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264 Enhanced Interleukin-1β Release and Longevity of Glioma-associated Peripheral Blood Monocytes in Vitro
Experimental Study

AUTHOR(S): Fries, Georg, M.D.; Perneczky, Axel, M.D.; Kempski, Oliver, M.D.

Department of Neurosurgery (GF, AP) and Institute for Neurosurgical Pathophysiology (OK), University of Mainz, Medical School, Mainz, Germany

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ABSTRACT: INTERLEUKIN-1 (IL-1) PLAYS A controversial role in the immune response. Besides its activation of immune cells and juvenile central nervous system cells, monocyte-derived IL-1 may be able to stimulate the malignant transformation and proliferation of glial brain tumor cells expressing IL-1 receptors. The aim of this study was to determine the growth pattern and the IL-1β release of long-term cultured peripheral blood monocytes of glioma patients. At 6- to 7-day intervals, the vital monocytes, characterized by CD14 immunophenotyping, were counted. By the use of a specific IL-1β enzyme-linked immunosorbent assay, the IL-1β content of monocyte culture supernatants derived from 13 subjects with glioma and from 12 controls were compared at Days 7, 21, and 100 of culture. Cell clusters of monocytes derived from glioblastoma patients survived more than 250 days in culture, whereas control monocytes survived only up to 114 days. The IL-1β release of glioma-associated peripheral blood monocyte cultures was about 50 times higher as compared with control monocyte cultures. Dexamethasone treatment at the time of blood sampling and recurrences of the gliomas did not influence the increase in the IL-1β expression of glioma monocytes. It seemed that at least subsets of glioma-associated blood monocytes, although they had been removed from the circulation, remained activated for a long period of time. We conclude that increased IL-1β production of glioma-associated peripheral blood monocytes and their longevity in vitro may be features of aberrant immune cell subsets. In future studies, the exact phenotyping of monocyte subsets will be mandatory.

KEY WORDS: Cytokine; Glioma; Immunosuppression; Interleukin-1β; Macrophage; Monocyte

In physiological and pathological conditions, peripheral blood monocytes are known to leave the cerebral circulation and become brain tissue macrophages, i.e., microglia [8,10,11,13,16,17,20,22]. In addition, there is evidence that microglial cells may enter the circulation and thus become peripheral blood monocytes again [8,13,16].

Monocytes/macrophages play a central role in the glioma-associated immune response because of their capability of tumor antigen incorporation, antigen processing, and antigen presentation to T lymphocytes. After contact with macrophage membrane-bound tumor antigen, T lymphocytes only proliferate and lyse tumor cells if they get activated by interleukin-1 (IL-1). This cytokine exists in two forms, membrane-bound IL-1α and free IL-1β, which is released into the extracellular space [2]. IL-1β activates circulating monocytes and influences their apoptotic cell death [23]. IL-1 and its receptors are produced by cells of both the immune system (monocytes, macrophages, microglia) and the nervous system (neurons, astrocytes) [12]. The receptors for IL-1 (IL-1R) also exist in at least two forms. One form of the IL-1R is present on T cells, fibroblasts, and epithelial cells. An alternative IL-1R has been determined on B cells, neutrophils, monocytes, macrophages, and microglia [18].

Recent studies [3,4,7,11,21,31] have provided strong evidence of IL-1 release and of IL-1R expression not only in immune cells and normal central nervous system cells but also in glioma cells. Thus, IL-1 plays a controversial role in the glioma-associated immune response. On the one hand, this cytokine is necessary to induce the activation and proliferation of immune cells and juvenile central nervous system cells; on the other hand, it is able to stimulate the proliferation of brain tumor cells. For instance, it was reported that IL-1 has mitogenic properties in fibroblasts and astrocytes because these cells possess at least one type of IL-1R [31]. Giulian and Lachman [11] demonstrated that IL-1 stimulated astroglial proliferation after brain injury. Bertoglia et al. [3] found that IL-1 derived from a B cell line promoted the proliferation of an astrocytoma cell line in vitro. Taken together, these studies indicate that glioma cells and tumor-associated monocytes/macrophages may stimulate each other via IL-1β in an autocrine or paracrine manner [23]. The aim of this study was to determine the growth pattern and the IL-1β release of cultured monocytes of patients with primary and recurrent glioblastoma multiforme.

PATIENTS AND METHODS

Composition of the study groups

The peripheral blood monocytes of 14 patients who underwent open or stereotactic surgery for primary glioblastoma multiforme (n = 7) and recurrent glioblastoma (n = 7) were investigated (*Table 1*). The grading of the tumors was based on histological criteria according to the World Health Organization classification of brain tumors [32].

One of seven patients with a primary manifestation of the tumor was on oral dexamethasone (12 mg/d). Six of seven patients with recurrent glioblastoma multiforme were on oral dexamethasone (dosage ranging from 3 to 12 mg/d). Six of seven patients with a primary manifestation of the tumor had not received any dexamethasone at the time of blood sampling.

Blood samples were taken by peripheral venous puncture at 1 to 4 days before surgery. The peripheral blood monocytes of five patients who had suffered subarachnoid hemorrhage, cer-ebral vasospasm, and

subsequent brain infarction as well as monocytes from seven healthy donors formed the control group (*Table 1*).

