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Review

Microglia in pediatric brain tumors: The missing link to successful immunotherapy

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SUMMARY

Brain tumors are the leading cause of cancer-related mortality in children. Despite the development of immunotherapeutic strategies for adult brain tumors, progress in pediatric neuro-oncology has been hindered by the complex and poorly understood nature of the brain's immune system during early development, a phase that is critical for the onset of many pediatric brain tumors. A defining characteristic of these tumors is the abundance of microglia, the resident immune cells of the central nervous system. In this review, we explore the concept of microglial diversity across brain regions and throughout development and discuss how their maturation stage may contribute to tumor growth in children. We also summarize the current knowledge on the roles of microglia in common pediatric brain tumor entities and provide examples of myeloid-based immunotherapeutic strategies. Our review underscores the importance of microglial plasticity in pediatric brain tumors and its significance for developing effective immunotherapeutic strategies.

INTRODUCTION

Microglia are the main resident myeloid cells of the central nervous system (CNS) and play a crucial role in the surveillance, development, and maintenance of the brain. As the primary immune sentinel of the brain, microglia are largely responsible for orchestrating immune responses against pathogens or damaged cells.[1] Although historically considered "the macrophages of the brain," microglia arise from distinct precursor cells compared with bone marrow-derived macrophages and perform tasks well beyond the innate immune cell realm.[2–4] Precursor microglia originate in the yolk sac and colonize the CNS during embryogenesis, where they form the predominant source from which mature microglia arise through life. Microglia reach their peak numbers during fetal development and early infancy, where they execute essential neurodevelopmental tasks, such as phagocytosis of neurotoxic waste, pruning of neuronal networks, and astrocyte modulation.[5] As a result, children below the age of five harbor a distinct microglial landscape when compared with older ages, and therefore CNS malignancies that develop prenatally or during early infancy are likely to encounter divergent microglial subpopulations that may play a driving role in tumor development.[6–8] However, the role of developmental microglia in pediatric brain tumors is not well understood, despite a noteworthy increase in interest in this topic. This is likely due to the limited tools and model systems to study microglia during brain development. Furthermore, microglia are influenced by a large variety of extrinsic factors of the cellular microenvironment and show different phenotypes depending on their location in the brain, even further increasing the complexity of pediatric brain tumor-associated microglia.[9,10] Grasping these immune dynamics in pediatric brain tumors is crucial for the design of immunotherapeutic approaches tailored to this patient population.

In this review, we explore the current comprehension of microglia's involvement in pediatric brain tumors and the potential therapeutic implications of targeting these cells. We discuss shared characteristics and distinctions among various tumor types, including medulloblastoma, atypical teratoid/rhabdoid tumor (AT/RT), ependymoma, pediatric Histone 3 (H3)-wild-type high-grade glioma (HGG), diffuse midline glioma (DMG), and other (low-grade) pediatric gliomas. Furthermore, we underscore prospective research directions crucial for unraveling the intricate role of microglia within the tumor's immune microenvironment.

MICROGLIAL DIVERSITY ACROSS THE DEVELOPMENTAL STAGES OF THE BRAIN

Microglia exhibit a broad functional diversity and can be classified into multiple subtypes based on their spatial and temporal characteristics.[4,6–8,10–12] This functional diversity is particularly evident during embryonic brain development, when microglia undergo various stages before reaching their mature state after birth.[13,14] Studies in rodents have shown that microglia stem from the yolk sac and colonize the CNS through the first primitive hematopoietic wave at embryonic day 7.5 (E7.5), independent from monocyte precursors.[15] This early colonization of the neuroepithelium sets microglia apart from tissue-resident macrophages, which arise at E10.5 from the transient definitive

