1β and GAPDH was measured by a charge-coupled device (CCD) imaging system (Densitograph AE-6900-F; Atto Corp., Tokyo). For the quantitative study of IL-1β mRNA, the intensity of the EtBr fluorescence of each band was measured by the CCD imaging system. To normalize the conditions of gel staining and CCD imaging, a constant amount of control DNA solution (IL-1β cDNA, GAPDH cDNA) was electrophoresed in one lane every time. For 20–35 cycles, the PCR products did not reach a plateau. The relative amount of mRNA was calculated by correcting the fluorescence of the control DNA to 1. The ratio was calculated by dividing the value of IL-1β by that of the internal control, GAPDH. The statistical comparison of the level of IL-1β mRNA was done with a nonparametric Kruskal-Wallis analysis followed by Fish-

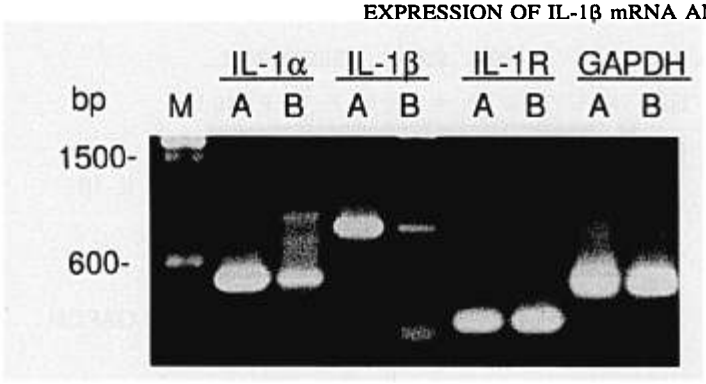


Fig. 1. Transcription of the IL-1α, IL-1β, and IL-1R genes in glioblastoma cell lines. The 2 glioblastoma cell lines (A: TATE-87, B: CGNH-89) were tested by RT-PCR with primers for IL-1α, IL-1β, IL-1R, and GAPDH. The number of PCR cycles was 35. M, size marker; bp, base pair.

er's protected least square difference procedure. Correlations with $p < 0.05$ were considered significant.

Immunohistochemistry

In the glioma tissues, paraffin sections were cut 3- to 4-μm thick, and the frozen sections were cut 6- to 8-μm thick. Both types of sections were mounted on silane-coated glass slides. The mounted frozen sections were fixed in periodate-lysine-paraformaldehyde (PLP) for 30 minutes (min) at 4°C. The specific characteristics of each of the primary reagents used in this study were as follows: rabbit anti-human IL-1α polyclonal antibody (PAb) (1:100, Genzyme, Cambridge, Mass), rabbit anti-human IL-1β PAb (1:200, Genzyme), mouse anti-human IL-1β monoclonal antibody (MAb) (1:100, Genzyme), Ki-M1P MAb (CD 68; 1:100, Seikagaku Corp, Tokyo), and rabbit anti-GFAP PAb (1:1000, Nakazato) (28). For the detection of IL-1α and IL-1β, the paraffin-embedded tissue sections were pretreated in an autoclave (Tomy, Tokyo) for 5 min at 121°C in phosphate buffer. Immunostaining was performed using a Vectastain ABC elite kit (Vector, Burlingame, Calif) or the biotin-streptavidine immunoperoxidase method (Nichirei Corp., Tokyo), as described previously (29). The immunoreaction was developed in a solution of diaminobenzidine, and then briefly counterstained with hematoxylin. Specimens were considered positive for IL-1α or IL-1β when the cytoplasm or cell membrane was unequivocally stained.

In paraffin sections of 3 glioma cases (cases 7, 9, 19), we used double-label indirect immunofluorescence with combinations of antibodies against IL-1β/GFAP and IL-1β/Ki-M1P. The staining procedures were performed essentially as described above until the incubation of the primary antibodies, and were then performed as follows: (a) incubation (overnight) with IL-1β–specific PAb (1:100) plus GFAP-specific MAb (1:4, IBL, Gunma, Japan) or Ki-M1P (1:100); (b) incubation (2 hours) with FITC-labeled anti-rabbit IgG (1:20) plus TRITC-labeled anti-mouse IgG (1:30). Both secondary antibodies were purchased from Jackson Immunoresearch Laboratories, Inc. (West Grove, Pa). After incubations with the antibodies, the glass slides were covered with phosphate-buffered saline/glycerol, overlaid with glass coverslips, and sealed with fingernail polish.

For the glioma cell lines, cells grown on coverslips were fixed in 4% paraformaldehyde for 30 min at room temperature, and stained by indirect immunofluorescence with IL-1α or IL-1β antibodies essentially described as above.