Separation of monocytes

Twenty-four-milliliter samples of citrated venous blood were mixed gently with the same aliquot of Hanks' Balanced Salt Solution (HBSS) (Sigma, Deisenhofen, Germany). This mixture underwent density gradient centrifugation (room temperature, 15 min, 300*g*) over Ficoll (Biochrom KG, Berlin, Germany). After centrifugation, the buffy coats containing lymphocytes, monocytes, and platelets were collected and washed twice in 10 ml of HBSS (centrifugation for 10 min at 100*g* each) to separate mononuclear cells from contaminating platelets. Cell viability was over 98% as assessed by trypan blue incorporation.

At the same time, differential white blood cell counts were performed. Blood samples were mounted on glass coverslips, air dried, and stained with May-Grünwald and Giemsa stains. The mean values of lymphocyte and monocyte numbers, determined by the counting of at least 600 white blood cells by two different investigators, served to represent the ratio of lymphocytes to monocytes in the peripheral venous blood of each subject. This ratio was used to calculate the number of monocytes seeded into cell culture dishes (35 and 100 mm in diameter; Falcon, Becton Dickinson GmbH, Heidelberg, Germany).

One hundred thousand monocytes per 35-mm dish and 5×10^5 monocytes per 100-mm dish were cultured in RPMI 1640 (Sigma) supplemented with 20 mmol of glutamine, penicillin, streptomycin, and 5% fetal calf serum (endotoxine content, less than 2.5 U/ml; Gibco/BRL Life Technologies, Eggenstein, Germany). Multiple 35-mm dishes were used to compensate for any accidental loss of 100-mm dishes, in which the experiments were carried out. Incubation was at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

After 48 hours, the dishes were washed twice with HBSS to remove nonadherent lymphocytes. The adherent monocytes were used for the in vitro experiments described in this study.

Culture conditions

The monocyte cultures were kept in an incubator (Nunc, Wiesbaden, Germany) under constant conditions (37°C; 5% CO_2) in a humidified atmosphere. At every 7th day, the monocyte cultures were washed twice with HBSS to remove cell debris and nonadherent dead cells. The cell death of the decanted mononuclear cells was proved by trypan blue incorporation. After the washing procedure, each culture received 2 ml (35-mm dishes) or 10 ml (100-mm dishes) of fresh culture medium.

Determination of cell numbers and of cell culture survival time

At 6- to 7-day intervals, the monocyte culture dishes of 14 patients with glioblastoma and of 12 controls were screened for adherent vital cells. The number of surviving monocytes in each culture dish was assessed by the counting of the adherent cells within five randomized optical fields of a phase contrast inversion microscope (Leitz, Wetzlar, Germany) at a magnification ×100.

Each culture was brought to a termination when cell numbers had reduced to fewer than 10 in each of the 5 optical fields. By this time, the culture was defined as avital. Kaplan-Meier curves [19] were calculated from the survival periods of culture dishes derived from 7 patients with primary glioblastoma, 7 patients with recurrent glioblastoma, and 12 controls.

Determination of cell type and Interleukin-1β release

Culture supernatants were obtained before the washing procedure at Days 7 and 21 and, if cells were still vital, at Day 100. After centrifugation (room temperature, 5 min, 400*g*), the material was immediately frozen and stored at -80°C. Randomized samples of culture supernatants of each subject were quantitatively analyzed for soluble CD14 (sCD14), a surface antigen restricted to monocytes and immature macrophages. Analysis was performed with a commercially available specific enzyme-linked immunosorbent assay (ELISA) detecting sCD14 (IBL, Hamburg, Germany).

In addition, the immunophenotype of cultured cells was determined by immunofluorescence staining at randomized intervals. Cells were incubated in the dark with fluorescein isothiocyanate-conjugated mouse antihuman Leu-M3 monoclonal antibodies at saturating amounts at 4°C for 20 minutes and were subsequently washed with ice-cold phosphate-buffered saline. The Leu-M3 monoclonal antibodies (Becton-Dickinson, Heidelberg, Germany) were directed against the monocyte/macrophage phenotype antigen CD14. Microphotographs were taken in a semidark chamber with a fluorescence microscope (Zeiss, Oberkochen, Germany) and Kodak Ektachrome 64 Professional Tungsten films (Eastman Kodak, Rochester, NY). The C6 rat glioma cell line served as a nonmonocyte control cell line.

Additionally, the culture supernatants were analyzed for the cytokine IL-1β at Days 7, 21, and 100. A commercially available IL-1β-specific ELISA (Hermann Biermann GmbH Diagnostica, Bad Nauheim, Germany) was used.

Statistical analysis

The cell numbers in each culture dish were used to calculate the concentration of sCD14 and IL-1β per 10^3 monocytes. Mean values and standard errors of sCD14 and IL-1β release per 10^3 cells each were calculated for the following experimental groups: all glioblastoma, primary glioblastoma, recurrent glioblastoma, glioblastoma without dexamethasone therapy, glioblastoma with dexamethasone therapy, and controls. Differences between the groups were evaluated by use of the Kruskal-Wallis test with multiple comparisons on ranks of several independent samples [30].

Survival curves were drawn by the use of the Kaplan-Meier product-limit method [19]. The log-rank or Mantel-Haenszel test was applied to evaluate

differences between the survival curves.

RESULTS

Survival of cultured monocytes

Survival data for all monocyte cultures are shown in *Figure 1*. All Kaplan-Meier curves of survival probability had an almost sigmoidal shape, with a shift to the right of the curves derived from glioblastoma-associated monocytes, indicating a delay of cell death in the latter. Typically, in those cultures, clusters of macrophages remained viable for long periods, a feature never observed in controls.

