# An Overview the Role of the CCL2/CCR2/IL-6 Axis in Glioma Progression: Mechanisms, Microglial Reprogramming, and Therapeutic Implications

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**Abstract**:

Gliomas are the most frequently occurring brain cancers defined by aggressive, invasive, and chemoresistant growth patterns. These tumors are responsible for a complex immunosuppressive tumor microenvironment mainly dictated by tumor-associated macrophages, including brain-resident microglia and bone-marrow-derived macrophages. Gliomas utilize the CCL2/CCR2/IL-6 axis to recruit and reprogram microglia to a tumor-supportive phenotype. This review article investigates the role of the CCL2/CCR2/IL-6 axis in glioma pathophysiology through elaborate in vitro, in vivo, and transcriptomic methodologies. Therapy targeting this pathway has yielded promising results so far. For instance, preclinical studies revealed that pharmacological inhibition of either CCR2 or IL-6R reduces tumor-associated macrophages (TAMs) recruitment and impairs glioma growth. Nevertheless, several hurdles remain, including species differences that limit the translation of findings to humans due to inherent differences in immune system regulation and tumor biology between commonly used animal models, such as rodents, and humans. Furthermore, incomplete differentiation between microglia and bone marrow-derived macrophages (BMDMs), and systemic effects due to inhibition of these pathways pose significant challenges. Further studies using human-derived models, spatial transcriptomics, and personalized therapies hold future promise for better treatment modalities for glioma.

## 1. Introduction

Gliomas are the most prevalent primary brain tumors accounting for 30% of all brain tumors and 80% of all malignancies (Zhou et al. 1). They are characterized by aggressive growth, invasiveness, and resistance to standard therapies, including chemotherapy, radiation, and immunotherapy, which leads to high incidence and mortality rates. Brain tumors are usually caused by cancers originating outside of the central nervous system. However, gliomas are primary brain tumors that originate in brain tissues (Zhou et al. 2). ***Figure 1*** down below illustrates the contrast between a healthy brain and glioblastoma.

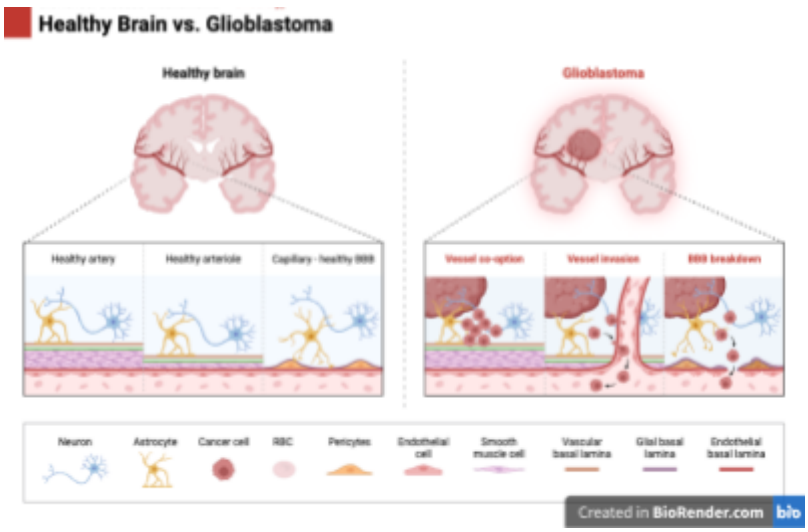

Figure 1. Glioblastoma versus Healthy Brain

The figure above shows the pathological mechanisms of glioblastoma compared to a normal, healthy brain. It emphasizes the structural and cellular differences between a healthy brain and glioblastoma. A glioblastoma is a grade IV glioma, and is highly aggressive. In the healthy brain, blood vessels, neurons, and astrocytes function normally, maintaining the integrity of the blood-brain barrier (BBB). In glioblastomas, key pathological processes occur, including vessel co-option, vessel invasion, and BBB breakdown, contributing to tumor progression and immune evasion. The disruption of the BBB allows immune cells such as bone marrow-derived macrophages (BMDMs) to infiltrate the brain, supporting tumor growth and creating an immunosuppressive tumor microenvironment.

Current treatment options for gliomas typically involve a combination of surgery, radiation therapy, and chemotherapy. Despite these interventions, the prognosis for patients with high-grade gliomas, particularly glioblastoma, remains poor. The median survival for glioblastoma patients is approximately 12-15 months with standard treatment (Hanif et al. 3). Treatment is particularly challenging due to the tumor's location, its ability to infiltrate surrounding brain tissue, and its resistance to conventional therapies. Side effects of current treatments can be severe, including cognitive impairment, fatigue, and decreased quality of life. As seen in ***Figure 1,*** the BBB poses a significant obstacle to drug delivery, making gliomas particularly hard to treat. Therefore, due to the limited efficacy of existing treatments and their associated side effects, there is an urgent need to better understand the biology of gliomas and develop more effective, targeted therapies to improve patient outcomes and reduce mortality rates.

