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Regulation of IL-10 expression by upstream stimulating factor (USF-1) in glioma-associated microglia ☆

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Abstract

Understanding the local CNS immune response to neoplasms is essential in the development of immune-based treatments for malignant brain tumors. Using rodent glioma models, we have recently found tumor-associated microglia/macrophages (MG/MP) to be less responsive to known MG/MP activators such as CpG, LPS and IFN-γ. To understand the mechanism of MG/MP suppression, nuclear extracts from rodent intracranial C6 gliomas, C6 glioma-associated MG/MP, normal brain, and normal MG/MP were obtained and studied using Electrophoretic Mobility Shift Assay (EMSA). Among the nuclear factors studied (AP-1, IRF, USF-1 and Stat-1) only USF-1, which is constitutively expressed in most cells, was down-regulated in tumor-associated MG/MP, but not normal MG/MP. Because tumor-associated MG/MP had higher expression of IL-10 (but not TNF-α or TGF-β), we evaluated the role of USF-1 on IL-10 expression. siRNA mediated inhibition of USF-1 expression in primary MG/MP cultures resulted in up-regulation of IL-10 mRNA but not TNF-α or TGF-β. These findings suggest that USF-1 may play a role in IL-10 regulation in MG/MP in brain tumors.

Keywords: Microglia; Macrophage; Glioma; IL-10; USF-1; E-box

1. Introduction

Malignant brain tumors are among the most fatal cancers. Even with aggressive surgical intervention and adjunct therapy with irradiation and chemotherapy, few patients with such tumors survive longer than two years after diagnosis (Prados and Levin, 2000). Although immunotherapy is actively being studied as a possible therapy for treatment of systemic cancer, its success in the treatment of intracranial tumors has been limited. Many factors likely play a role in this failure. For example, many tumors including gliomas, have been shown to secrete immunosuppressive cytokines, which inhibit the cytotoxic function of T cells (Roszman et al., 1991). Although T cells may be present in gliomas, they appear to have Th2 and Th3 related functions and are unable to mount a sufficient anti-tumor response (Hao et al., 2002).

In addition to lymphocytes, histological analysis of gliomas has revealed a significant (as much as 60%) macrophage (MP) and microglia (MG) infiltration (Badie and Schartner, 2000; Roggendorf et al., 1996; Shinonaga et al., 1988; Streit, 1994; Wierzba-Bobrowicz et al., 1994). Some studies have even suggested a direct correlation between the glioma grade and the magnitude of MG/MP invasion (Roggendorf et al., 1996). Moreover, MG/MP infiltration is more pronounced in gliomas than in metastatic brain tumors, suggesting gliomas may play an active role in MG/MP recruitment (Morioka et al., 1992). Studies by us and others have supported this hypothesis and indicate that gliomas are capable of secreting chemokines and growth factors that attract and support the growth of MG/MP in brain tumors (Alterman et al., 1994; Badie et al., 1999; Galasso et al., 2000; Kielian et al., 2002). In spite of this, the biological role of MG/MP in gliomas remains unknown.

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MG and MP are capable of potent suppression and stimulation of the local immune system (Guillemin et al., 2004). When activated, MG release reactive oxygen intermediates and proteinases, and become capable of phagocytosis, MHC expression, and lymphocyte activation (Moore et al., 1996). However, MG have also been shown to secrete anti-inflammatory factors. These include transforming growth factor (TGF)-β, Interleukin (IL)-10, and Prostaglandin E_2 (PGE_2), which inhibit MG antigen presentation function and IL-12 secretion (Aloisi and Ria 1999; Aloisi and De Simone 1999). Using rodent models, we have recently demonstrated that MG/MP in brain tumor models are more resistant to stimulation when exposed to known MG/MP activators such as interferon (IFN)-γ, LPS and CpG oligodeoxynucleotide (ODN) (Schartner et al., 2005). This relative impairment may be due to MG/MP inactivation in response to local suppressive factors.

To better understand the mechanism of MG/MP immune suppression in brain tumors, the expression of MG/MP nuclear and transcription factors were studied. Among the nuclear factors examined, AP-1, IRF, USF-1 and Stat-1, only USF-1, which is constitutively expressed in most cells, was down-regulated in tumor MG/MP as compared to normal brain MG/MP. Upstream stimulating factor-1 (USF-1), a transcription factor belonging to the basic helix–loop–helix–leucine zipper family (Galasso et al., 2000), interacts with high affinity to cognate E-box regulatory elements which are particularly represented over the genome (Kielian et al., 2002). Binding of USF-1 to E-box results in the regulation of many genes, such as the expression of Cox2 and Osteopontin (Guillemin and Brew, 2004; Moore and Thanos, 1996). Transcriptional activity and DNA-binding of the USF proteins can be modulated by multiple ways, including DNA modification of the E-box binding motif, phosphorylation by distinct kinases, and regulation by the extracellular signal-regulated kinase (ERK)1/2 pathway (Donnelly et al. 1999; Lang 2005; Zhang et al. 2006).