The median survival for control cultures was 61 days, and, for glioma-associated cultures, it was 100 and 107 days (primary and recurrent glioma, respectively). By Day 114, all control monocyte cultures had died, whereas 14.3% of monocyte cell cultures derived from glioblastoma patients survived for more than 250 days. This difference was significant ($P < 0.001$, log-rank test). Even at Day 250 of culture, when the culture experiments for this study were terminated, some glioma-associated monocytes depicted protruding growth cones, active migration, and cell-to-cell contacts with neighboring monocytes (*Fig. 2*).

CD14 monocyte surface antigen expression

The CD14 monocyte phenotype marker, as determined by immunostaining and with a specific sCD14 ELISA in the supernatants of cultures, did not change throughout the time of observation both in cultures of glioma patients and in those of control subjects (*Fig. 3*). This demonstrates that cultures derived both from glioma patients and from controls mainly retained cells of the monocyte phenotype. At Day 7, the supernatants of glioblastoma monocyte cultures contained 0.55 ± 0.04 (mean ± standard error) ng of sCD14/10^3 monocytes and those of control cultures contained 0.38 ± 0.04 ng of sCD14/10^3 monocytes. At Day 21, the sCD14 content of supernatants was 0.52 ± 0.06 ng/10^3 monocytes derived from tumor patients and 0.51 ± 0.08 ng/10^3 monocytes derived from control subjects. At Day 100, the sCD14 content of culture supernatants was 0.48 ± 0.03 ng/10^3 monocytes derived from tumor patients. At Day 100, only one control culture retained vital cells left that had not undergone cell death, and the sCD14 content of this control supernatant was below the level of detection. In contrast, at this time, 57% of glioma monocyte cultures contained many cells growing in cluster-like formations.

There were no significant differences of sCD14 content between monocyte cultures derived from patients with primary tumors and those with recurrent glioblastomas. The dexamethasone treatment of glioblastoma patients at the time of blood sampling also did not significantly influence the sCD14 content of the monocyte cultures.

Interleukin-1β release of cultured monocytes

With regard to IL-1β release, there was a significant difference between cultured monocytes of glioblastoma multiforme patients and control monocytes throughout the time of culture (*Fig. 4*). At Day 7, the IL-1β content of glioblastoma monocyte culture supernatants was 1.01 ± 0.11 (mean ± standard error) pg/10^3 cells as compared with 0.21 ± 0.06 pg/10^3 control monocytes. This difference was significant ($P \leq 0.01$). At Day 21, the IL-1β content of supernatants increased to 5.02 ± 0.60 pg/10^3 monocytes derived from tumor patients and decreased to only 0.10 ± 0.04 pg/10^3 monocytes derived from control subjects. Statistical analysis showed a significant difference ($P \leq 0.001$) of IL-1β content between both groups at Day 21. At Day 100, the IL-1β content of culture supernatants was 3.31 ± 0.38 pg/10^3 monocytes derived from brain tumor patients. At Day 100, the IL-1β content of one control supernatant was below the level of detection.

There was no significant difference of IL-1β content between monocyte cultures derived from patients with primary tumors and those with recurrent glioblastoma (*Fig. 5*). At Day 7, the IL-1β content was 1.13 ± 0.38 pg/10^3 monocytes derived from subjects with primary lesions and 0.91 ± 0.04 pg/10^3 monocytes from patients with recurrent glioblastoma. At Day 21, the IL-1β content increased to 5.19 ± 1.48 and 4.85 ± 1.01 pg/10^3 monocytes, respectively. At Day 100, the IL-1β content of primary glioblastoma monocyte cultures was 3.29 ± 1.65 pg/10^3 cells, and the IL-1β content of recurrent glioblastoma monocyte cultures was 3.34 ± 0.55 pg/10^3 cells.

The dexamethasone treatment of glioblastoma patients at the time of blood sampling also did not significantly influence the IL-1β content of monocyte cultures (*Fig. 6*). At Day 7, the IL-1β content of culture supernatants was 0.82 ± 0.12 pg/10^3 monocytes derived from patients treated with dexamethasone and 1.24 ± 0.46 pg/10^3 monocytes from brain tumor patients without dexamethasone treatment. At Day 21 of culture, the IL-1β content was 4.38 ± 1.34 pg/10^3 monocytes from subjects with dexamethasone and 5.66 ± 1.78 pg/10^3 monocytes from subjects without dexamethasone. At Day 100, the IL-1β content of monocyte culture supernatants was 3.26 ± 0.96 pg/10^3 cells from glioblastoma patients with dexamethasone and 3.37 ± 1.33 pg/10^3 monocytic cells from glioblastoma patients without dexamethasone.

DISCUSSION

Tumor cells and tumor-associated monocytes release Interleukin-1β

At Day 21, the supernatants of cultured peripheral blood monocytes of glioblastoma patients contained about 50 times more IL-1β than did those of healthy donors as well as those of patients with subarachnoid hemorrhage, vasospasm, and brain infarction. Moreover, the IL-1β content of culture supernatants was not influenced by the dexamethasone treatment of patients at the time of blood sampling. In addition, the IL-1β production of cultured monocytes did not differ between cells derived from subjects with primary glioma and those with recurrent tumors.