These tumors generate a highly complex, immunosuppressive tumor microenvironment (TME ) in the presence of several immune cells, including tumor-associated macrophages (TAMs) (Zhou et al., 2023). TAMs are made up of both brain-resident microglia, the immune cells of the brain that support development, and bone marrow-derived macrophages (BMDMs). The latter comes from circulating monocytes that infiltrate the brain because of a torn blood-brain barrier, seen in ***Figure 1***, which gliomas cause. Microglia are the central nervous system's (CNS) primary immune cells  and have a dual role in brain health and disease. Under physiological conditions, the wild-type (WT) microglia perform various homeostatic functions such as phagocytosis and synaptic pruning (Recasens et al. 3). However, glioma-derived signals can reprogram these cells into reactive microglia adopting a tumor-supportive phenotype. (Joly-Amado et al. 2).

Microglial activation has been classically described as either classically activated (M1) or alternatively activated (M2). ***Figure 2*** illustrates the classical (M1) activation pathway, in which resting microglia are simulated by microbial-associated molecular patterns (MAMPs) and cytokines to form proinflammatory M1 microglia.

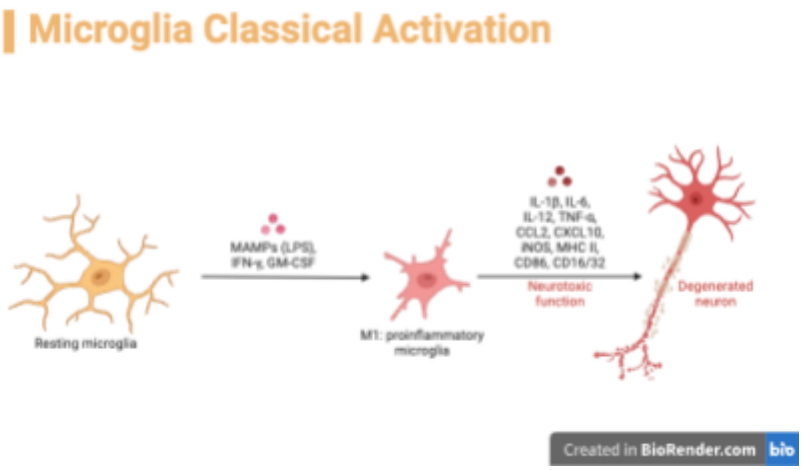


Figure 2. Microglia Classical (M1) Activation and Neurotoxicity

These activated microglia release neurotoxic cytokines which contribute to neuronal degeneration. M1 microglia are considered proinflammatory and neurotoxic, M2s, conversely, are more linked to anti-inflammatory, tissue-repairing activities and secrete factors that will support healing and regeneration. However, it is currently being suggested that such a binary model is too simplistic, for microglia may cover a broad range of activation states with overlapping characteristics (Strizova et al. 1067). Microglial cells play a major role in early-stage gliomas, while in high-grade gliomas, blood-derived monocytes or macrophages dominate microglial contribution (Hanahan & Weinberg; Selenica et al. 2). Gliomas hijack two types of TAMs by reprogramming them through IL-6 and CCL2 cytokine and chemokine signaling, to assume the pro-tumorigenic functions promoting tumor cell proliferation, invasion, and immune evasion (Recasens et al. 5; Zhou et al. 6). The transition of microglia into an M1 proinflammatory state, as depicted in ***Figure 2***, plays a role in shaping the tumor microenvironment, facilitating glioma progression.

**2. Wild-Type (WT) vs Reactive Microglia**

Wild-type  microglia are resident CNS immune cells. They are responsible for maintenance of tissue homoeostasis through phagocytosis, immune surveillance, and regulatory inflammatory responses. Under normal circumstances, these WT microglia would express low levels of inflammatory markers and play a key role supporting brain health and maintenance of the blood-brain barrier (Recasens et al. 2). In the context of glioma, however, tumor-derived factors induce a phenotypic switch and cause these cells to develop into reactive microglia with tumor-supportive characteristics (Joly-Amado et al. 2; Zhou et al. 2).

Among their most common secreted molecules are vascular endothelial growth factor A (VEGF-A), Interleukin-10 (IL-10), and Matrix Metalloproteinases (MMPs). VEGF-A is a secreted ligand that promotes angiogenesis, the process of transforming pre-existing blood vessels into new ones. This process is essential for providing tumors with the oxygen and nutrients needed for rapid growth. VEGF-A secretion by reactive microglia facilitates an environment conducive to glioma progression (Joly-Amado et al. 4). IL-10 is an anti-inflammatory cytokine that suppresses immune response. However, elevated levels contribute to immune evasion by downregulating the activity of other immune components critical for anti-tumor immunity (Recasens et al. 5). MMPs are enzymes that degrade extracellular matrix components. This degradation enables tumor cells to invade tissues and metastasize, enhancing glioma invasiveness and progression (Gruol et al. 2) These molecules, combined with transcriptional changes driven by signaling pathways such as Signal Transducer and Activator of Transcription 3 (STAT3), further exacerbate the tumor-promoting functions of reactive microglia. STAT3 activation, often induced by cytokines like IL-10 and growth factors such as VEGF-A, drives the expression of genes that support immune suppression, angiogenesis, and extracellular matrix remodeling. By sustaining an anti-inflammatory, immune-evasive state and enhancing tumor invasion, STAT3 signaling reinforces the glioma-supportive phenotype of reactive microglia, further facilitating tumor progression.