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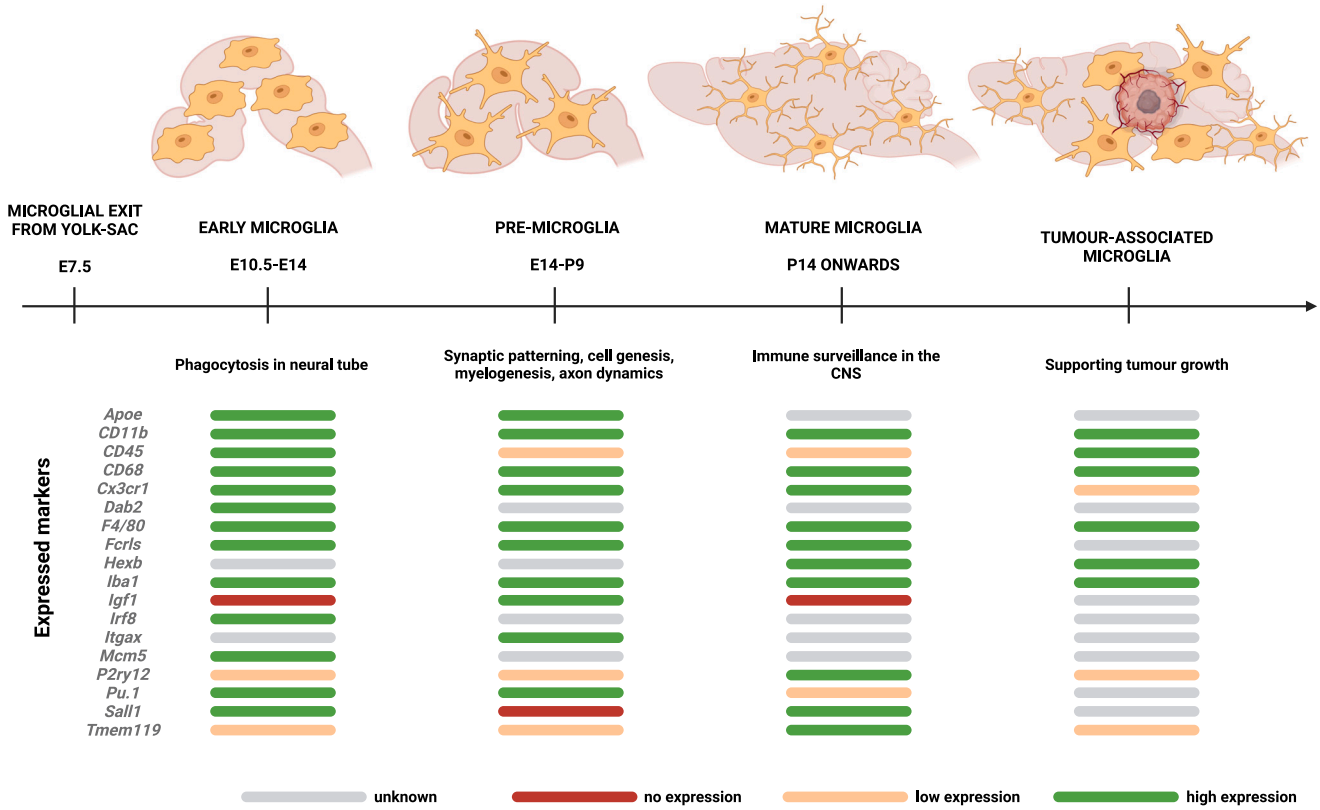

Figure 1. Phenotypic heterogeneity of microglia across brain development in mice

Following the exit of the yolk sac on embryonic day 7.5 (E7.5), the first microglial cells populate the murine brain. On E10.5–E14, microglia have an ameboid morphology and are predominantly involved in phagocytosis of the neural tube (early microglia). From E14 to postnatal day 9 (P9), microglia adopt a transient phenotype and are focused on synaptic and axon dynamics (premicroglia). Microglia from P14 present a mature ramified phenotype and are now able to perform immune surveillance tasks as the main immune effector the CNS (mature microglia). Also represented are some of the markers commonly expressed by microglia in different stages of brain development and beyond. With the occurrence of a CNS malignancy, microglia often adopt a more immature, ameboid morphology; lose some of their phenotypical markers; and largely support tumor growth.

wave of hematopoiesis.[16] Microglia initially colonize the subventricular zones, which contain a high number of neural stem cells, and then migrate toward the periphery of the brain. During this process, microglia harbor sequential developmental gene expression signatures, each associated with their corresponding roles throughout brain development (Figure 1).[17] Ameboid early microglia, predominantly found from E10.5 to E14, are thick, short-branched cells that are mainly involved in phagocytosis of debris of apoptotic neurons in the developing brain. Transitional premicroglia are found from E14 to postnatal day 9 (P9) and are mainly involved in neuronal developmental processes such as synaptic patterning, myelinogenesis, and axon dynamics. Microglia reach peak numbers at P14 at the subventricular zones in the midline structures of the brain and then disperse throughout the rest of the CNS, reaching a mature load and a resting (ramified) phenotype from P28 onward.[5,18,19] Ramified microglia have small cellular bodies and long branching arms, which continuously survey the surroundings for injuries or infections. When encountering pathogens or internal stressors, ramified microglia become activated (or reactive), adopt a more ameboid morphology, and rapidly orchestrate a local immune response. However, microglia activity is not simply an on or off switch but rather a spectrum of different functional states that can be influenced by various cues from the microenvironment.[9,10] Microglia can adopt a range of phenotypes, each associated with the expression of different markers, secretion of different factors, and extent of phagocytic activity. With the help of improved single-cell sequencing techniques, additional subtypes and phenotypic states of microglia are being identified, and more are expected to be discovered in the future.