Because it is well known that IL-1β has a central function in the autoregulatory circuits of immune cell proliferation, the 50-fold increase in IL-1β content in

culture supernatants and the pronounced longevity of subpopulations of glioma-associated monocytes in this study may indicate an imbalance of immune cell regulation leading to aberrant macrophages in glioma patients. Recently, it has been shown that malignant tumors not only induce an increase in IL-1β production by monocytes/macrophages, but also that glioblastoma cells in vitro and in vivo synthesize messenger ribonucleic acid encoding for IL-1β and for IL-6 [21] and express IL-1β, IL-6, transforming growth factor-β, and tumor necrosis factor proteins [4,9]. Similar findings were provided by Evans et al. [7], who reported that progressing murine sarcomas not only produce IL-1 but also may induce high levels of IL-1 systemically in association with macrophages. In addition, those authors [7] found progressively growing tumors to release other biologically active molecules into the circulation that induce IL-1α, IL-1β, tumor necrosis factor-α, and IL-6 gene expression in macrophages. One could speculate whether substances produced by glioma cells are able to induce and sustain IL-1β gene expression in glioma-associated monocyte subsets as well [15].

Autocrine/paracrine stimulation of monocytes by Interleukin-1β

The increased IL-1β production of nonstimulated, long-term cultured blood monocytes of glioblastoma patients in our study could be a feature of selectively activated monocyte subsets. This finding is not contradictory to data demonstrating that short-term cultured glioma-associated monocytes produce IL-1 levels comparable with those released by monocytes from healthy persons when activated by lipopolysaccharides [6]. The synthesis of our data and those presented by Elliott et al. [6] corroborates the assumption that glioblastoma-associated circulating monocytes are maximally activated cells. This kind of activation seems to be related to the inflammatory response against the tumor, because such activated cells could not be found in cultures derived from control subjects with brain infarction, although quite frequently, in pathological studies, an inflammatory border zone around infarcted brain tissue can be seen.

It seems possible that the pronounced delay of monocyte cell death observed in our experiments at least partially was caused by an autocrine or paracrine stimulation via IL-1β. Because monocytes are known to express IL-1R [2,18,23], an autocrine stimulation would lead to evolutionary advantages of monocytes with a high IL-1β production. Such autocrine or paracrine stimulation may be the reason that about 14% of glioblastoma-associated monocyte cell cultures survived for more than 250 days after having been removed from the circulation and from any effect of substances shed by the tumor. Therefore, the longevity as well as the increase in the IL-1β production of glioma-associated monocytes from Days 7 to 21 of culture might be due to a selective loss of suppressor monocytes in these cultures.

The significance of such monocyte subsets in vivo may be their ability to stimulate the growth of glioma cells carrying IL-1β receptors, provided that these monocytes infiltrate into the tumors. If they do, the enhanced IL-1β release of such monocytes may be part of an explanation for the not entirely consistent findings showing malignant tumors, such as glioblastoma multiforme, on the one hand retaining more tumor-infiltrating macrophages than benign lesions [4,6,14,26,28] and, on the other hand, inducing immunosuppression [1,5,6,15,24-27,29]. Hence, in future studies, it will be valuable to characterize the peripheral blood monocytes and the cerebral tissue macrophages of glioma patients.

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Reprint requests: Georg Fries, M.D., Neurochirurgische Universitätsklinik, Langenbeckstr. 1, 55101 Mainz, Germany.

REFERENCES: [1-32]

1. Barna BP, Rogers L, Thomassen MJ, Barnett GH, Estes ML: Monocyte tumoricidal activity and tumor necrosis factor production in patients with malignant brain tumors. **Cancer Immunol Immunother** 33:314-318, 1991.
2. Barrett JT: Macrophages and antigen-processing and -presenting cells, in Bircher S (ed); *Textbook of Immunology*. St. Louis, The C.V. Mosby Company, 1988, pp 84-106.
3. Bertoglia JA, Rimsky L, Kleinerman ES, Lachman LB: B-cell line-derived interleukin 1 is cytotoxic for melanoma cells and promotes the proliferation of an astrocytoma cell line. **Lymphokine Res** 6:83-91, 1987.
4. Black KL, Chen K, Becker DP, Merrill JE: Inflammatory leukocytes associated with increased immunosuppression by glioblastoma. **J Neurosurg** 77:120-126, 1992.
5. Chen TC, Hinton DR, Apuzzo MLJ, Hofman FM: Differential effects of tumor necrosis factor-alpha on proliferation, cell surface antigen expression, and cytokine interactions in malignant gliomas. **Neurosurgery** 32:85-94, 1993.
6. Elliott LH, Brooks WH, Roszman TL: Cytokinetic basis for the impaired activation of lymphocytes from patients with primary intracranial tumors. **J Immunol** 132:1208-1215, 1984.
7. Evans R, Kamdar SJ, Duffy TM: Tumor-derived products induce IL-1α, IL-1β, TNF-a, and IL-6 gene expression in murine macrophages: Distinctions between tumor- and bacterial endotoxin-induced gene expression. **J Leukocyte Biol** 49:474-482, 1991.
8. Finsen BR, Jorgensen MB, Diemer NH, Zimmer J: Microglial MHC antigen