### 3. CCL2/CCR2/IL-6 Pathway

Glioma cells manipulate microglia through the CCL2/CCR2/IL-6 signaling axis to create a favorable microenvironment for tumor growth. ***Figure 3*** illustrates how the tumor microenvironment (TME) influences tumor-associated macrophages (TAMs) by polarizing them into either M1 or M2 phenotypes, with M1 macrophages exhibiting pro-inflammatory functions that can suppress tumors, while M2 macrophages promote tumor growth by facilitating tissue repair, angiogenesis, and immunomodulation.

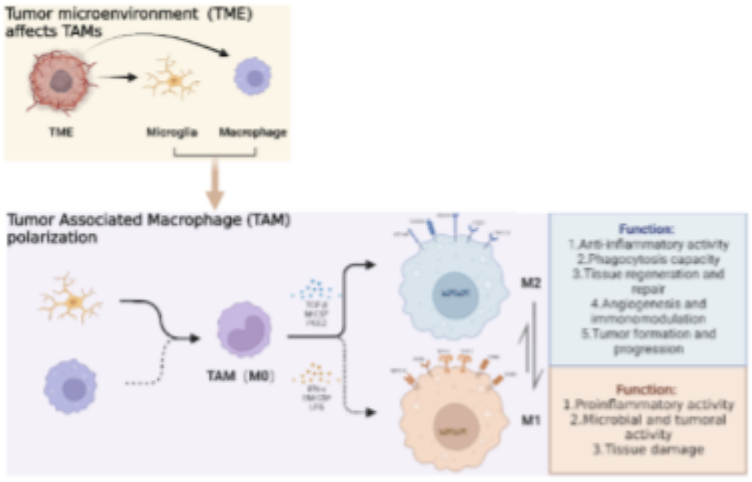


Figure 3. Tumor microenvironment-mediated macrophage polarization in gliomas. (Image from Zhou et al.).

CCL2 or monocyte chemoattractant protein-1 is a chemokine secreted by these glioma cells which binds CCR2 receptors on microglia and monocytes to facilitate recruitment of these immune cells into the tumor site. There, it reprograms them into pro tumor-associated TAMs (Selenica et al. 3; Joly-Amado et al. 3). Simultaneously, glioma cells secrete IL-6, which binds to its receptors IL6R on microglia for activation of the Signal Transducer and Activator of Transcription 3 (STAT3) pathway. This activation leads to transcription of genes suppressing anti-tumor immunity while facilitating angiogenesis and growth of the tumor (Gruol et al. 2; Recasens et al. 6). Given its central role in shaping an immune-suppressive and tumor-supportive microenvironment, the CCL2/CCR2/IL-6 axis has garnered significant attention as a potential therapeutic target, with efforts focused on disrupting this signaling cascade to restore anti-tumor immune responses and inhibit glioma progression. This review aims to elucidate the research studying this axis to highlight a potential avenue to investigate gliomas.

### A. Interaction Between IL-6 and CCR2 Pathways

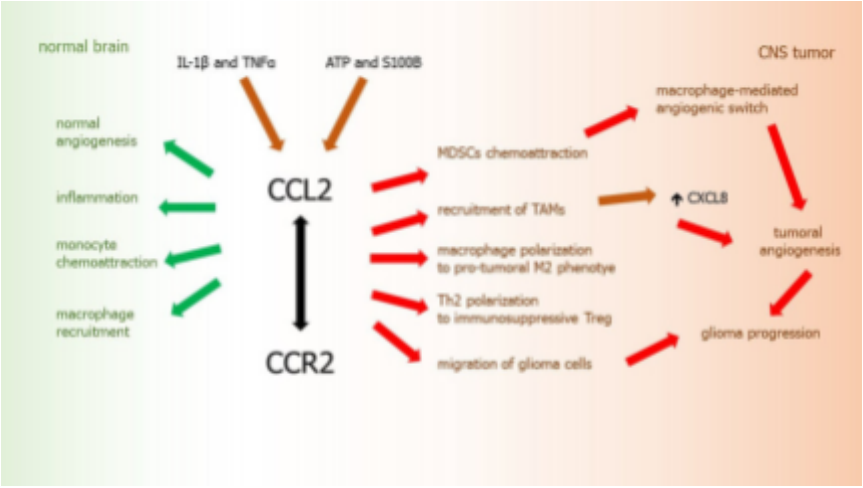


Figure 4. The CCL2/CCR2 Pathway in Glioma Progression
(Image from Groblewski et al.)