DISCRIMINATING BETWEEN MACROPHAGES AND MICROGLIA REMAINS A TOPIC OF DEBATE

Microglia and macrophages are often studied as one cell population based on the assumption that both cell types have the same function in the brain tumor immune microenvironment. However, the spatial distribution and role of these cell types within tumors is debated,[20,21] and studies have called into question the traditional markers used to differentiate between the two populations.[10,22–27] The distinction between the two is not clear-cut, as both can adopt similar morphologies and gene expression profiles, particularly when faced with the same pathological

burden. For example, the field of neuro-oncology has traditionally relied on the expression of CD45 and CD11b to separate CD11b/CD45^{low} microglia from CD11b/CD45^{high} macrophages. However, microglia have been shown to increase CD45 expression in conditions of disease and to become indistinguishable from innately CD45^{high} macrophages.[28] This is particularly relevant in neuro-oncology, as microglia upregulate CD45 expression in hypoxic environments, which are common in high-grade brain tumors.[29] More recently, advanced profiling analyses have introduced other microglia-specific markers, like TMEM119 and P2RY12, which are currently two of the most commonly used markers to discriminate resident microglia from blood-derived macrophages in the human and murine brain.[30–34] However, emerging evidence casts doubt on their reliability, given that both microglia and macrophages can alter the expression of these markers under specific pathological circumstances, such as integration into the tumor environment.[29,35–37] As additional markers are continuously being discovered, it is important to note that a substantial portion of these markers are identified under the assumption, subject to ongoing debate, that the expression patterns of conventional markers like CD45 differentiate between microglia and brain-infiltrating macrophages. Moreover, this delineation often lacks a direct comparison between microglia and the macrophages that infiltrate the brain.[29] It is important to establish better expression profiles of myeloid subpopulations in pediatric brain tumors and to consider the model systems used when interpreting results from previous studies. The field of research is still in its infancy, and the in-depth functional differences between the two types of myeloid cells in the tumor are not covered in this review, but the importance of such a distinction is acknowledged for future studies.

PRENATAL OR EARLY POSTNATAL MICROGLIA MAY BE CRITICAL FOR THE ONSET OF CHILDHOOD BRAIN TUMORS

Many pediatric brain tumors are thought to arise during prenatal brain development due to genetic perturbances during critical developmental windows.[38–41] Coincidentally, these pediatric CNS tumors emerge during a period where unique immature microglia subpopulations are abundantly represented, suggesting a potential role in tumor onset and progression. Additionally, many pediatric brain tumors are characterized by high numbers of tumor-associated microglia, often with aberrant morphologies and phenotypes, suggestive of a crucial role in the tumor immune microenvironment (Figure 1). Developmental tracing studies have shown that microglia reach their peak concentration in the midline of the brain in newborns, with many microglia in this region still in an embryonic, immature state.[14,42] This could have significant implications for understanding the biology of brain tumors in young children, which often occur in midline structures. Therefore, the role of immature microglia in the tumor immune microenvironment of pediatric CNS malignancies is of great interest.

An illustration of the potential involvement of distinct immature microglial subgroups in the onset of pediatric brain tumors can be observed in medulloblastoma and DMG. In medulloblastoma, microglia found in the cerebellum and corpus callosum at P3–P5 have been shown to highly express IGF1, promoting the expansion and survival of cerebellar granule neural precursor cells, which are precursors of sonic hedgehog (SHH)-subtype medulloblastoma.[1,43,44] As such, these studies put forward this subtype of developmental microglia as a catalyst for SHH medulloblastoma development in young children. On the contrary, microglia beyond this developmental stage have been shown to exhibit anti-tumoral activity in SHH medulloblastoma. Tumor-associated myeloid cells isolated from a fully developed murine SHH medulloblastoma model (NeuroD2:SmoA1) were found to increase tumor cell killing.[45] This suggests that microglia can adopt either an anti- or pro-tumor phenotype depending on their maturation stage, with immature microglia favoring tumor initiation and progression, while myeloid cells present at a later stage favor tumor cell killing. While briefly introduced in this section, a comprehensive exploration of medulloblastoma subtypes and their immune microenvironments is subsequently provided in the ensuing sections of this review.

