CXCR4 Is a Major Chemokine Receptor on Glioma Cells and Mediates Their Survival*

Yan Zhou‡, Peter H. Larsen‡, Chunhai Hao§, and V. Wee Yong¶

From the ¶Departments of Oncology and Clinical Neurosciences, University of Calgary, Calgary, Alberta T2N 4N1, Canada and §Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

Chemokines were described originally in the context of providing migrational cues for leukocytes. They are now known to have broader activities, including those that favor tumor growth. We addressed whether and which chemokines may be important promoters of the growth of the incurable brain neoplasm, malignant gliomas. Analyses of 16 human glioma lines for the expression of chemokine receptors belonging to the CXCR and CCR series revealed low to negligible levels of all receptors, with the exception of CXCR4 that was expressed by 13 of 16 lines. All six resected human glioma specimens showed similarly high CXCR4 expression. The CXCR4 on glioma lines is a signaling receptor in that its agonist, stromal cell-derived factor-1 (SDF-1; CXCL12), produced rapid phosphorylation of mitogen-activated protein kinases. Furthermore, SDF-1 induced the phosphorylation of Akt (protein kinase B), a kinase associated with survival, and prevented the apoptosis of glioma cells when serum was withdrawn from the culture medium. SDF-1 also mediated glioma chemotaxis, in accordance with this better known role of chemokines. We conclude that glioma cells express a predominant chemokine receptor, CXCR4, and that this functions to regulate survival in part through activating pathways such as Akt.

Chemokines are a family of molecules that regulate the chemotaxis of leukocytes in tissues (1). Chemokines have other roles, including promoting mitosis and the modulation of apoptosis, survival, and angiogenesis (reviewed in Ref. 2). These functions are beneficial for tumor growth, and it is now appreciated that chemokines are expressed by many tumor types. Indeed, several chemokines have been purified and characterized from tumor sources (3). Recently, it was discovered that the ability of breast cancer cells to metastasize to particular tissues was the result of the expression of a specific chemokine receptor, CXCR4, on breast cancer cells; coordinatealy, its ligand, stromal cell-derived factor-1a (SDF-1α; CXCL12) was expressed in target tissues (4). The SDF-1/CXCR4 pathway has also been implicated in the metastasis of prostate cancer cells to bone (5).

The chemokine family now consists of over 40 members that are subdivided into groups based on the motifs of their first two N terminus cysteine residues (6, 7). The CXC chemokines have an amino acid separating the first two cysteine residues whereas the CC chemokines do not. The CX3C subgroup, with three intervening amino acids, has a single member, as does the C subfamily with one cysteine residue at the N terminus. Chemokines bind to G protein-coupled receptors that are segregated on the basis of the chemokine subgrouping (CXCRs, CCRs, CX3R1, XCR1).

Malignant gliomas are brain neoplasms that account for more than 50% of tumors that arise within the central nervous system (CNS). They are highly proliferative and angiogenic and are locally very invasive within the CNS. The median survival of patients with malignant glioma is less than 1 year, and only 2% survive beyond 3 years (8). A better understanding of glioma biology to derive effective therapeutics for this incurable neoplasm is indicated clearly.

Several chemokines have now been described on glioma cells in situ and in vitro, and these include macrophage chemotactic protein-1 (CCL2) (9–11), interleukin-8 (CXCL8) (12, 13), RANTES (regulated on activation normal T cell expressed and secreted) (CCL5) (14), and SDF-1 (CXCL12) (15). The function of these proteins in glioma biology remains uncertain, but they could account for the increased malignancy of glioma cells. For example, a role for chemokines in regulating angiogenesis is suggested by the observation that hypoxic/anoxic insults to glioma cells in vitro induced an increase in IL-8 mRNA (16). Also, SDF-1 and CXCR4 are co-localized to regions of angiogenesis in glioma specimens in situ (15).

To better understand whether and which chemokines may be important promoters of the growth of glioma cells, we have performed a systematic analysis of the chemokine receptors that may be over-represented in the majority of glioma cell lines. Furthermore, we examined biochemical signaling pathways that could be triggered by such chemokine receptors. Our results reveal the predominance of expression of CXCR4, and we further demonstrate that its ligand, SDF-1, activated MAP kinases and Akt and is a survival and chemotactic factor for glioma cells in vitro.