The IL-6 and CCR2 pathways work together to sustain glioma progression. IL-6 enhances the production of CCL2 in glioma cells and microglia, increasing the recruitment of $CCR2^{+}$ immune cells to the tumor microenvironment. As shown in ***Figure 4***, the recruited microglia undergo polarizations into TAMs, which can differentiate into either M1 or M2 macrophages depending on the cytokine signals present in the tumor microenvironment. The CCL2/CCR2 axis is critical in glioma progression by mediating immune cell recruitment and polarization within the tumor microenvironment. CCL2, produced by glioma cells and tumor-associated microglia, recruits CCR2+ monocytes and myeloid-derived suppressor cells (MDSCs) into the glioma niche. These immune cells contribute to an immunosuppressive environment by differentiating into tumor-associated macrophages (TAMs), which predominantly polarize toward the M2 phenotype, promoting glioma growth through angiogenesis and immunomodulation. IL-1β and TNFα upregulate CCL2 expression, amplifying immune cell infiltration, while ATP and S100B further drive monocyte recruitment. This pathway is closely

intertwined with the IL-6 signaling cascade, as IL-6 stimulation enhances CCL2 expression, increasing immune infiltration and reinforcing the positive feedback loop that sustains glioma progression.

***Figure 5*** further highlights how IL-6 signaling amplifies this process through STAT3 activation, which reinforced the recruitment of immune cells and the maintenance of a tumor-supportive microenvironment. M2 polarization, which supports tumor progression, is driven by transforming growth factor-beta (TGF-β), macrophage colony-stimulating factor (M-CSF), and prostaglandin E2 (PGE2). In contrast, M1 polarization, which is associated with anti-tumor activity, is induced by interferon-gamma (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and lipopolysaccharide (LPS). IL-6 signaling plays a pivotal role in glioma progression by activating the JAK/STAT3 and MAPK pathways. Binding of IL-6 to its receptor (IL-6R) leads to STAT3 phosphorylation and translocation into the nucleus, where it drives the transcription of tumor-promoting genes, including CCL2. This transcriptional activation further amplifies the recruitment of CCR2+ immune cells to the glioma microenvironment, linking the IL-6 and CCL2/CCR2 pathways in a reinforcing loop. Additionally, IL-6-mediated STAT3 activation enhances the polarization of TAMs toward the M2 phenotype, amplifying the IL-6 signaling by secreting factors that reinforce STAT3 activation. This creates a positive feedback loop that accelerates microglial recruitment, microglial polarization, and glioma progression (Recasens et al. 7; Gruol et al. 5).

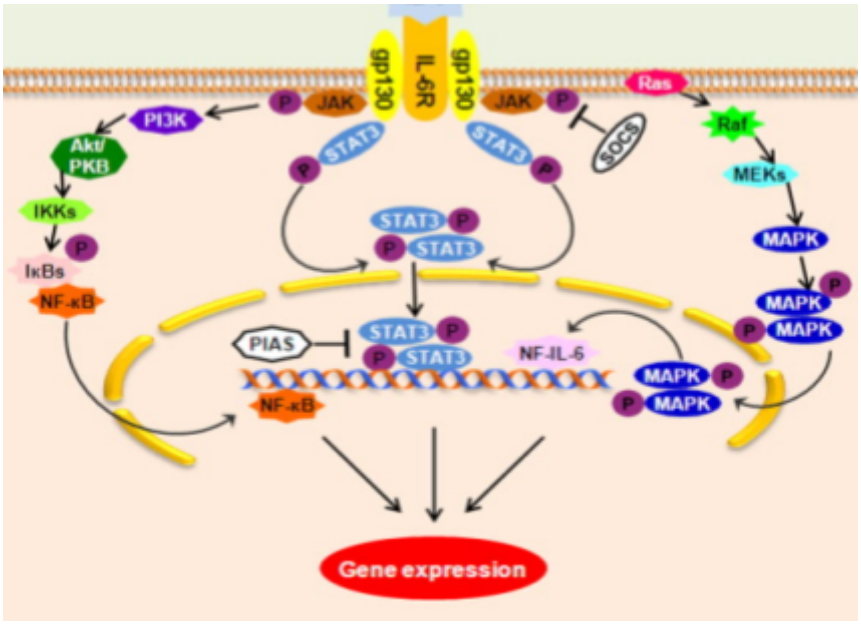


Figure 5. The IL-6 Signaling Pathway in Glioma Progression (Image from Ataei-Kachoei et al.)