expression after ischemic and kainic acid lesions of the adult rat hippocampus. **Glia** 7:41-49, 1993.
9. Fontana A, Hengartner H, de Tribolet N, Weber E: Glioblastoma cells release interleukin 1 and factors inhibiting interleukin 2-mediated effects. **J Immunol** 132:1837-1844, 1984.
10. Fujita S, Tsuchihashi Y, Kitamura T: Origin, morphology and function of the microglia. **Prog Clin Biol Res** 59:141-169, 1981.
11. Giulian D, Lachman LB: Interleukin-1 stimulation of astroglial proliferation after brain injury. **Science** 228:497-499, 1985.
12. Hetier E, Ayala J, Denefle P, Bousseau A, Rouget P, Mallat M, Prochiantz A: Brain macrophages synthesize interleukin-1 and interleukin-1 mRNA in vitro. **J Neurosci Res** 21:391-397, 1988.
13. Hickey WF, Kimura H: Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. **Science** 239:290-292, 1988.
14. Ibayashi Y, Uede T, Uede T, Kikuchi K: Functional analysis of mononuclear cells infiltrating into tumors: Differential cytotoxicity of mononuclear cells from tumors of immune and nonimmune rats. **J Immunol** 134:648-653, 1985.
15. Ikeda M, Sakagami H, Fukushima Y: Effect of sera of brain tumor patients on cytokine-induced human myelogenous leukemic cell differentiation. **Anticancer Res** 11:331-336, 1991.
16. Imamoto K: Origin of microglia: Cell transformation from blood monocytes into macrophagic ameboid cells and microglia. **Prog Clin Biol Res** 59A:125-139, 1981.
17. Jordan FL, Thomas WE: Brain macrophages: Questions of origin and interrelationship. **Brain Res** 13:165-178, 1988.
18. Ju G, Labriola-Tompkins E, Campen CA, Benjamin WR, Karas J, Plocinski J, Biondi D, Kaffka KL, Kilian PL, Eisenberg SP, Evans RJ: Conversion of the interleukin 1 receptor antagonist into an agonist by site-specific mutagenesis. **Proc Natl Acad Sci USA** 88:2658-2662, 1991.
19. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. **J Am Stat Assoc** 53:475-481, 1958.
20. Lassmann H, Schmied M, Vass K, Hickey WF: Bone marrow derived elements and resident microglia in brain inflammation. **Glia** 7:19-24, 1993.
21. Lichtor T, Dohrmann GJ, Gurney ME: Cytokine gene expression by human gliomas. **Neurosurgery** 26:788-792, 1990.
22. Ling E-A, Wong W-C: The origin and nature of ramified and amoeboid microglia: A historical review and current concepts. **Glia** 7:9-18, 1993.
23. Mangan DF, Welch GR, Wahl SM: Lipopolysaccharide, tumor necrosis factor-alpha, and IL-1βeta prevent programmed cell death (apoptosis) in human peripheral blood monocytes. **J Immunol** 146:1541-1546, 1991.
24. Maxwell M, Galanopoulos T, Neville-Golden J, Antoniades HN: Effect of the expression of transforming growth factor-beta2 in primary human glioblastomas on immunosuppression and loss of immune surveillance. **J Neurosurg** 76:799-804, 1992.
25. McVicar DW, Davis DF, Merchant RE: In vitro analysis of the proliferative potential of T cells from patients with brain tumor: Glioma-associated immunosuppression unrelated to intrinsic cellular defect. **J Neurosurg** 76:251-260, 1992.
26. Milas L, Wike J, Hunter N, Volpe J, Basic I: Macrophage content of murine sarcomas and carcinomas: Associations with tumor growth parameters and tumor radiocurability. **Cancer Res** 47:1069-1075, 1987.
27. Neuwelt EA, Kikuchi K, Hill S, Lipsky P, Frenkel EP: Immune response in patients with brain tumors. **Cancer** 51:248-255, 1983.
28. Rossi ML, Buller J, Heath SA: Monocyte/macrophage infiltrate in 41 oligodendrogliomas. **Pathol Res Pract** 187:166-169, 1991.
29. Schneider J, Hofman FM, Apuzzo MLJ, Hinton DR: Cytokines and immunoregulatory molecules in malignant glial neoplasms. **J Neurosurg** 77:265-273, 1992.
30. Theodorsson-Norheim E: Kruskal-Wallis test: BASIC computer program to perform nonparametric one-way analysis of variance and multiple comparisons on ranks of several independent samples. **Comput Method Program Biomed** 23:57-62, 1986.
31. Wallis RS, Fayen J, Wiblin TS, Fujiwara H, Ellner JJ: Direct mitogenic properties of interleukin-1. **J Lab Clin Med** 117:234-240, 1991.
32. Zülch KJ: Histological Typing of Tumours of *the Central Nervous System.* Geneva, World Health Organization, 1979.

COMMENTS

Using tissue culture flasks, the authors have observed a differential in the survival of monocytes derived from the peripheral blood of glioblastoma patients versus control patients. At the same time, the former cells were shown to produce much greater quantities of interleukin-1β.

The observation that the monocytes derived from glioblastoma patients produce greater quantities of interleukin-1β than do control monocytes is not surprising, considering the immunocompromised status of glioblastoma patients. It is, however, surprising to see that the altered expression of interleukin-1β by monocytes is maintained in vitro for such a long period of time; this indicates, most likely, an alteration of the monocytes at the genome level. It will be critical in future studies to confirm

this hypothesis and especially to elucidate the precise mechanisms through which the brain tumor can affect the genetic expression of peripheral blood cells.

