Expression and Release of Interleukin-1 by Human Glioblastoma Cells in vitro and in vivo

T. Gauthier, M.-F. Hamou, L. Monod, P. Gallay, St. Carrel, and N. de Tribolet

Neurosurgical Service, Centre Hospitalier Universitaire Vaudois, Lausanne, Institute of Biochemistry, University of Lausanne, Epalinges, and Ludwig Institute for Cancer Research, Lausanne Branch, Epalinges, Switzerland

Summary

The present study demonstrates interleukin-1 (IL-1) production by human glioblastoma cells both in vitro and in vivo. The presence of IL-1α and IL-1β transcripts was analyzed in 4 cell lines. IL-1α mRNA was expressed constitutively in one cell line whereas constitutive IL-1β mRNA could not be detected in any of the cell lines. IL-1α transcripts could be induced with phorbol myristate acetate (PMA) or PMA plus lipopolysaccharide (LPS) in 2 of 4 cell lines and IL-1β mRNA in 2 of 4 cell lines. Culture fluid from these cell lines was tested for the presence of IL-1 using a specific radio-immuno-assay for either IL-1α or IL-1β. In agreement with the results on RNA, one of 4 cell lines was found to constitutively produce IL-1α but not IL-1β. After treatment with PMA and LPS, IL-1α was detected in the culture fluid from two other lines and IL-1β in the medium from three lines. That the IL-1 produced by these cell lines was biologically active was confirmed in a two step thymocyte proliferation assay. IL-1 like activity was detected in all samples that were positive in the radio-immuno-assay.

Finally, immunohistological analysis on fresh frozen tumour sections provided evidence for IL-1 production by glioblastoma cells in vivo. Fourteen out of 28 glioblastomas were stained with an anti-IL-1α monoclonal antibody while none of them was stained with an anti-IL-1β antibody.

Keywords: Brain; glioblastoma; interleukin-1; neoplasm.

Abbreviations: GFAP glial fibrillary acidic protein; MAb monoclonal antibody; IL-1 interleukin-1; PMA phorbol myristate acetate; LPS lipopolysaccharide; RIA radio-immuno-assay.

Introduction

The lack of major success with chemotherapy and radiation therapy in the treatment of glioblastoma has led a number of investigators to study the interaction between tumour cells and the host immune system. Communication between cells may occur either through direct intercellular contacts, or by release of soluble factors such as interleukins. In malignant brain tumours, MHC class I and II antigens expressed on glioma cells are involved in direct cell-cell interactions between tumour cells and lymphoid cells. Furthermore, glioblastoma cells have been reported to secrete cytokines such as interferon-β, GM-CSF, and TGF-β, which may regulate the activation and/or differentiation of lymphocytes invading the tumour site.

IL-1 represents a pleiotropic cytokine produced predominantly by monocytes and macrophages but also by a wide variety of other cell types. Both astrocytes and microglial cells from the brain produce IL-1 when stimulated with endotoxin in vitro. Two different forms of IL-1, IL-1α and IL-1β, have been characterized. Although they have only 26% amino acid sequence homology, they bind with the same receptor and both play a fundamental role in inflammation and the regulation of the immune system.

Fontana et al. reported an IL-1-like activity released by the glioblastoma cell line LN 308. These results however require further investigation since the bioassay used in the study could not discriminate between IL-1 and other cytokines such as IL-6, which can be released by some glioblastoma cell lines. Other reports indicate that glioblastoma cells to produce IL-1 in vitro. Lichtor et al. investigated the IL-1β mRNA level in 2 unstimulated glioma cell lines and 4 fresh glial tumours, whereas Lee et al. studied the presence of IL-1α and IL-1β both at the level of specific transcripts and protein synthesis in one cell line either unstimulated or stimulated with PMA only.

In the present report we investigated whether glioblastoma cells exhibit the capacity to release the immunoregulatory cytokine IL-1 both in vitro and in vivo.
Methods and Material

Cell Lines

All human glioblastoma cell lines used were established and characterized in our laboratory. They were grown as monolayer cultures in RPMI 1640 medium (Gibco, Basel, Switzerland) supplemented with 5% foetal calf serum (FCS), L-glutamine, 100 IU/ml penicillin, and 100 mg/ml streptomycin (Seromed, Basel, Switzerland). The cells were harvested using 0.05% trypsin 0.02% EDTA. All cells were shown to contain GFAP indicating their astrocytic origin.

Monoclonal Antibodies

In the present study we used MAbs against IL-1α (F11) and IL-1β (11C2) as well as a polyclonal rabbit anti-IL-1 serum prepared in our laboratory as described previously. In addition, anti-IL-1α and anti-IL-1β were obtained from Oneogene Sciences Inc., Manhasset, NY, U.S.A., and an anti-GFAP MAb from Serotec, Oxford, England.