Microglial polarization is the process by which microglia adopts an activation state, commonly referred to as an M1 or M2 phenotype. M1 microglia are typically pro-inflammatory and can have anti-tumoral effects, while M2 microglia are associated with anti-inflammatory responses that promote tumor growth. As ***Figure 4*** and ***Figure 5*** emphasize, the bidirectional interaction between these pathways maintains a pro-tumor environment, making the CCL2/CCR2/IL-6 axis a critical therapeutic target. Due to this positive feedback loop and its role in maintaining a pro-tumor microenvironment, the CCL2/CCR2/IL-6 Axis is a potential therapeutic target to mitigate glioma progression. Preclinical studies using CCR2-knockout mice and pharmacological inhibitors have shown reduced microglial recruitment, impaired M2 polarization, and decreased glioma growth (***Figure 4***; Selenica et al. 4). Similarly, IL-6R antagonists like Tocilizumab have demonstrated the ability to block STAT3 activation and suppress other tumor-promoting functions (***Figure 5***; Gruol et al. 4).

Despite these advances, significant gaps remain in understanding the mechanisms that transition the WT microglia into reactive states. Current studies also often rely on murine models, which do not fully recapitulate human glioma biology and the complexities of these signaling pathways which pose challenges for clinical translation. Further research should prioritize patient-derived models to develop targeted therapies that disrupt the CCL2/CCR2/IL-6 axis effectively while minimizing off-target effects.

**B. Common Methodology used to Investigate the CCL2/CCR2/IL-6 Axis**

The methodologies used to study the CCL2/CCR2/IL-6 axis vary significantly in their approaches. As summarized in ***Table 1,*** cellular and molecular studies provide details about gene expression and signaling mechanisms, while animal models offer critical information on *in vivo* tumor behavior and treatment efficacy.

Table 1: Common Methodologies used to Investigate the CCL2/CCR2/IL-6 Axis

| Methodology | Description | Key Experiences | Limitations |
|---|---|---|---|
| **Glioma-conditioned media** | Microglia are cultured with glioma-secreted factors to observe changes in gene expression and phenotype. This simulates tumor-microglia interactions in vitro. | Joly-Amado et al. (Frontiers in Immunology): Exposed microglia to tumor-secreted factors and observed M2 polarization, increased VEGF-A, and IL6R expression. | Does not replicate the complexity of in vivo interactions; lacks contributions from other immune cells and the full tumor microenvironment. |
| **Gene expression analysis (RT-qPCR, single-cell RNA sequencing)** | Measures the expression of target genes related to inflammation, immune suppression, and tumor progression using qPCR techniques or high-throughput single-cell sequencing. | Recasens et al. (Journal of Neuroinflammation): Used RT-qPCR and RNA-seq to identify SOCS3 dysregulation and STAT3 pathway activation in glioma-associated microglia. | Provides molecular-level insights but lacks spatial and temporal information on immune cell interactions; does not account for tumor behavior in vivo. |
| **Animal models (CCR2-knockout mice)** | Mice lacking CCR2 are injected with glioma cells. TAM recruitment, tumor growth, and immune responses are analyzed to determine CCR2's role in glioma progression. | Selenica et al. (Journal of Neuroinflammation): Studied glioma progression in CCR2-deficient mice, showing reduced TAM infiltration, lower IL-6 secretion, and smaller tumors. | Challenges in distinguishing resident microglia from infiltrating bone marrow-derived macrophages (BMDMs) due to shared CCR2 expression; lineage-tracing needed. |
| **Transgenic mouse models (IL-6 and CCL2 overexpression)** | Genetically modified mice overexpress IL-6 and CCL2 under the GFAP promoter, allowing for the study of localized cytokine effects in different brain regions on glioma behavior. | Gruol et al. (Frontiers in Cellular Neuroscience): Used IL-6 and CCL2 overexpression models to analyze glioma invasiveness, regional immune responses, and microglial activation. | Forced overexpression may not reflect physiological conditions; cytokine effects could be exaggerated compared to natural glioma development. |
| **Glioma implantation in immunocompetent mice** | Human or murine glioma cells are implanted in immunocompetent mice to create a tumor microenvironment similar to human gliomas, allowing analysis of immune responses and therapeutic interventions. | Zhou et al. (Frontiers in Immunology): Implanted glioma cells in mice, used pharmacological inhibitors of CCR2 and IL6R to evaluate their role in TAM recruitment and tumor suppression. | Pharmacological inhibitors may have off-target effects; immune system variability in different mouse strains can influence glioma progression outcomes. |

Additionally, studies have used imaging and localization techniques to further clarify the spatial distribution of TAMs within the glioma environment. Glioma-conditioned media experiments, such as those conducted by *Joly-Amado et al.,* revealed that exposing microglia to tumor-secreted factors induces phenotypic shifts towards an M2-like state. These studies demonstrated the upregulation of pro-tumor genes like VEGF-A and IL6R (Joly-Amado et al. 4). While these experiments were conducted in vivo using a mouse model, a key limitation is that glioma-conditioned media studies often isolate specific tumor-secreted factors, which do not fully replicate the complexity of cell-cell interactions, extracellular matrix influences, or vascular components found in a living tumor microenvironment. Although the use of an in vivo model provides physiological relevance, conditioned-media-based approaches may not completely capture the dynamic interplay between glioma cells and surrounding immune components within the intact brain.