Raymond Sawaya
Houston, Texas

The role of interleukin-1β in the growth of a variety of neoplasms has been of significant interest. This is one of a series of cytokines that appears to have multiple roles in directly and indirectly affecting cells of the immune system as well as tumor cells.

In this study, interleukin-1β production has been found to be increased in the peripheral blood monocytes of glioma patients. The relationship between the production of this cytokine interleukin-1β and glioma growth represents an intriguing issue in understanding the role of cytokines in glioma patients.

Paul L. Kornblith
Pittsburgh, Pennsylvania

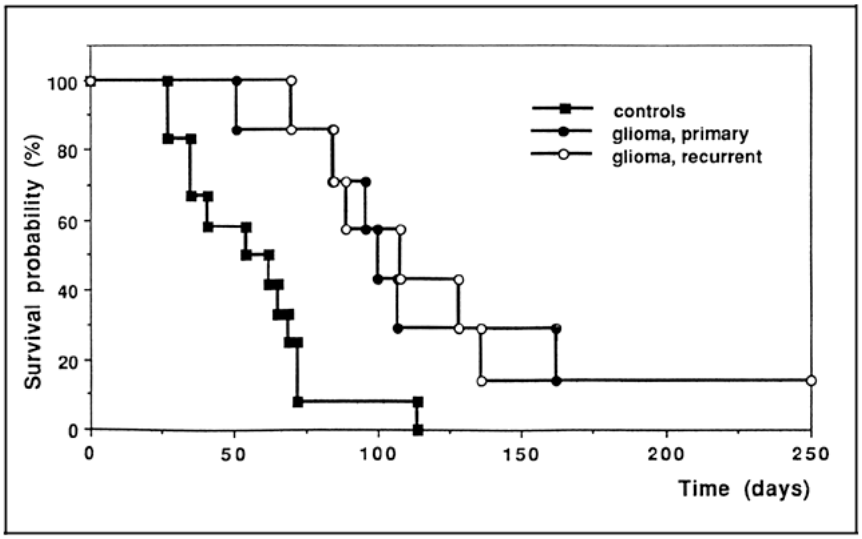

Figure 1. Graph showing survival data of peripheral blood monocyte cultures of 12 nontumor controls and of 14 patients with glioblastoma (7 primary, 7 recurrent) by the use of the Kaplan-Meier method (19). Note the almost sigmoidal shape of the survival curves and the shift to the right of curves derived from glioblastoma-associated monocytes, indicating delay of in vitro cell death of these monocytes. The difference in survival periods between controls and glioma-associated monocytes was significant ($P < 0.001$, log-rank test).

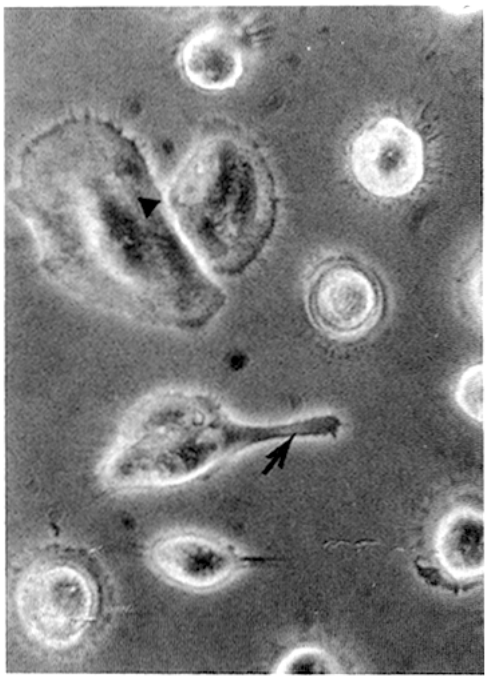

Figure 2. Photomicrograph (original magnification ×400) of long-term cultured peripheral blood monocytes derived from a 61-year-old woman with glioblastoma (Tumor Patient 9 in *Table 1*). Subpopulations of these monocytes did not undergo cell death until Day 250 of culture, when the experiments of this study were terminated. Note the protruding growth cone (*arrow*), indicating migration, and the contact of neighboring monocytes (*triangle*).