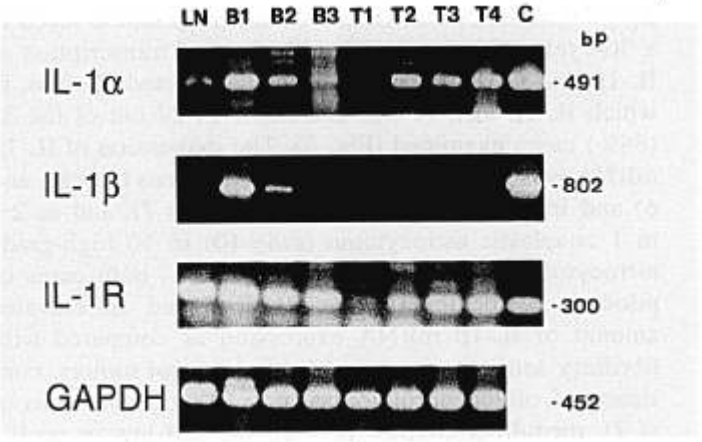


Fig. 2. RT-PCR analysis of the transcription of the IL-1α, IL-1β, and IL-1R genes in biopsied (B1-3) human gliomas (cases 33–35) and subcutaneously transplanted (T1–4) malignant gliomas of nude mice. One μg of total RNA was reverse-transcribed into cDNA, and assayed by 35 cycles of PCR with each primer. LN, human lymph nodes; C, positive DNA control; B1, glioblastoma; B2, glioblastoma; B3, anaplastic astrocytoma; T1, KYG (glioblastoma); T2, TYG (glioblastoma); T3, NNE (ependymoblastoma); T4, YAG (glioblastoma).

RESULTS

Glioblastoma Cell Lines

Under optimal PCR conditions (35 cycles), both of the glioblastoma cell lines (TATE-87, CGNH-89) expressed mRNA for IL-1β, as well as for IL-1α and IL-1R (Fig. 1). To determine whether these glioblastoma cells produce IL-1, immunofluorescent staining was performed with anti-GFAP, anti–IL-1α, and anti–IL-1β Abs (Fig. 6A–C). Intense immunofluorescence was observed in the cytoplasm of the glioblastoma cells, providing evidence of IL-1α and IL-1β production by glioblastoma cells in vitro.

Human Malignant Glioma Xenografts

Of the 3 glioblastomas (KYG, TYG, YAG) transplanted into nude mice, IL-1β was positive in 1 (YAG), IL-1α in 2 (TYG,YAG), and IL-1R in 2 (TYG, YAG) by RT-PCR. In the transplanted ependymoblastoma (NNE), IL1-β mRNA was absent, while both IL-1α and IL-1R mRNA were present (Fig. 2).

Primary Human Gliomas

All 3 high-grade astrocytomas (cases 33–35) expressed mRNA for IL-1β, IL-1α, and IL-1R after 35 cycles of RT-PCR (Fig. 2). The bands for the IL-1α and IL-1R transcripts were consistently strong, while the band for IL-1β mRNA showed varied in intensities from moderate to strong. For the subsequent comparison of the expressions of IL-1β mRNA in the primary human gliomas, 0.5

μg of total RNA was reverse-transcribed and assayed in a 30-cycle PCR for the identification of transcription of IL-1β and GAPDH in 32 surgically resected gliomas, in which IL-1β mRNA was detectable in 27 out of the 32 (84%) cases examined (Fig. 3). The expression of IL-1β mRNA was graded as 3+ in 2 glioblastomas (cases 1 and 6) and in 1 anaplastic astrocytoma (case 7), and as 2+ in 1 anaplastic astrocytoma (case 10) in 10 high-grade astrocytomas. In low-grade astrocytomas, both cases of pilocytic astrocytoma (n = 2) displayed an elevated amount of IL-1β mRNA expression as compared with fibrillary astrocytomas (n = 8). A group of tumors, consisting of oligodendrogliomas (n = 7), ependymomas (n = 2), medulloblastomas (n = 2), showed low or no IL-1β transcription. Quantitatively, the IL-1β mRNA levels in the grade 3 and 4 astrocytomas were significantly higher than those in the grade 2 astrocytomas and oligodendrogliomas ($p < 0.05$) (Fig. 4).