EXPERIMENTAL PROCEDURES

Glioma Cell Lines, Primary CNS Cultures, and Resected Glioma Specimens—The human glioma cell lines U251N, U373, and U87 were obtained from the American Type Culture Collection (Manassas, VA) and have been described elsewhere (17). Lines LN18, LN71, LN215, LN229, LN308, LN340, LN405, LN427, LN428, LN443, LN464, LN827 and LN992 (18) were obtained from Dr. Erwin Van Meir (Emory University, Atlanta, GA). Cells were maintained in feeding medium containing 10% fetal calf serum (FCS) as described previously (19).

This paper is available on line at http://www.jbc.org

Received for publication, June 21, 2002, and in revised form, October 10, 2002
Published, JBC Papers in Press, October 17, 2002, DOI 10.1074/jbc.M206222200
Chemokines and Glioma

Primary cultures of astrocytes (over 95% pure) and neurons (purity of 90% or higher) were isolated from the brains of human fetuses as detailed before (20). The use of these therapeutic abortion specimens has been approved by local institutional human ethics committees.

Glioma samples were kindly provided by the London (Ontario) Brain Tumor Tissue Bank in the London Health Sciences Center. The six cases used in this study were diagnosed histologically as glioblastoma multiforme (World Health Organization Grade IV). RNase Protection Assay (RPA)—Cells at 80% confluence in 100-mm diameter dishes were harvested by applying 1 ml of Trizol® (Invitrogen) per dish. Total RNA was extracted, and 15 μg was used for each RPA. Multiprobe template sets for chemokine receptors (hCR-5, hCR-6) were obtained from Pharmingen (Riboquant™). The method for RPA was as detailed by the manufacturer. In an attempt to establish if secreted cytokines were bound or bound to the extracellular matrix, dishes were treated with IL-1β (2 ng/ml), tumor necrosis factor-α (TNF-α; 100 units/ml), or their combination for 18 h, and cells were then harvested for RPA.

Assessment of CXCR4 Expression by Flow Cytometry and Immunohistochemistry—Cells at 80% confluence were scraped off 100-mm dishes using a rubber policeman, stained with a monoclonal anti-human CXCR4-fluorescein isothiocyanate (R&D Systems, Minneapolis, MN) for 45 min, washed, fixed, and analyzed by flow cytometry. Alternately, 10,000 cells were plated onto glass coverslips and were incubated with a monoclonal antibody to CXCR4 (clone 44708.111; R&D Systems) a day later. Following the application of goat anti-mouse Ig conjugated to Cy5 and fixation, coverslips were analyzed by fluorescence microscopy.

Activation of MAP Kinases and Akt—To determine whether CXCR4 on glioma cell lines was functionally active, glioma cells were treated with SDF-1α (20 ng/ml) for 15, 30, or 60 min. Lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 2.5 mM EGTA, 0.1% Tween 20, 1% Nonidet P-40, 0.2% SDS, 10% glycerol, and complemented with 1 mM dithiothreitol, 10 mM β-glycerophosphate, 1 mM NaF, 0.1 mM sodium orthovanadate, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml pepstatin A, and 1 mM phenylmethylsulfonyl fluoride) was then added. The lysate was collected, freeze-thawed twice, and spun at 10,000 rpm in a microfuge for 5 min. The supernatant was analyzed for total MAP kinase activity using the Bio-Rad kinase assay. Fifty percent of total protein was added to each lane of a 12.5% SDS-PAGE and resolved. Proteins were transferred on to an Immobilon™ membrane (Millipore Corporation, Bedford, MA), which was then blocked for nonspecific binding (using phosphate-buffered saline containing 0.1% Tween 20 and 10% milk). A primary antibody to the extracellular signal-regulated kinases (ERKs) (either rabbit anti-ERK (1:1000) or rabbit anti-phosphorylated ERK (1:1000); New England Biolabs) was then applied overnight at 4°C. A secondary antibody of anti-rabbit horseradish peroxidase (1:10,000) was then added at room temperature for 1 h. Membranes were then probed with enhanced chemiluminescence reagent (ECL Western blotting detection system; Amersham Biosciences). In other experiments, cell lysates were probed for the activated Akt/protein kinase B, a kinase that down-regulates apoptosis by phosphorylating various substrates including caspase-9 and forkhead transcription factors. The antibodies utilized were rabbit anti-phospho-Akt (Ser-473) and rabbit anti-Akt (both from New England Biolabs).