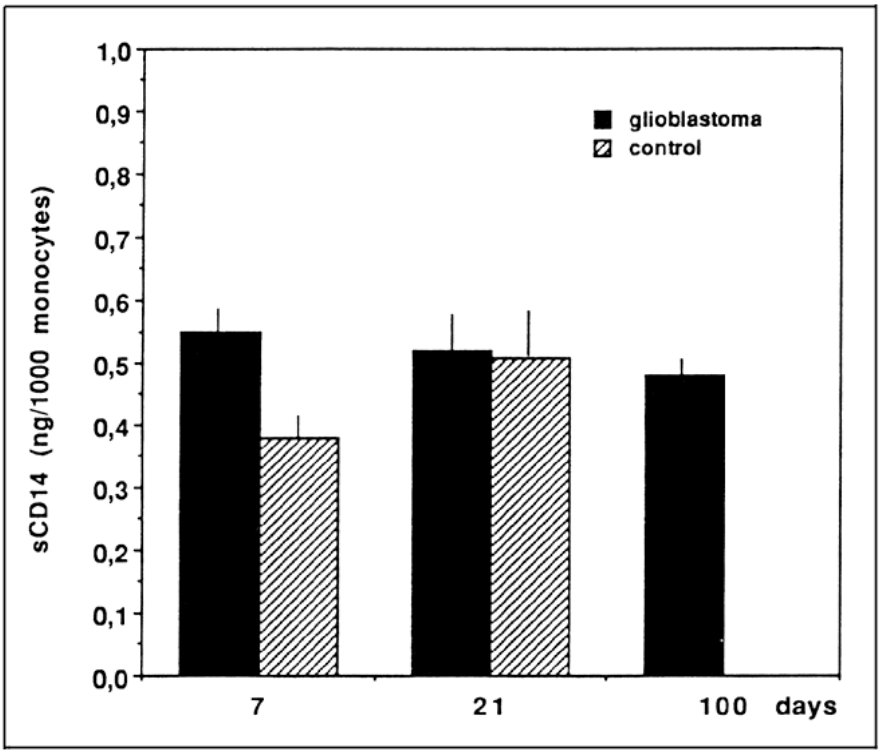

Figure 3. Graph showing sCD14 monocyte/macrophage lineage marker content per 10^3 cells at Days 7, 21, and 100 in culture supernatants of peripheral blood monocytes. The monocytes were derived from control subjects and from glioblastoma patients. There were no significant differences between both groups, and there was no significant change of sCD14 content throughout the time of culture. This demonstrates that the cultured cells of both groups were white blood cells of the monocyte/macrophage lineage.

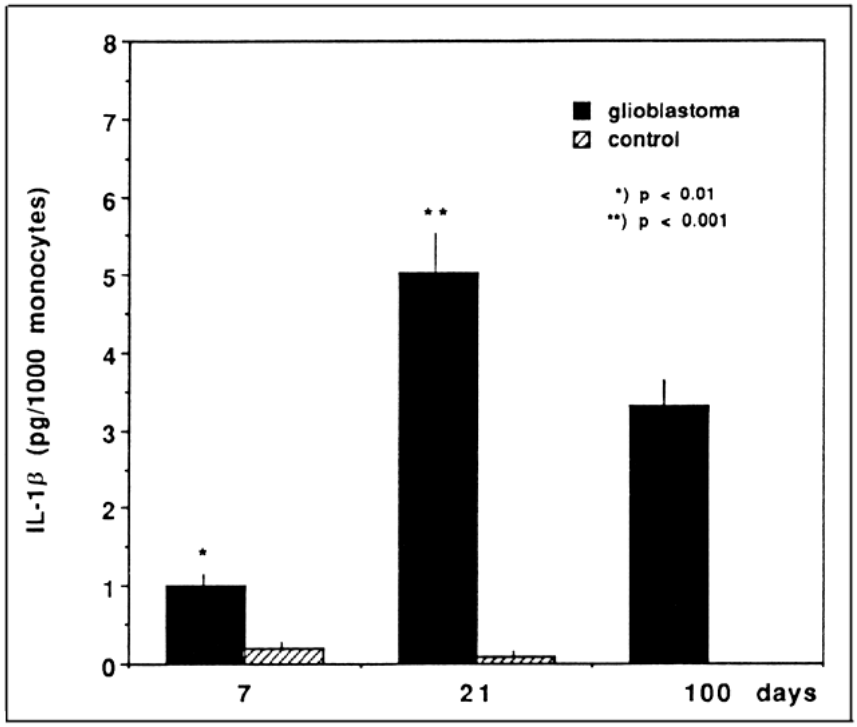

Figure 4. Graph showing the IL-1β content per 10^3 cells at Days 7, 21, and 100 in culture supernatants of peripheral blood monocytes. The monocytes were derived from control subjects and from glioblastoma patients. At Day 7, the IL-1β content of glioblastoma monocyte culture supernatants was about 5 times higher (*$P \leq 0.01$, Kruskal-Wallis test) and at Day 21 was about 50 times higher (**$P \leq 0.001$) as compared with control cultures.

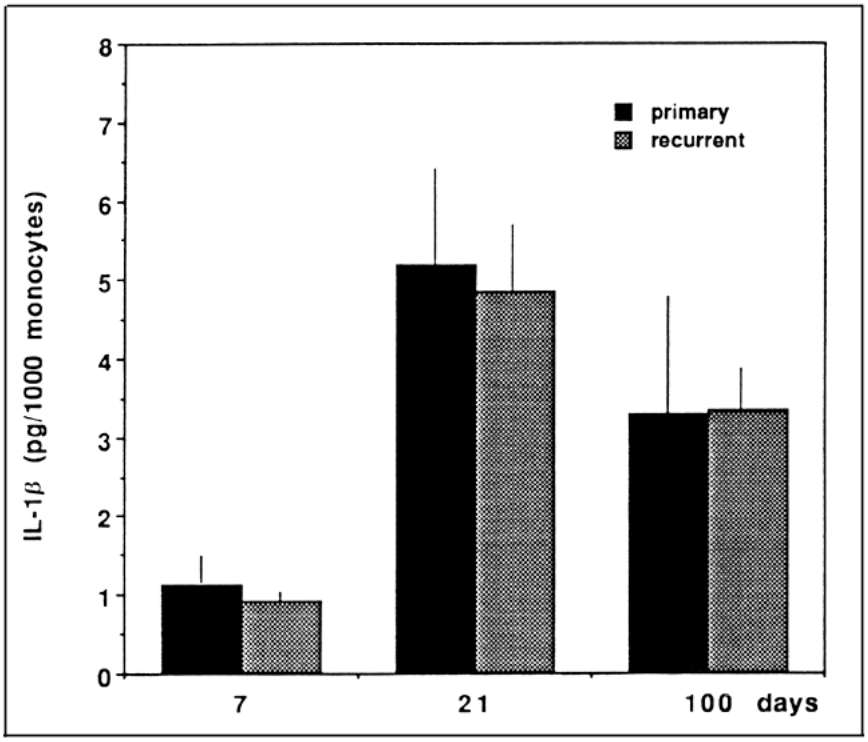

Figure 5. Graph showing the IL-1β content per 10^3 cells at Days 7, 21, and 100 in culture supernatants of peripheral blood monocytes. The monocytes were derived from subjects with primary glioblastoma and recurrences. Statistical analysis revealed the differences between both groups to be not significant.