To determine the localization of IL-1β protein in the glioma tissues, immunohistochemical staining was performed with monoclonal and polyclonal Abs against IL-1β. In both the frozen and permanent sections, the polyclonal Ab was more sensitive than the monoclonal Ab of the 2 anti–IL-1β Abs. The frozen sections and paraffin sections showed the same results, and the polyclonal Ab was used on all of the specimens. Immunostaining using polyclonal rabbit antiserum to IL-1β showed a positive reaction in 90% of the astrocytic tumors (19 of 21 biopsied cases), in which IL-1β–positive cells were morphologically identified as macrophages and astrocytoma cells. Three of 10 high-grade astrocytomas (cases 3, 5, 7) were graded as 2+ or 3+ for IL-1β in neoplastic cells, while all other cases of astrocytic tumors showed no or faint staining in the neoplastic astrocytes. In these three cases, the cytoplasm and the plasma membrane of large tumor cells were intensely stained with anti–IL-1β (Fig. 5A, B), whereas small anaplastic cells were nonreactive (Fig. 5C). In an anaplastic astrocytoma (case 36), IL-1β–positive macrophages were seen in the tumor areas with lymphocytic infiltrates (Fig. 5D). In an autopsied case of the glioblastoma (case 56), phagocytic macrophages seen in necrotic areas showed a strong expression of IL-1β (Fig. 5E). The immunohistochemical analysis of the pilocytic astrocytoma showed that IL-1β-positive cells were scattered (Fig. 5F, case 60). Of the oligodendrogliomas, the IL-1β immunostaining showed little or no expression in the neoplastic oligodendroglia (Fig. 5G, case 25). The endothelial cells of blood vessels, including hyperplastic vessels, occasionally showed weak immunostaining. At the border zone or in the surrounding brain, IL-1β immunoreactivity was often localized in activated microglia (Fig. 5H, case 56), but rarely in reactive astrocytes or cortical neurons.

To more precisely identify the cell type of the IL-1β-positive cells, we used double-label indirect immunoflu-

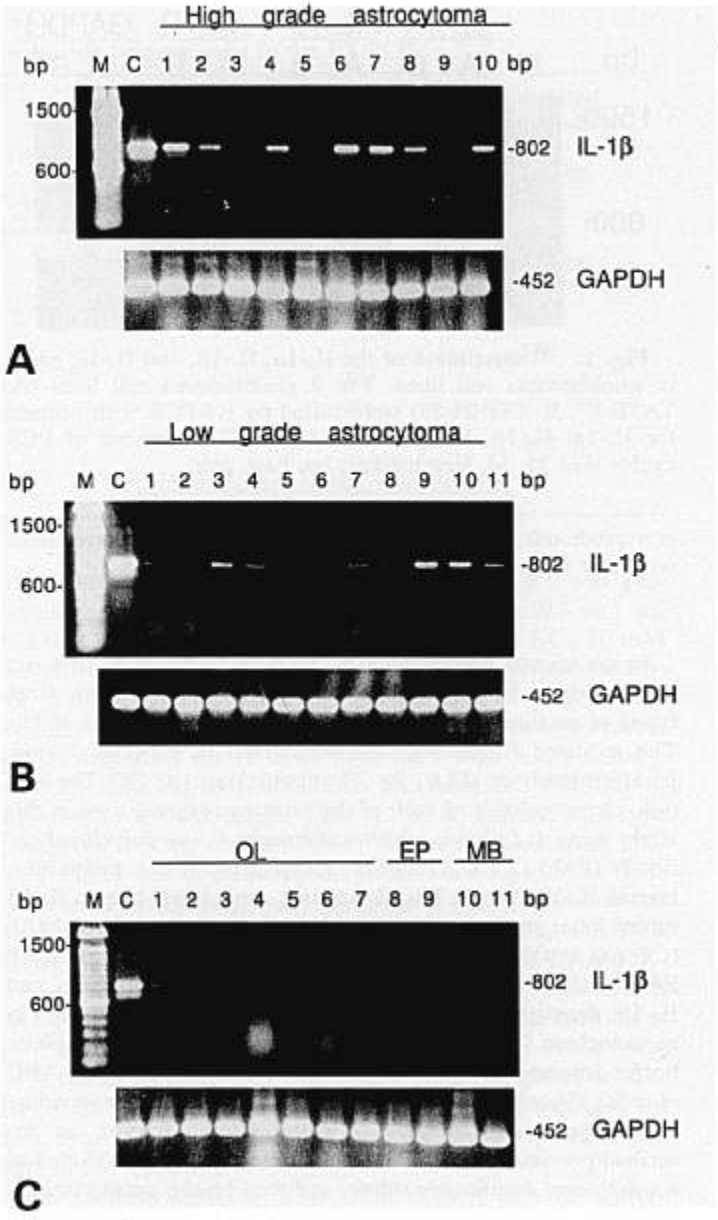


Fig. 3. IL-1β mRNA expression determined by RT-PCR in high-grade astrocytomas (A), low-grade astrocytomas (B), and other gliomas (C). Here, 0.5 μg of total RNA was reverse-transcribed and assayed in a 30-cycle PCR to identify the transcription of IL-1β and GAPDH. A: The high-grade astrocytomas are shown in lanes 1–6 (glioblastomas, cases 1–6) and lanes 7–10 (anaplastic astrocytomas, cases 7–10). B: The low-grade astrocytomas are shown in lanes 1–8 (astrocytomas, cases 11–18), lane 9 and 10 (pilocytic astrocytomas, cases 19 and 20), and lane 11 (pleomorphic xanthoastrocytoma, case 21). C: OL, oligodendroglioma (lanes 1–7, cases 22–28); EP, ependymoma (lanes 8 and 9, cases 29 and 30); MB, medulloblastoma (lanes 10 and 11, cases 31 and 32). M, size marker; C, positive control cDNA fragments.