SDF-1 Enzyme-linked Immunosorbent Assay—50,000 glioma cells were seeded into each well of a 24-well plate. The amount of SDF-1 secreted into the medium after 48 h of culture was analyzed using 100 μl of media. The upper and lower chambers were separated by a polycarbonate filter (8-μm pore; polyvinylpyrrolidone (PVP)-free). The filled chamber was incubated at 37°C for 96 h. A filter which the cells that had not traveled to the lower chamber were scrapped off. The filter was fixed in acid alcohol (5% acetic acid, 95% ethanol) for 15 min, and the cells were stained with hematoxylin. The migrated cells were then counted using a microscope.

Determination of Proliferation Rate—10,000 cells plated on glass coverslips were incubated with test agents for 24 h; 10 μm bromodeoxyuridine (BrdUrd) was added during the last hour. Cells were then fixed with 70% ethanol and stained for nuclear incorporation of BrdUrd as described previously (23). This was followed by counter staining for 3 min with the DNA binding Hoechst dye (Hoechst 33852, Sigma) to label all cells. Using an immunofluorescence microscope, the number of Hoechst-positive cells was counted per field to obtain the total number of cells in that field. Of these, the number of cells that were BrdUrd-positive was evaluated to obtain the percentage of cells in the S phase of the cell cycle.

Cell Death Assays—A nucleosome enzyme-linked immunosorbent assay (Roche Diagnostics) was used to determine cell death in response to serum deprivation. The method relies on the detection of cytoplasmic histone-associate DNA fragments (mono- and oligonucleosomes) that are generated in apoptotic cells. 10,000 cells were placed into each well of a 96-well plate. Cells were plated in feeding medium containing 10% FCS. 4–5 h later, when the majority of cells had adhered, the medium for most experimental groups was changed over to an identical one, except that this contained 0.1% FCS. Some cells continued to be maintained in 10% FCS. In wells that were kept in the 0.1% FCS, SDF-1 was applied to specified groups. Two days later, the cell lysate was collected, and the amount of cell death was evaluated using the nucleosome assay as described in detail by the manufacturer.

Statistical Analysis—When two groups were compared, the unpaired Student’s t test was applied. When multiple groups were evaluated, the one-way analysis of variance test with post-hoc Student-Newman-Keuls multiple comparisons was used. Statistical significance was set at p < 0.05.

RESULTS

Expression of Chemokine Receptors by Glioma Cell Lines—Sixteen human glioma cell lines were analyzed for the expression of chemokine receptors using RPA. Of the CXCR series, CXCR4 was expressed by 13 of 16 lines, albeit at low levels in some lines (e.g., lines 7, 9, 12, 15, and 16), whereas CXCR3 was undetectable in all samples. Of the CCR series (1, 2a, 2b, 3, 4, 5, and 8), except for low CCR1 expression by 4 of 16 lines, these were uniformly absent in gliomas (data not shown). Collectively, these results demonstrate that the CXCR4 receptor is a major chemokine receptor expressed by glioma cells in culture.

Six glioblastoma specimens were analyzed, and the predominant expression of CXCR4 was also noted in these samples (Fig. 2). Subsequent experiments therefore focused on glioma lines to elucidate the biology of CXCR4 in gliomas.

A potent stimulus of the expression of chemokines or their receptors is inflammatory cytokines, particularly IL-1 and TNF-α (10). Because glioma cells in situ are in close contact with microglia (24), which are principal sources of IL-1 and TNF-α in the CNS (25), we evaluated whether these cytokines, or their combination, could alter the profile of chemokine receptor expression by glioma cells in vitro. Fig. 3 reveals that treatment with IL-1β and TNF-α, alone or in combination, did not alter the profile of chemokine receptor expression. Thus,
IL-1β or TNF-α did not elevate CXCR4 transcript levels in lines (LN827, LN427, and LN992) that express CXCR4 under basal culture conditions and did not induce CXCR4 in a line that is without CXCR4 basally (LN229). Similarly, cytokine treatment did not promote the expression of CXCR1, CXCR2, and CXCR3 in all four lines tested (Fig. 3). The virtual absence of the CCR series was not up-regulated by IL-1β and/or TNF-α treatment (data not shown).

To confirm that the transcripts encoding CXCR4 were translated into receptor protein expression, the LN827 line was analyzed by flow cytometry following staining for the CXCR4 receptor. Fig. 4 indicates that although the CXCR4 receptor expression at the level of protein tended to be low, this was definitively present on the LN827 line. The expression of CXCR4 protein was also confirmed by cell surface immunofluorescence microscopy analysis (Fig. 4).