For double staining experiments, polyclonal rabbit anti-GFAP serum from Dako, Dakopatts, Denmark, and goat anti-rabbit serum from Nordic, The Netherlands, were used.

Tumour Specimens

Fresh human glioblastoma tissue was obtained from patients undergoing tumour resection. Surgically removed tumours were snapfrozen in isopentane cooled in liquid nitrogen and stored in airtight plastic tube at −80°C until used for immunohistochemistry. Part of the tissue was used in routine histological procedures for diagnosis and classification. The tumour diagnosis was made by the Institute of Neuropathology, University of Lausanne, using the WHO grading system.

Immunohistochemical Staining

Slides were stained with anti-IL-1α or anti-IL-1β MAbs at a 1/10 dilution and with an anti-GFAP MAb at a 1/20 dilution. After 3 washings with PBS they were stained with immunoperoxidase as described previously. Double staining with commercial rabbit anti-GFAP antisem and commercial mouse monoclonal anti-IL-1 were prepared as described elsewhere. IL-1 positive cells were visualized with amino-ethyl carbazol (AEC), which stains brown red, whereas GFAP positive cells, revealed with chloronaphtol, stained dark blue. The counterstaining step was omitted.

For the immunohistochemical staining of cell lines, 13 glioblastoma cell lines were cultured on glass coverslips as described elsewhere. They were then stained with anti-IL-1α or anti-IL-1β MAb at a 1/10 dilution, or by an irrelevant antibody, PX 63, as described previously. Positive cells were visualized with AEC.

RNA Isolation and Hybridization

Human glioblastoma cells from the various lines used in this study were seeded at a concentration of 1 x 10^6 cells/ml and grown until confluent in 75 cm² flasks. PMA (50 ng/ml), or PMA + LPS (10 µg/ml) was added to the cultures 3 hours before harvesting. Northern blot analysis was performed as described. The 450 base pair Eco RI/Pst I cDNA fragment of the IL-1α coding region and the 300 base pair Eco RI/Pst I fragment of the IL-1β coding region were used as probes for IL-1α and IL-1β respectively (generously provided by Dr. Alan Shaw, Glaxo, Geneva, Switzerland).

Production of Conditioned Medium

Cells were incubated in medium with 5% FCS and when subconfluent the medium was changed and PMA (50 ng/ml) or PMA (50 ng/ml) + LPS (10 µg/ml) was added. After 5 days, the supernatants were removed and centrifuged. The samples were concentrated 4 times by ultrafiltration (Amicon) and stored frozen at −70°C.

Solid-Phase Radio-Immuno-Assay (RIA)

For the detection of IL-1α and IL-1β released into the culture fluid by the glioblastoma cell lines a recently developed solid phase RIA was used. The detection limit was in the range of 2.5–10 pg/300 µl of cell free supernatant.

Bioassay

The biological activity of IL-1 released in the conditioned medium was assessed by a two step assay as described elsewhere, using first cells from the NOB-1 subclone of the mouse EL-4 line which constitutively produces very little IL-2 but in response to IL-1 produces high concentrations of IL-2. In the present study NOB-1 cells were incubated for 3 days at 1.5 x 10^6 cells/well in microculture plates in the presence of various dilutions of glioma cell-derived supernatants.

The IL-2 activity in cell-free supernatants from such cultures was then assessed using the murine IL-2 dependent cell line CTLL-20 and the hexamidin assay.

Results

IL-1 Transcripts

The presence of RNA transcripts was tested by Northern blot analysis in 4 human glioblastoma cell lines (LN 229, LN 308, LN 382, and LN 428) with cDNA probes for IL-1α and IL-1β. Constitutive expression of IL-1α in the cell lines was not detectable (Fig. 1), except for LN 428, where a faint IL-1α message was observed in overexposed autoradiograms (not shown). None of the cell lines showed constitutive IL-1β mRNA expression (Fig. 1). After stimulation with either PMA alone or PMA plus LPS, the 2.2 kb mRNA for IL-1α was enhanced strongly in LN 428 cells and weakly in cells from two other lines (LN 229, 308), while the 1.4 kb mRNA for IL-1β was clearly expressed in cells from line LN 428 and weakly in cells from line LN 229.

Detection of IL-1 Protein

The presence of IL-1 in the supernatants of glioblastoma cultures was investigated by a solid-phase RIA on 4-fold concentrated samples. One of four glioblastoma cell lines (LN 428) constitutively released low amounts of IL-1α (40–60 pg/0.3 ml), but not IL-1β (Fig. 2). Different treatments were tested for their effect on the induction of IL-1 release. Maximal release of