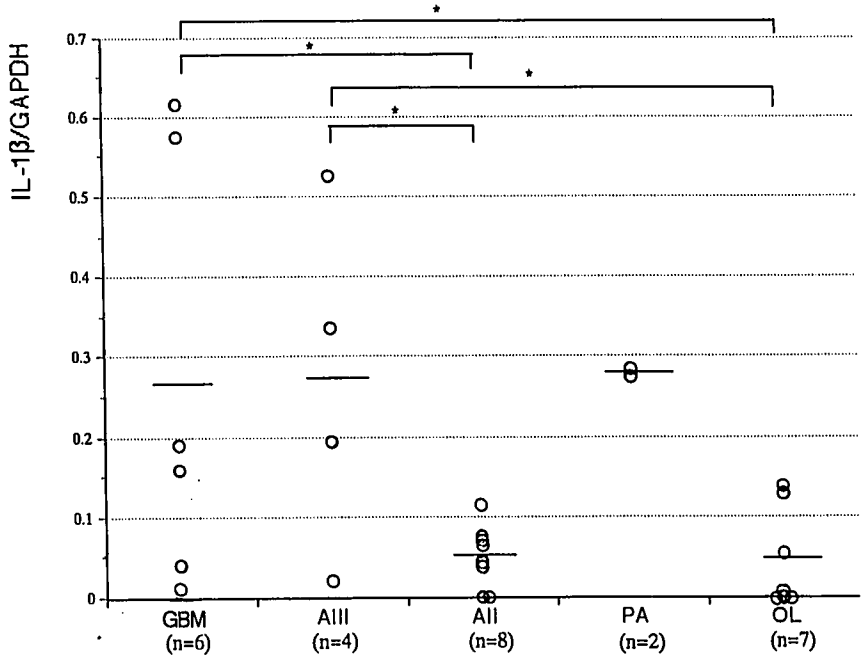


Fig. 4. Quantitation of RT-PCR for IL-1β mRNA in glioma subtypes. Horizontal bars denote mean values. * $p < 0.05$ between the indicated groups. GBM, glioblastoma; AIII, anaplastic astrocytoma; AII, astrocytoma; PA, pilocytic astrocytoma; OL, oligodendroglioma.

orescence with combinations of antibodies against IL-1β/GFAP and IL-1β/Ki-M1P. In a case of anaplastic astrocytoma (case 7) in which an abundant macrophages infiltration was demonstrated with the Ki-M1P staining, the double-label indirect immunofluorescence showed that IL-1β was expressed in numerous neoplastic astrocytes (Fig. 6D, E) and some macrophages (Fig. 6F, G). In a case of glioblastoma (case 9), the IL-1β immunofluorescence of macrophages was higher than that of neoplastic astrocytes. Most of the high-grade astrocytomas contained many tumor-associated macrophages. The relationship between macrophage infiltration and IL-1β expression was not evident. The double immunofluorescent staining of a case of pilocytic astrocytoma (case 19) with the Ki-M1P and anti–IL-1β showed that some microglia/macrophages are intensely positive for IL-1β (Fig. 6H, I).