In summary, of the multitude of chemokine receptors that were analyzed, the majority of glioma cell lines express a major subtype, CXCR4. This and other receptors were not up-regulated by IL-1β or TNF-α.

The CXCR4 Receptor on Glioma Cells Is a Signaling Receptor—To evaluate whether the presence of CXCR4 on glioma cells contributes to the phenotype of glioma cells, it was necessary to demonstrate that the receptor was functional. The only ligand that is known currently for the CXCR4 receptor is SDF-1. Thus, glioma cells lines were treated with SDF-1 and harvested 15, 30, and 60 min thereafter to evaluate whether cellular activation had occurred. Because SDF-1 is known to activate the ERK pathway (26), cell lysates were investigated for the phosphorylation of ERKs. Fig. 5 demonstrates that shortly after the addition of SDF-1, whereas the total amount of ERKs (p42 and p44 MAP kinase) remained unchanged, there was a rapid induction of ERK phosphorylation. Thus, the
CXCR4 receptor on glioma cell lines is a signaling receptor.

**SDF-1α Does Not Appear to Be a Glioma Autocrine Factor**—Glioma cells have a propensity to produce autocrine factors for their own growth and survival. We evaluated whether SDF-1 was produced by glioma cells to interact with their CXCR4 receptors for autocrine functions. Because SDF-1 is a secreted product, we measured the cell-conditioned medium for the amount of this protein. Non-transformed astrocytes were also used, as these are known sources of SDF-1 (27). Fig. 6 reveals that the LN427 and U251N lines secreted minimal amounts of SDF-1 under basal culture condition, and this was not further up-regulated by treatment with IL-1β. Two other lines, U87 and U373, had similarly minimal SDF-1 levels under basal or cytokine-stimulated conditions (data not shown). In the case of the LN827 line, some SDF-1 was detected in basal culture, and this was up-regulated in response to IL-1β. Notably, the amount of SDF-1 in cytokine-treated LN827 culture was similar in all time points analyzed, compared with controls (Fig. 7A). The LN427 and U251N lines did not express SDF-1, and neither did the U87 and U373 lines (data not shown). Finally, the LN827 line was found to produce some SDF-1 under basal culture conditions, and this was promoted by IL-1β. ***, p < 0.001 compared with basal levels of the same line.

**Functions of CXCR4 on Glioma Cell Lines**—To investigate the functional significance of CXCR4 on glioma cells, we focused on the LN827 line and treated its cells with SDF-1α to determine whether a specific phenotype would manifest. It should be noted that all results were reproduced in another glioma line, LN427 (data not shown). Fig. 7A demonstrates that the amount of MMP-2 did not increase following treatment with SDF-1α. In correspondence, the number of cells that transmigrated across matrigel, an indicator of invasiveness that is linked to MMP-2 levels (21), was not different between SDF-1α-treated cells and controls (Fig. 7B).

The mechanisms underlying the invasiveness of cells across matrigel include the degradation of matrigel and associated protease activity (28). These are related but distinct from those that regulate the motility of cells in a two-dimensional manner. The latter involves the formation of focal adhesion complexes and the activation of signaling receptors such as integrins (22, 29, 30). Thus, we used a scratch assay to evaluate whether SDF-1α could regulate the motility of glioma cells. Upon the removal, by scratching, of half a monolayer of cells on coverslips, the cells in the remaining half monolayer migrated promptly across into the barren substratum (22). Hepatocyte growth factor increased the distance that remaining cells migrated across the scratch line in comparison to controls (Fig. 7C), in concordance with the reports that hepatocyte growth factor is a motility factor for glioma cells (31, 32). In contrast, SDF-1α did not increase their motility (Fig. 7C).

Although SDF-1 applied directly to glioma cells did not increase their motility in a two-dimensional assay, it did not
Chemokines and Glioma

![Figure 7: Assessment of glioma phenotype following SDF-1α treatment.](image)

**Panel A** shows that SDF-1 treatment for 24 h did not increase the amount of pro-MMP-2 that is produced. Similarly, the number of transmigrated cells across matrigel was not affected by SDF-1 (panel B). Although hepatocyte growth factor increased glioma motility across a scratch line, SDF-1α did not (panel C). Finally, the proliferation rate of glioma cells was not influenced by SDF-1α (panel D). All values are mean ± S.D. of triplicate analysis and have been replicated in at least two separate experiments and in another cell line, LN427. *****, *p < 0.001 compared with controls.