To study the role of USF-1 on cytokine expression, USF-1 expression was inhibited using siRNA transfection of tumor-associated primary MG/MP cultures (PMG); which expressed higher levels of this nuclear factor when expanded in vitro compared to tumor-associated primary MG/MP studied ex vivo. USF-1 inhibition in these cells resulted in up-regulation of IL-10 mRNA expression but not TNF-α or TGF-β. These findings suggest that USF-1 plays a role in IL-10 regulation in MG/MP in brain tumors. Considering its immunosuppressive properties, IL-10 expression by MG/MP may be important in MG/MP inactivation, and possibly play a role in the inhibition of the CNS immune response to tumors.

2. Materials and methods

2.1. Tumor implantation

All animals were housed and handled in accordance with the University of Wisconsin Research Animal Resources Center guidelines. The rat cell line C6 was obtained from ATCC and maintained in DMEM medium (Life Technologies, Gaithersburg, MD) supplemented with 10% heat-inactivated FBS (BioWhittaker, Walkersville, MD), 100 U/ml penicillin-G, 100 ug/ml streptomycin and 0.01 M hepes (Life Technologies, Gaithersburg, MD) (culture media). Intracranial tumor implantation was performed as described previously (Badie and Schartner, 2000). Cells were harvested by trypsinization and were counted and resuspended in 1.2% methylcellulose. Wistar rats (Harlan, Indianapolis, IN) weighing 100–200 g were anesthetized by intraperitoneal administration of ketamine (50–90 mg/kg) and xylazine (10 mg/kg), and immobilized in a stereotactic head frame. Through a small burr hole, 5 ul of methylcellulose containing 1×10^6 tumor cells was injected bilaterally at the coronal suture, 4 mm lateral to the midline and 5 mm deep into the left and right frontal lobes, using a Hamilton syringe (Fischer Scientific, Tustin, CA). The C6 glioma cell line, an allogen model, results in a significant MG/MP infiltration compared to other rodent gliomas (Badie and Schartner, 2000). This feature makes the C6 glioma model ideal for MG/MP isolation and purification from adult rodents.

2.2. Microglia isolation and cultures

Primary MG cultures (PMG) were generated from Wistar rats harboring C6 brain tumors as described previously (Badie and Shartner, 2001; Badie et al., 2003). After 2–3 weeks, when animals showed signs of raised intracranial pressure, they were sacrificed and the brain was removed. Tumor and the immediate tumor periphery from 5–7 rats were isolated from surrounding brain tissue, minced, triturated, and incubated in 0.125% trypsin (Life Technologies, Gaithersburg, MD) containing 2% Chick Serum (Sigma Aldrich, St. Louis, MO) for 10 min at 37 °C, and centrifuged at 250 ×*g* for 2 min. The supernatant containing fully digested tissue was removed and added to the culture media. The sample was then centrifuged at 500 ×*g* for 3 min, resuspended in 10 ml of culture media, and placed on ice. An additional 10 ml trypsin with chick serum was added to the remaining tumor fragments and triturated 10–20 times. The sample was incubated at 37 °C for 10 min, and the above procedure was repeated until all tumor fragments were completely digested.

After digestion was completed, cells were passed through a sterile 40 μm filter and washed twice in media at 500 ×*g*. The pellet was resuspended in 15 ml of 70% isotonic Percoll/PBS in the bottom of a 50 ml tube and overlaid with 25 ml of 35% Percoll/PBS and 10 ml of sterile PBS. The tubes were centrifuged at 4 °C at 500 ×*g* for 45 min with no break. The 35/70 fraction of cells containing MG/MP were collected, washed once in media and used for studies described below. To develop PMG, cells were plated in culture media (DMEM with 0.1% FBS). Cells were allowed to attach for 24 hr, after which the adherent cells were washed twice in media and allowed to expand in the culture medium.

2.3. Preparation of nuclear extracts for gel shift assays

For in vitro experiments, cultured cells and PMG were washed with cold PBS and detached using a cell scraper. The