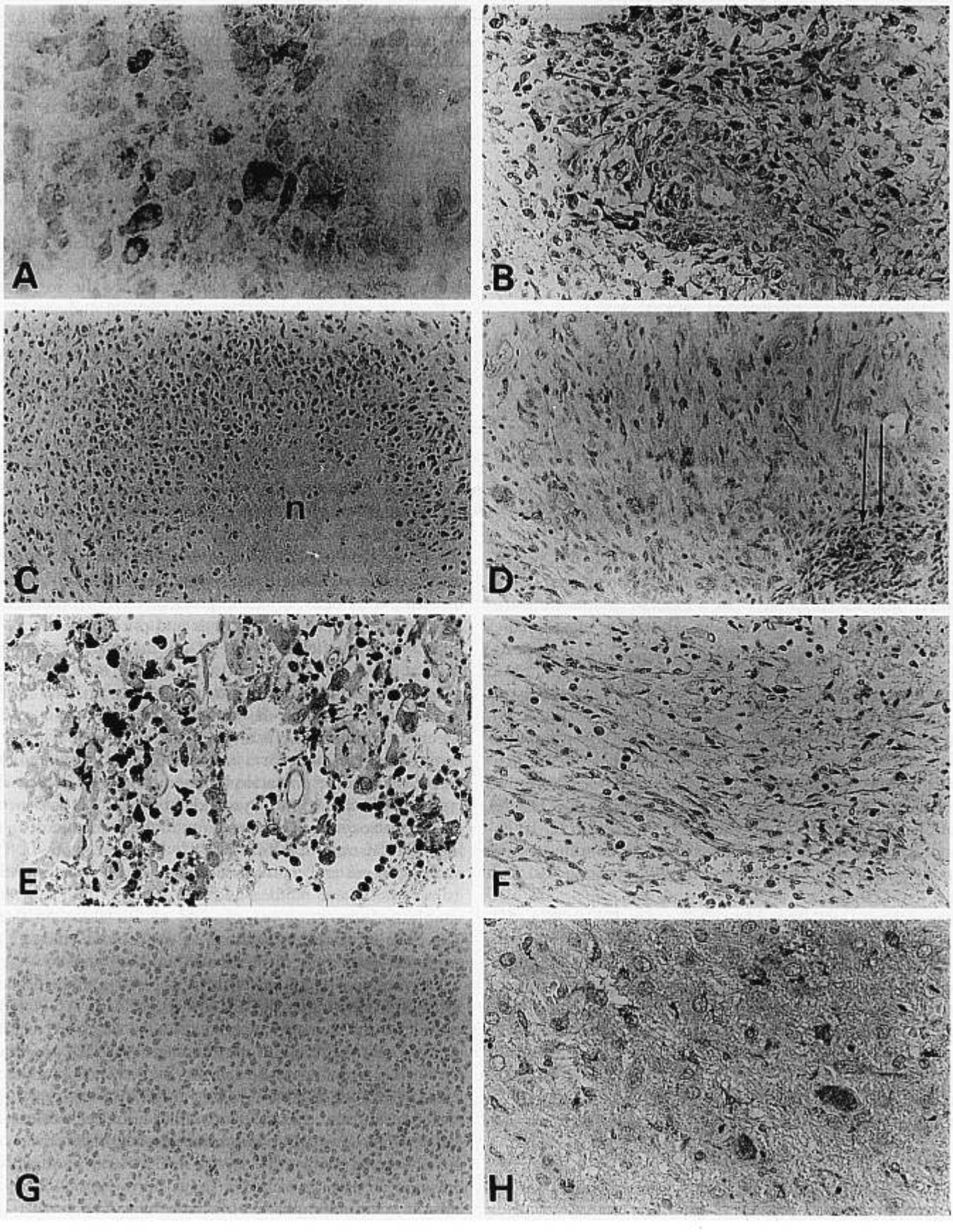
The level of IL-1β mRNA and the IL-1β immunoreactivity of 32 surgical biopsy specimens from gliomas were investigated using the same frozen specimens (Table 2). Most of the astrocytic tumors (19/21) showed IL-1β expression at both the mRNA and protein levels, although the level of IL-1β mRNA demonstrated in quantitative RT-PCR was not always identical to the IL-1β immunohistochemical result.

DISCUSSION

In this study, 4 major findings were obtained: (a) IL-1β production was present in glioblastoma cells both in vitro and in vivo; (b) the initial sources of IL-1β production in the glioma tissues were macrophages and astrocytic tumor cells; (c) the expression of IL-1β was often high in high-grade astrocytomas and pilocytic astrocytomas, but low in other types of gliomas; (d) IL-1β was present less frequently than IL-1α and IL-1R in malignant gliomas transplanted into nude mice at the mRNA level.

The polyclonal anti–IL-1β antibody used in this study specifically binds IL-1β and its precursor, and has been shown to be reliable for use in the immunocytochemical detection of IL-1β in various human tissues, such as skeletal muscle tissue (30), lymphoid tissues (31) and neoplastic astrocytes (24). Our immunohistochemical findings demonstrate that this antibody recognizes pro-IL-1β in the cytosol and membrane-bound IL-1β in neoplastic astrocytes. These studies and our findings indicate that neoplastic astrocytes are capable of IL-1β production. The lack of IL-1β in gliomas in a previous study (23) might be due to the difference of antibody used.

We assayed IL-1β mRNA in biopsied glioma tissues using the quantitative RT-PCR method. This RT-PCR is useful for the quantification of mRNA, since the results obtained by this method are the same as those obtained by Northern blot analysis, and this method is nonradioactive (32, 33). Elevated cytokine mRNA levels typically correlate with protein products, although a lack of translation of IL-1β mRNA in monocytes has been observed previously in vitro (34). IL-1β mRNA and protein expression increase in ischemic tissue during permanent focal (35, 36) and global (14) ischemia. The pro-IL-1β should be proteolytically cleaved into 17.5 kDa mature



IL-1β to be secreted from cells. The finding that the RT-PCR results were not always identical to those of cellular IL-1β immunoreactivity might be related to the metabolism of IL-1β, or to differences in the detection sensitivity of the 2 methods.