**DISCUSSION**

Chemokines have been investigated extensively in the context of providing migratory and directional cues for the trafficking of leukocytes. However, these molecules also regulate other important cellular functions such as survival, granule exocytosis, angiogenesis, cell cycle progression, growth, and cell-cell interaction (2, 7, 33). Indeed, chemokines and their receptor systems are being pursued actively as novel cancer therapeutics (34). An important role for chemokines in the CNS is suggested by the early findings that astrocytes are sources of chemokines in experimental autoimmune encephalomyelitis (35) and brain neoplasms (12, 36). Increasingly, it is appreciated that chemokines are found in the normal CNS. Indeed, several chemokines and their receptor pairs are found on different neural cell types, where they are thought to provide for intercellular communication between neurons and glia (reviewed in Ref. 37). This is exemplified in the case of fractalkine (CX3CL1), which has been localized to neurons, whereas its receptor, CX3CR1, has been localized in on microglia (38). After a facial motor nerve axotomy, the number of perineuronal microglia expressing CX3CR1 increased, inviting the possibility that neuronal-derived fractalkine may mediate interactions between neurons and microglia (39).

Because many tumor cell types exploit their environment for their growth, survival, and invasive capacity (40), and given that chemokines are involved in cell-cell communication in the CNS and in tumorigenesis, we have investigated whether specific chemokine receptors may be over-represented in glioma specimens. We have chosen to focus on receptors, rather than on chemokines themselves, because receptor expression on glioma cells would best guide the analyses of which chemokines were important for glioma biology. By using a broad analysis for multiple chemokine receptors, we have found that CXCR4 is a major chemokine receptor on 13 of 16 glioma lines and in six of six resected specimens. CXCR1 and CXCR2 were expressed at lower detection levels, in concordance with previous reports of the detection of IL-8 receptors (CXCR1 and CXCR2) on glioma lines when polymerase chain reaction methods were used for analyses (16). Other receptors, particularly CXCR3

exclude the possibility that SDF-1 would act as a chemotactic factor if introduced as a gradient for glioma cells to migrate toward. Thus, we used a conventional chemotaxis assay where SDF-1α added to the lower chamber was used to attract glioma cells seeded onto the upper chamber; such an assay did not contain extracellular matrix barriers to avoid the need for proteases for invasion. Under these conditions, SDF-1α was chemotactic for the migration of glioma cells (Fig. 8A).

Finally, we addressed whether SDF-1α modulates glioma cell proliferation by examining the proportion of cells that incorporated BrdUrd. Although an average of 28% of control LN827 glioma cells incorporated BrdUrd over 1 h, the treatment of glioma cells with SDF-1α for 24 h did not increase the proportion of glioma cells with BrdUrd (Fig. 7D). Thus, SDF-1α did not regulate glioma cell proliferation. Collectively, these results indicate that signaling through CXCR4 on glioma cells does not regulate glioma motility, invasiveness, and their cell cycle; however, SDF-1α enabled chemotaxis of glioma cells, demonstrating that CXCR4 functions to sense this chemokine for glioma cells to migrate toward.

**SDF-1α and Glioma Survival—An important determinant of tumorigenic potential is the ability of transformed cells to regulate their survival.** We addressed whether SDF-1α could alter cell death following the deprivation of FCS, and its attendant growth factors, from glioma cell medium. Although glioma cells are known to produce a variety of autocrine survival factors, many cells do undergo apoptosis when deprived of the rich growth conditions afforded by 10% FCS in the culture medium, as noted in Fig. 8B. Interestingly, the addition of SDF-1α to the cultures upon FCS removal attenuated cell death. Indeed, the level of apoptosis in SDF-1α (10 and 50 ng/ml)-treated cultures was similar to the low level that was found in 10% FCS medium (Fig. 8B). To support these results, there were noticeably less glioma cells present in serum-free medium in the absence of SDF-1α than in its presence (data not shown).

Thus, SDF-1α is a survival factor for glioma cells. In support, SDF-1α induced a prolonged activation of the survival kinase, Akt, in glioma cells (Fig. 9).
and CCR1 to CCR5, were noticeably absent from glioma specimens.