Gene expression analyses, including reverse transcription quantitative real-time PCR (RT-qPCR) and single-cell RNA sequencing, provide a more detailed view of the transcriptional changes that occur in glioma-associated microglia. For example, *Recasens et al.* identified the dysregulation of suppressor of cytokine signaling 3 (SOCS3) as a key regulatory failure in reactive microglia, leading to sustained STAT3 activation and chronic inflammation (Recasens et al. 9). However, it is important to note that conditioned-media models and gene expression analysis are not mutually exclusive. As ***Table 1*** outlines, gene expression studies can be applied to multiple model systems, including in vitro cell cultures, in vivo harvested tissues, and computational models. While glioma-conditioned media studies simulate tumor-microglia interaction, gene expression analysis helps to pinpoint the specific molecular disruptions driving these changes, regardless of the model system used. Future studies should integrate both approaches to validate findings across different experimental conditions and provide a more comprehensive understanding of microglial reprogramming in glioma progression.

Animal models have been most instrumental in clarifying the role of the CCL2/CCR2/IL-6 axis in living organisms by incorporating both genetic manipulations and pharmacological interventions to study the progression of gliomas. *Selenica et al.,* used CCR2-knockout mice to investigate the dependency of glioma progression on the CCL2/CCR2 axis. Glioma cells were injected into murine brains, and tumor growth and immune cell infiltration were monitored. The results showed a significantly reduced amount of TAM recruitment, diminished IL-6 secretion, and decreased tumor size in CCR2-deficient mice compared to the wild-type controls (Selenica et al. 4). These findings highlighted the crucial role CCL2 plays in TAM recruitment and glioma progression. As noted in ***Table 1,*** a key challenge lies in differentiating the specific contributions of resident microglia versus infiltrating BMDMs due to shared CCR2 expression.This limitation could be addressed by employing single-cell RNA sequencing or lineage-tracing techniques to better differentiate between these cell types based on their unique molecular signatures or developmental origins. Additionally, temporal studies examining immune cell dynamics during glioma progression could provide further insights into how microglia and BMDMs contribute at different stages.

The study conducted by *Gruol et al.* employed transgenic mice engineered to overexpress IL-6 and CCL2 in astrocytes under the glia fibril acid protein (GFAP) promoter. This approach allowed the examination of region-specific cytokine effects within the CNS. In these models, elevated IL-6 expression was associated with increased glioma invasiveness and altered microglial activation in the cerebellum, while CCL2 overexpression primarily affected the hippocampus, leading to TAM infiltration and angiogenesis (Gruol et al. 3). This methodology could be used to study microglia as well; however, ***Table 1*** notes that forced overexpression models may exaggerate cytokine effects, making it difficult to interpret their physiological relevance in glioma development.

In another approach , *Zhou et al.*, demonstrated that glioma cells derived from human or murine origins were implanted into immunocompetent mice to mimic the natural tumor microenvironment. These models enabled researchers to study specifically how much CCL2 and IL-6 influence tumor growth and immune cell recruitment. Pharmacological inhibition of CCR2 or IL6R in these models reduced TAM recruitment and reprogrammed microglia towards an anti-tumor phenotype while suppressing tumor progression (Zhou et al. 5). However, ***Table 1*** explains that these pharmacological inhibitors may have off-target effects, and immune variability across mouse strains can influence glioma progression outcomes, which is a possible limitation.

**4. Conclusion, Discussion and Future Directions**

This review paper investigates the role of the CCL2/CCR2/IL-6 axis in glioma-microglia interactions and its impact on tumor progression. A comprehensive review of current literature and methodologies identifies that gliomas have actively exploited this signaling pathway to recruit and reprogram microglia into tumor-supportive phenotypes. In particular, studies using experiments with glioma-conditioned media like *Joly-Amado et al.*, have demonstrated that glioma-derived factors induces the polarization of microglia towards the M2-like phenotype, creating an immunosuppressive and tumor-supportive environment. Furthermore, gene expression studies highlight key transcriptional changes, including the activation of STAT3 and upregulation of VEGF-A, IL-10, and MMPs, which further consolidates microglial polarization (Recasens et al. 9). Moreover, models without CCR2 have reduced recruitment of glioma-associated macrophages (GAM) and impaired glioma progression (Selenica et al. 4). Pharmacological inhibition of IL-6 and CCR2 also demonstrates a decrease in the recruitment of tumor-associated macrophage (TAM), thus highlighting the therapeutic potential involved in targeting this pathway (Gruol et al. 3).