The high-grade astrocytomas examined here often showed a high expression of IL-1β, as previously demonstrated (22, 24). The various types of tumors other than astrocytic tumors showed little or no expression of IL-1β at both the mRNA and protein levels. The characteristics of IL-1β expression among the histological types of gliomas might be explained by the previous findings that the IL-1β expression in glial cells of the normal CNS is limited to microglia and astrocytes (15, 16), or that macrophage/microglia are most abundant in high-grade astrocytomas (6). Our results are the first demonstration in gliomas that pilocytic astrocytomas show relatively high IL-1β expression, although further studies with larger samples are needed for the conclusion. The cause and function of this expression remain unclear, but might be related to the pathogenesis of microvascular proliferation. Microvascular proliferation is not uncommon in pilocytic astrocytomas (37). Many cytokines, including IL-1β, are associated with endothelial cell proliferation and the promotion of neovascularization (38–40). There is recent evidence that vascular endothelial growth factor (VEGF) and the VEGF receptor each play an important role in angiogenesis in malignant gliomas (41). We observed that hyperplastic vessels in glioblastomas were surrounded by IL-1β–positive tumor cells. Our results suggest that IL-1β might participate in angiogenesis as a positive regulator in malignant gliomas and pilocytic astrocytomas.

We observed the IL-1β mRNA expression of malignant gliomas subcutaneously transplanted into nude mice. The morphological and phenotypical characteristics of original surgical specimens have been shown to be generally retained in the subcutaneous transplants of mice (26, 27). No study concerning the IL-1 expression of transplanted glioma tissues has been reported to date. It is a well-known fact that stromal components including inflammatory infiltrates are changed into mouse-derived cells in transplanted tumors. Therefore, human microglia/macrophages could be absent in the transplanted tumors. The possible explanation of the present findings that IL-1β mRNA in transplanted tumors was detected in only 1 of 3 glioblastoma cases could be due to the absence of human microglia/macrophages or the decrease of expression by neoplastic cells associated with the transplantation.

The function of IL-1β in glioma tissues remains speculative. However, the results of the present study suggest that IL-1β might be involved in different mechanisms, such as angiogenesis, inflammation/immune reaction, and tumor growth stimulation. Locally, IL-1 facilitates neutrophil and macrophage emigration well by inducing adhesion molecule expression on vascular endothelial cells and the release of chemokines. IL-1 also strongly enhances the proliferation of ramified microglia (42). Mononuclear cell infiltration in brain tumors (especially in gliomas) has been extensively investigated, and in addition to the lymphocyte subpopulation, microglia/macrophage infiltration has recently received attention (6–10). However, the mechanisms of the recruitment of these cells remain unclear. In our study, the principal inflammatory cells were CD68-positive microglia/macrophages, and the largest number of these cells were seen in the high-grade astrocytomas, as shown in previous studies (6, 7). We found the highest level of IL-1β mRNA and protein in high-grade astrocytomas, and we observed IL-1β immunoreactivity in tumor areas close to the lymphocytic infiltration in a case of anaplastic astrocytoma. A recent study (43) proposed that macrophage recruitment is affected by monocyte chemoattractant protein-1 (MCP-1), which is produced by tumor cells and tumor-associated macrophages. Desbaillets et al (44) showed an increased expression of MCP-1 transcripts by glioblastoma cell lines upon stimulation with IL-1β. Thus, IL-1β and MCP-1 may give rise to a positive amplification circuit for macrophage recruitment.

IL-1β was reported to induce proliferation and growth in the glioma cell line U373MG (18, 19). Thus, it is of particular interest to establish whether IL-1β participates in tumor initiation and progression of gliomas in vivo. The results of our study showed a significant increase of IL-1β in 70% of the high-grade astrocytomas at the mRNA level, and in 30% of the high-grade astrocytomas at the protein level, compared with the grade 2 astrocytomas. Thus, we suggest that IL-1β may contribute to tumor progression by autocrine or paracrine loops in a subpopulation of astrocytic tumors.

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Fig. 5. Immunohistochemical staining of IL-1β in biopsied (A-D, G) or autopsied (E, F, H) brains. Numerous neoplastic cells exhibiting pleomorphism were stained with anti–IL-1β antiserum in the frozen sections (A) and paraffin sections (B) of a case of anaplastic astrocytoma (case 7). There was no definite expression of IL-1β in small anaplastic cells around the necrotic areas (n) in a glioblastoma (C, case 3). IL-1β-positive macrophages were seen in the tumor areas with lymphocytic infiltrate (arrows, D, case 36). Phagocytic macrophages were intensely stained with anti–IL-1β monoclonal antibody (E, case 56). IL-1β–positive cells were scattered in a pilocytic astrocytoma (F, case 60), while no IL-1β immunoreactivity was found in an oligodendroglioma (G, case 25). In the surrounding brain in the autopsied glioblastoma (case 56), IL-1β immunoreactivity was localized in microglial cells (H). A, H: ×340; B–G: ×170.