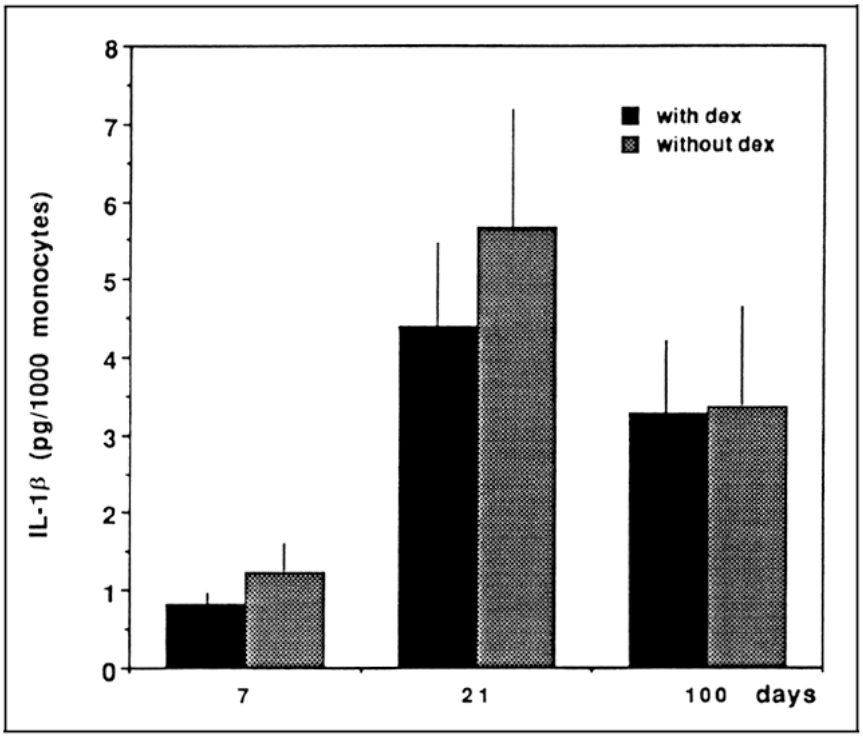

Figure 6. Graph showing the IL-1β content per 10^3 cells at Days 7, 21, and 100 in culture supernatants of peripheral blood monocytes. The monocytes were derived from glioblastoma patients with dexamethasone (*dex*) treatment and without dexamethasone at the time of blood sampling. There were no differences between the two groups.

Patient No.	Age (yr)/Sex	Tumor Classification/Remarks	Dex	LY/100	MO/100
Tumor patients					
1	50/M	Glioblastoma, recurrent	Yes	25	7
2	27/F	Glioblastoma, recurrent	Yes	29	6
3	49/F	Glioblastoma, recurrent	No	22	3
4	56/F	Glioblastoma, recurrent	Yes	29	7
5	62/F	Glioblastoma, recurrent	Yes	34	1
6	30/M	Gliosarcoma, recurrent	Yes	23	5
7	43/M	Glioblastoma	No	20	6
8	60/M	Glioblastoma	Yes	25	7
9	61/F	Glioblastoma	No	31	2
10	49/F	Glioblastoma	No	24	3
11	67/F	Glioblastoma	No	18	5
12	58/M	Glioblastoma	No	23	7
13	41/M	Glioblastoma	No	30	5
14	48/M	Glioblastoma, recurrent	Yes	32	4
Controls					
1	32/M	SAH, aneurysm, vasospasm	Yes	26	5
2	51/M	SAH, aneurysm, vasospasm	Yes	37	2
3	44/F	SAH, aneurysm, vasospasm	Yes	38	5
4	56/F	SAH, aneurysm, vasospasm	Yes	21	7
5	68/F	SAH, aneurysm, vasospasm	Yes	34	7
6	24/F	Healthy donor	No	42	5
7	34/M	Healthy donor	No	43	3
8	25/F	Healthy donor	No	31	8
9	30/M	Healthy donor	No	33	2
10	24/M	Healthy donor	No	32	6
11	50/F	Healthy donor	No	28	4
12	53/M	Healthy donor	No	38	5

[a] **Interleukin-1β (IL-1β) content of monocyte culture supernatants of 13 patients with glioblastoma (7 primary, 6 recurrent) and of 12 controls (5 SAH, 7 healthy donors) was determined. The monocytes of one additional patient (Patient 14) were cultured but were not tested for IL-1β release. Dex, dexamethasone treatment at the time of blood sampling; LY/100, average number of counted lymphocytes per 100 peripheral white blood cells; MO/100, average number of counted monocytes per 100 white blood cells; SAH, subarachnoid hemorrhage.**

Table 1. Composition of the Study Groups