### A. Interpretation of Findings

These findings provide strong evidence that the CCL2/CCR2/IL-6 axis is a key promoter of immune evasion and progression in glioma. The studies suggest that CCL2 secreted by gliomas plays a central part in the recruitment of microglia and monocyte-derived macrophages through the CCR2 receptor, which significantly adds to the population of tumor-associated macrophages (TAMs) within the tumor microenvironment. ***Figure 4*** and ***Figure 5*** demonstrate how this recruitment process is mediated by CCL2 signaling and is amplified by IL-6 driven STAT3 activation. These recruited microglia are subjected to IL-6-mediated activation that augments STAT3 signaling, promoting the secretion of pro-tumorigenic molecules such as VEGF-A and MMPS thereby facilitating angiogenesis and tumor invasiveness (Recasens et al. 6; Joly-Amado et al. 4). Together ***Figure 4*** and ***Figure 5*** illustrate the positive feedback loop between IL-6 and CCL2 that exacerbates glioma progression, solidifying its role as a viable therapeutic target.

Moreover, the research studies reviewed revealed that STAT3 activation in microglia leads to immune suppression by downregulating anti-tumorigenic M1-like microglia, while promoting M2-like functions at the same time (Gruol et al. 5). In addition, the pharmacological inhibition of IL-6R (e.g., Tocilizumab) has been shown to suppress STAT3 activation, suggesting potential avenues for therapeutic intervention (Gruol et al. 4). The results presented are consistent with earlier observations demonstrating that inhibition of STAT3 permits reprogramming of microglia towards an anti-tumorigenic phenotype (Gruol et al. 5). Further research is, however, required to establish the long-term consequences and specificity of such interventions.

### B. Implications of Findings

The data suggests that targeting the CCL2/CCR2/IL-6 axis could be a promising strategy for preventing glioma proliferation and spread. Because TAM-induced immune suppression is crucially dependent on gliomas, disrupting TAM recruitment and reprogramming can switch the TME to an anti-tumorigenic state. CCR2 inhibitors and IL-6 antagonists have been reported with promising outcomes in preclinical research, suggesting their potential inclusion in combination therapies alongside immune checkpoint inhibitors (ICIs) (Selenica et al. 5). Additionally, the findings highlight the importance of early intervention. Since microglial reprogramming takes place during the initial phases of glioma development, therapeutic approaches that focus on the inhibition of TAM recruitment through CCR2 blockade, could potentially have greater impacts than those that try to reverse M2 polarization after TAMs have already been established in the TME (Joly-Amado et al. 5). As such, patient stratification based on tumor stage and immune profile should be incorporated into the clinical trial design of CCL2/IL-6-targeting therapies.

### C. Limitations of the Current Methodologies

While potential information from such studies is encouraging, there are certain limitations that must be addressed: ***Table 2*** summarizes descriptions, advantages, and limitations of each approach, along with potential solutions to overcome key challenges. These methodologies range from in vitro models (e.g., glioma-conditioned media) to in vivo models (e.g., CCR2-knockout mice, transgenic models, patient-derived xenografts), providing different levels of insight into glioma progression and immune interactions.

Table 2: Overview of experimental methodologies used in glioma research to study the CCL2/CCR2/IL-6 axis.

| Methodology | Description | Advantages | Limitations | Potential Solutions |
|---|---|---|---|---|
| **Glioma-Conditioned Media Experiments** | In vitro model where microglia are exposed to glioma-secreted factors | Provides controlled conditions for studying molecular mechanisms | Lacks complexity of in vivo models, missing TME interactions, extracellular matrix influences, vascular components, and immune dynamics | Use in vivo models like GEMMs, patient-derived xenografts (PDXs), and humanized mouse models for better clinical relevance |
| **Gene Expression Analysis (RT-qPCR, Single-Cell RNA Sequencing)** | Identifies molecular and transcriptional changes in microglia | High precision in detecting gene regulation changes | Does not capture protein-level modifications or spatial interactions | Combine with proteomics, spatial transcriptomics, and advanced imaging techniques |
| **CCR2 Knockout (KO) Mouse Models** | Genetic knockout of CCR2 to assess its role in TAM recruitment | Demonstrates direct effect of CCR2 inhibition on TAM infiltration | Cannot differentiate between resident microglia and BMDMs, limiting insights into specific immune cell dynamics | Use single-cell lineage tracing and spatial transcriptomics to separate cell origins and analyze immune populations in gliomas |
| **IL-6 Overexpression Models (Transgenic Mice)** | Overexpresses IL-6 to study its effect on microglial activation | Helps in understanding IL-6's role in tumor progression | May overstate IL-6's relevance in gliomas, potentially exaggerating cytokine effects | Use inducible expression systems to regulate cytokine levels and avoid artificial overexpression artifacts |
| **Patient-Derived Xenografts (PDX)** | Human glioma cells implanted into mice | Closely mimics human glioma microenvironment | Expensive, limited availability of patient samples | Expand use of humanized mouse models to better replicate human gliomas and immune interactions |
| **Pharmacological Inhibition (CCR2, IL-6R Blockers)** | Uses small molecules or antibodies to block pathways | Demonstrates therapeutic potential of blocking the CCL2/IL-6 axis | May cause systemic immune suppression and off-target effects | Develop nanoparticle-based drug delivery for targeted inhibition while minimizing systemic toxicity |