It should be emphasized that malignant gliomas are remarkably heterogeneous and that mutations for particular proteins are often found in only a subset of tumors. For example, amplification of the epidermal growth factor receptor, considered a common finding in gliomas, is only found in 40–50% of specimens (41, 42). Thus, that 13 of 16 glioma lines (81%) expressed protein for CXCR4 in two glioma lines, CRT-J and U87 (26).

Recent report, expression of SDF-1 and CXCR4 was examined in resected glioma specimens. Both were co-localized in higher grade tumors to regions of angiogenesis, and the authors suggested a function for SDF-1/CXCR4 in gliomas, cells were treated with the α isofrom SDF-1; α and β SDF-1 isoforms are splice variants, but their functions appear similar. Also, SDF-1 is the only known ligand for CXCR4 (44, 45), and a similar profile of deficiencies is found in CXCR4- and SDF-1α-deficient mice (46, 47). We found that the CXCR4-bearing glioma lines enhanced their soft agar colony-forming capability, whereas the treatment with antibodies to CXCR4 and SDF-1α caused inhibition of proliferation. In a more recent report, expression of SDF-1 and CXCR4 was examined in resected glioma specimens. Both were co-localized in higher grade tumors to regions of angiogenesis, and the authors suggested a function for SDF-1/CXCR4 in blood vessel formation (15).

To investigate possible functions of CXCR4 in gliomas, cells were treated with the α isofrom SDF-1; α and β SDF-1 isoforms are splice variants, but their functions appear similar. Also, SDF-1 is the only known ligand for CXCR4 (44, 45), and a similar profile of deficiencies is found in CXCR4- and SDF-1α-deficient mice (46, 47). We found that the treatment of glioma cells with SDF-1α did not alter their proliferative, motility, or invasive properties; however, SDF-1α promoted the chemotaxis of glioma cells in accordance with this well-described property of chemokines. Moreover, we found that SDF-1α protected against apoptosis resulting from serum-deprivation suggesting that it could substitute for deprivation of growth factors when FCS is withdrawn from the culture medium. The mechanism of the SDF-1α-mediated survival involves at least the Akt pathway, because this survival kinase was activated in SDF-1α-treated cultures (Fig. 9). Akt signaling has been linked in many studies to increased cell survival and cancer progression (reviewed in Ref. 48).
the absence of an autocrine loop involving these molecules. This inference must be regarded with caution, because we did not rule out the possibility that amounts of SDF-1 undetected by our protocol may indeed be sufficient to trigger CXCR4 in an autocrine manner. Comparatively, however, astrocytes are a good source of SDF-1, and we postulate that the astrocyte-derived SDF-1 has the potential to activate CXCR4 on glioma cells to promote their survival. Expression of SDF-1α in the CNS in vitro and in vivo is detected predominantly on astrocytes (43, 55, 56), although this chemokine has also been detected in neurons (56). The latter, however, was not reproduced in this study in that fetal human neurons did not secrete SDF-1 into the culture medium. Because glioma cells are in close proximity to astrocytes in vitro, it is possible that these transformed cells activate astrocytes to produce SDF-1 to enhance their survival. This would be a good example of tumors exploiting their surroundings for their own growth advantage.

Nonetheless, this would be an important experiment to understand glioma cells from astrocytes in this mix; this is not a trivial experiment, because many astrocytes and glioma cells are indistinguishable morphologically from one another in culture. Nonetheless, this would be an important experiment to understand the in vivo role of astrocytes in glioma cell motility.

It would be desirable to test directly the hypothesis that astrocytes provide paracrine interaction with glioma cells in this mix; this is not a trivial experiment, because many astrocytes and glioma cells are indistinguishable morphologically from one another in culture. Nonetheless, this would be an important experiment to understand the in vivo role of astrocytes in glioma cell motility.

REFERENCES

1. Moser, B., and Loetscher, P. (2001) Nat. Immunol. 2, 123–128
2. Gerard, C., and Rollins, B. J. (2001) Nat. Immunol. 2, 884–888
3. Moore, M. H., and Armitage, J. N. (2001) Curr. Opin. Cell Biol. 13, 111–117
4. Sabin, A. J., Ganiats, T. G., and Aynardi, C. (2001) Fam. Pract. 18, 291–295
5. Moore, M. H., and Armitage, J. N. (2001) Curr. Opin. Cell Biol. 13, 111–117
6. Sabin, A. J., Ganiats, T. G., and Aynardi, C. (2001) Fam. Pract. 18, 291–295