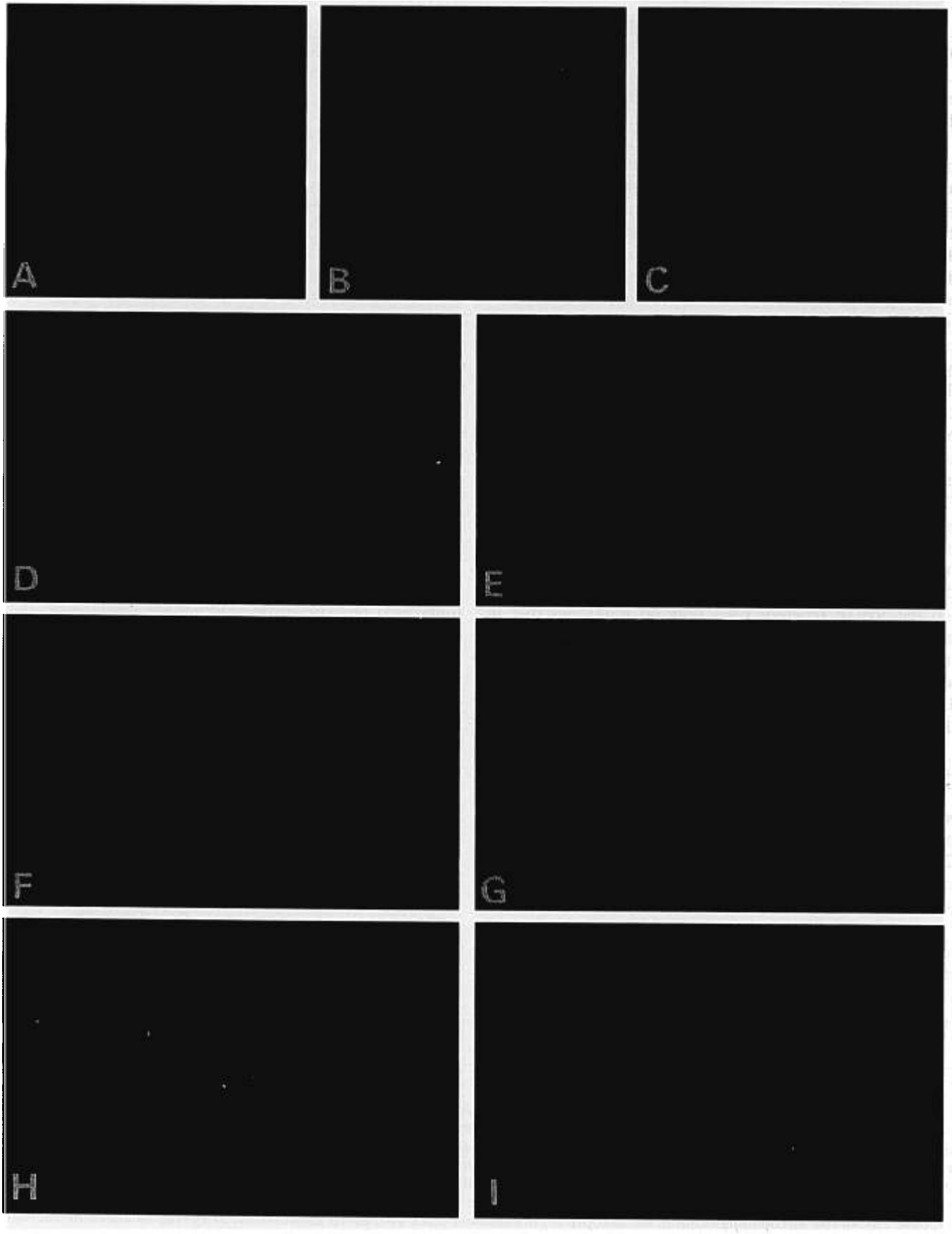

TABLE 2
Summary of IL-1β mRNA Expression and IL-1β Immunoreactivity in Freshly Excised Primary Gliomas

Case	Age	Sex	Location	Diagnosis	IL-1β mRNA[a] expression	IL-1β protein[b] expression
1	49	F	Frontal	GBM	+++	+
2	60	F	Temporal	GBM	+	+
3	51	F	Frontal	GBM	+	++
4	71	F	Parietal	GBM	+	+
5	66	F	Temporal	GBM	+	++
6	67	F	Temporal	GBM	+++	+
7	60	M	Parietal	AIII	+++	+++
8	70	F	Unknown	AIII	+	+
9	51	M	Temporal	AIII	+	+
10	12	F	Frontal	AIII	++	+
11	37	M	Insular	AII	+	+
12	20	M	Frontal	AII	+	+
13	24	M	Frontal	AII	+	+
14	61	F	Spinal	AII	+	+
15	36	M	Frontal	AII	−	−
16	47	M	Frontal	AII	−	−
17	28	M	Frontal	AII	+	+
18	13	F	Thalamus	AII	+	+
19	66	F	Cerebellum	PA	++	+
20	16	F	Frontoparietal	PA	++	+
21	12	F	Temporal	PXA	+	+
22	50	F	Frontal	OII	+	+
23	60	F	Frontal	OII	+	+
24	47	F	Frontal	OIII	−	+
25	41	F	Insular	OIII	+	−
26	55	M	Temporal	OIII	−	−
27	35	F	Temporal	OIII	+	−
28	40	F	Frontal	OIII	+	+
29	15	F	IVth ventricle	EP	+	+
30	1	M	IVth ventricle	EP	+	−
31	9	M	Cerebellum	MB	−	ND
32	19	F	Cerebellum	MB	+	ND