1. **Reductionist Nature of *In Vitro* Models**: Glioma-conditioned media-based experiments do not fully capture the complexity of the in vivo tumor microenvironment (TME), as they lack key factors such as cell-cell interactions, extracellular matrix influences, vascular components, and immune dynamics (Joly-Amado et al. 6). While these models provide controlled systems for studying molecular mechanisms, their clinical translatability remains limited. Even more advanced in vitro models, such as 3D organoids and patient-derived tumor explants, still fail to fully replicate the structural and cellular diversity of gliomas in living organisms. To address these limitations, researchers should prioritize in vivo studies using genetically engineered mouse models (GEMMs), patient-derived xenografts (PDXs), and humanized mouse models, which better recapitulate glioma progression and the interaction between tumor cells and the immune system. These models provide critical insights into the dynamics of TAM recruitment, microglial polarization, and drug responses within a physiologically relevant TME. Additionally, combining in vivo approaches with advanced imaging techniques and spatial transcriptomics can further elucidate the spatial organization and functional heterogeneity of glioma-associated immune cells, leading to more precise therapeutic targeting.

2. **Species-Specific Differences in Animal Models:** As noted in ***Table 2***, Murine models are informative, but they do not fully replicate the biology of human gliomas. The research covered here mostly used mice gliomas, which have different immune microenvironment dynamics than human tumors (Selenica et al. 4). A clinically relevant model such as patient-derived xenografts (PDX) or humanized mouse models, would better capture the heterogeneity of human gliomas and the complexity of immune interactions within the tumor microenvironment. These models would enable a more accurate assessment of how human TAMs respond to therapeutic interventions. Furthermore, integrated single-cell RNA sequencing in these models could provide deeper insights into patient-specific immune responses to refine the development of personalized therapies.
3. **Challenges in Microglial vs. Monocyte-Derived Macrophage Differentiation:** ***Table 2*** highlights that CCR2-knockout models effectively demonstrated reduced TAM recruitment but were not able to discern resident microglia and BMDMs (Selenica et al. 4). Single-cell lineage tracing along with spatial transcriptomics is a necessary step in future studies to precisely map immune cell populations. Spatial transcriptomics would allow researchers to determine the spatial organization of microglia and BMDMs within the glioma microenvironment, providing critical insights into how these populations interact with tumor cells and other immune components. By incorporating this technique, studies could identify distinct gene expression profiles unique to each cell type in their native context. This would help uncover novel molecular targets for therapeutic intervention. This approach would also improve the understanding of how TAM populations evolve during glioma progression.
4. **Systemic Effects of CCR2 and IL-6 Blockade:** Inhibitors of CCR2 and IL-6 are promising; however, total inhibition of these pathways will lead to unexpected side effects, i.e., immunosuppression or aberrant inflammatory responses (Gruol et al. 6). A complete knockout of these pathways may disrupt essential immune functions beyond the tumor microenvironment, so a knockdown approach would allow for partial suppression. This would reduce tumor-supportive signaling while preserving necessary immune activity. As indicated in ***Table 2,*** one potential solution is the development of nanoparticle-based drug delivery to enhance targeting, thereby minimizing off-target effects and systemic toxicity. Targeted treatment strategies help minimize off-target effects by concentrating therapeutic agents within the tumor site to reduce systemic toxicity. This enhances treatment efficacy by ensuring that glioma cells and their supportive microenvironment are selectively disrupted while sparing healthy tissues.

### D. Future Directions

Future studies should focus on enhancing human-like glioma models by employing an *ex-vivo* patient and mouse organoid-like system. These models would enable studying glioma's interaction with microglia and how drugs behave in a more physiologically relevant manner. In addition, the use of scRNA-seq and spatial transcriptomics will enable the clarification of how microglia actually become activated and how gliomas develop. Furthermore, exploration of combinatory treatments that merge CCR2/IL-6 blocking with immune checkpoint blockade, such as anti-programmed death-1 (PD1) therapy, can potentially render immunotherapy effective. Since gliomas are exceedingly heterogeneous, individualized medicine strategies must be aimed at categorizing patients based on their glioma immune microenvironment. Clinical trials must utilize CCL2 and IL-6 biomarkers, for personalizing treatments to specific patient populations, to minimize side effects, and improve treatment responses.

**E. Conclusion**

Major findings indicate that CCL2 attracts immune cells, and IL-6 stimulation of STAT3 enhances pro-tumor activity, such that this axis may represent a worthwhile therapeutic target. Preclinical studies demonstrate that CCR2 or IL-6R inhibition could reduce TAM recruitment and glioma expansion, though there are challenges in translating these findings to human treatments. There are still knowledge gaps regarding what makes microglia tumor permissive. Patient models, drug-delivery targeting, and combinatory regimens using CCR2/IL-6 inhibitors and checkpoint blockades are  areas of interest to be focused on with future research. An ideal treatment may lie in intervention at an earlier time point than this, and prior to TAM recruitment, given glioma aggressiveness. Developing personalized treatments based on biomarker-derived patient stratification may significantly improve glioma outcomes.

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