* GBM, glioblastoma; AIII, anaplastic astrocytoma; AII, astrocytoma grade 2; PA, pilocytic astrocytoma; PXA, pleomorphic xanthoastrocytoma; OII, oligodendroglioma; OIII, anaplastic oligodendroglioma; EP, ependymoma; MB, medulloblastoma. [a] IL-1β/GAPDH by RT-PCR: + < 0.25, ++ 0.25–0.5, +++ > 0.5. [b]Number of IL-1β-immunoreactive cells: + < 10%, ++ 10–50%, +++ > 50%. ND, not determined.

IL-1β is mainly produced by activated macrophages, but there is increasing evidence that nearly all nucleated cells are able to produce and release IL-1β (45). Glioma tissues are composed of many different cells, such as tumor cells, vascular cells (endothelium, smooth muscle cells), infiltrating cells (macrophages, lymphocytes), reactive astrocytes, and activated microglia. This study demonstrated that the predominant cells for IL-1β expression are astrocytic tumor cells, macrophages, and activated microglia. Our data confirmed that neoplastic astrocytes produce IL-1β in vitro and in vivo, and we found that astrocytic tumor cells with a large pleomorphic shape are more likely to express a high level of IL-1β. This finding is interesting, since these cells have been shown to express a high level of MCP-1 transcripts (43). The number of IL-1β–reactive, activated macrophages was not large in CD68- (a panmacrophage marker) reactive macrophages in glioma tissues. However, tumor-infiltrating macrophages and the phagocytic macrophages showed a strong expression of IL-1β antigen, and sometimes the

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Fig. 6. Indirect immunofluorescence in a glioblastoma cell line and primary gliomas. The immunofluorescent analysis of the glioblastoma cell line, TATE-87, provided evidence of GFAP (A), IL-1α (B), and IL-1β (C) production by glioblastoma cells in vitro. We use double-label indirect immunofluorescence with combinations of antibodies against IL-1β/GFAP (D, E) and IL-1β/Ki-M1P (F–I). In a glioblastoma (case 7), IL-1β (D, F) was expressed by GFAP (E)-positive neoplastic astrocytes and Ki-M1P (G)-positive macrophages. Double immunofluorescent staining with anti–IL-1β (H) and the Ki-M1P (I) in a pilocytic astrocytoma (case 19) showed some macrophages with IL-1β immunoreactivity. A, D–I: ×180; B, C: ×360.

intensity of the macrophages was stronger than that of neoplastic astrocytes. These data, along with the results of the transplanted gliomas, suggest that IL-1β production by macrophages might be more important than IL-1β production by glioma cells.

IL-1 is likely to be one of the most important cytokines in the proposed cytokine network in glioblastomas (46), since IL-1 can directly stimulate tumor cell proliferation and induce the expression of many other cytokines such as IL-6, IL-8, transforming growth factor (TGF)-β, and tumor necrosis factor. Deficiencies in cellular immune response were recognized early in glioma patients (47), and a considerable body of evidence implicates the TGFβs in this process (48). IL-1β has been shown to reduce the expression of MHC class II antigens in astrocytes, microglia/macrophages, and a glioma cell line (49). Our study indicates that IL-1α and IL-1R mRNA were expressed more frequently than IL-1β in malignant gliomas in vivo. Thus, IL-1 could be a key cytokine, and other secondary cytokines might act in a cascade. The RT-PCR and immunohistochemistry used in this study are useful for investigating the expression of other cytokines using biopsied tissue sections of human gliomas. Further study is necessary to elucidate the role of IL-1 in cytokine cascades in gliomas in vivo.

ACKNOWLEDGMENTS

We are grateful to Dr Tsutomu Uchida for his helpful comments. We also thank Professor Hiroo Hoshino and Dr Atsushi Jinno for use of their imaging facilities, and Professor Haruyasu Yamaguchi for the use of immunofluorescent microscopy. We greatly appreciate the statistical help of Drs Tomoyuki Kawada and Atsushi Saitoh, and the technical assistance of Masako Kohno and Kohji Isoda.

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Received January 23, 1998
Revision received March 31, 1998
Accepted April 3, 1998