Another illustration of how immature microglia may play a role in tumor growth is by mimicking epigenetic aberrations found in tumor cells, as seen in DMG, which is discussed in depth in the following sections of this review. DMG cells typically contain a mutation in lysine 27 of histone 3 (H3K27M), which leads to a loss of di-/trimethylation at this lysine position. As a result of this epigenetic modification, the chromatin structure changes, leading to sustained expression of stemness-related genes.[46] Recent studies found that microglia in DMG do not harbor the H3K27M mutation but do demonstrate the loss of lysine 27 methylation seen in DMG cells, thereby contributing to a more stem cell-like phenotype of these tumor-associated microglia.[47,48] In many cases, pediatric brain tumors develop a dependence on certain stem cell processes occurring during embryogenesis.[49] It may be worth investigating whether immature microglia mimicking epigenetic changes in tumor cells are involved in this process and thereby contribute to tumor development.

Currently, details on the interaction between microglia and brain tumors arising in the embryonic and early postnatal brain remain largely unknown. On the one hand, the lack of robust experimental setups, which include specific markers for microglia at different developmental stages, hinders the discovery of how this population of cells behaves when encountering a developing tumor. On the other hand, especially in humans, there is still uncertainty regarding the exact moment of tumor onset during early development because of the difficulty in detecting and studying tumor development *in utero*, although many studies now point toward a prenatal onset.[38–41,50,51] The possibility of tumor dormancy is another element to take into consideration, further complicating the identification of the moment in time that the immune system makes first contact with the tumor. Thus, scientists remain unknowing of the exact developmental stage in which microglia encounter the malignancy.

Thus, while research has provided some initial insight into the role of microglia in the development of pediatric CNS malignancies, more studies are needed to fully understand the intricate relationship between immature microglia and the development and progression of these diseases.

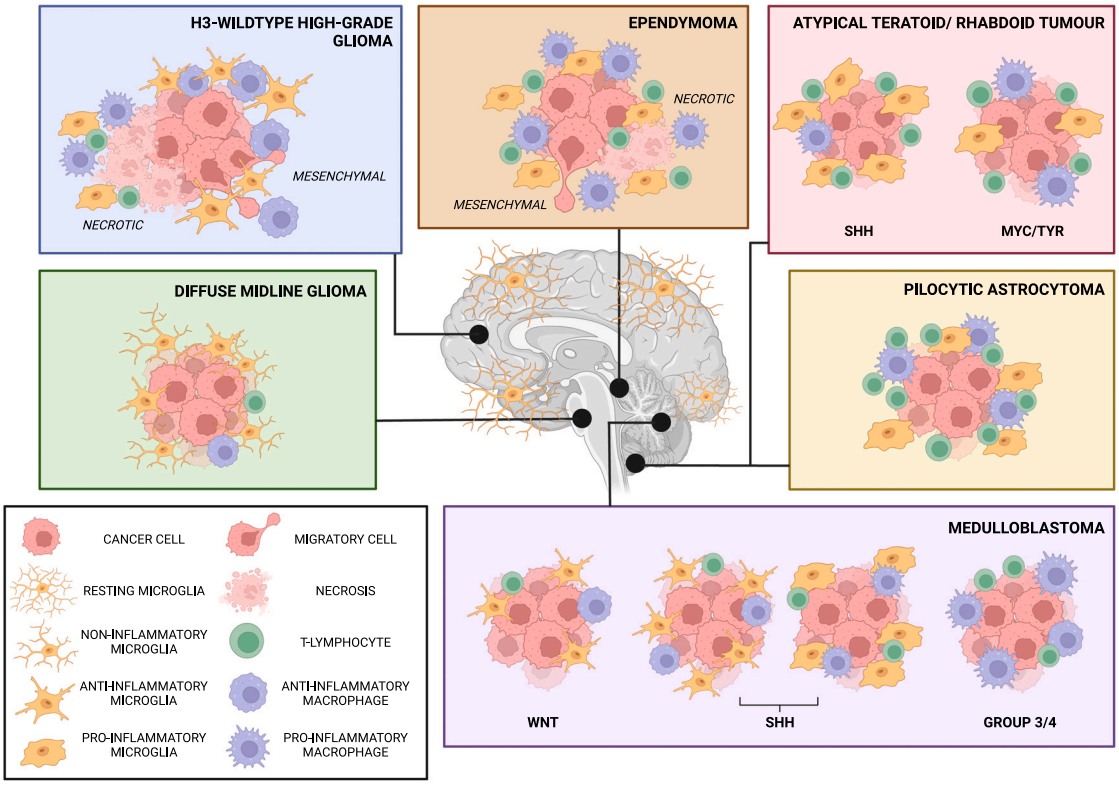

Figure 2. Overview of the immune microenvironment in different brain tumor entities

Ependymomas, pilocytic astrocytomas, and atypical teratoid/rhabdoid tumors typically present a more pro-inflammatory immune microenvironment with M1-like myeloid cells and considerable presence of T lymphocytes. Pediatric H3-wild-type high-grade gliomas (or glioblastomas) and medulloblastomas, on the other hand, are generally more anti-inflammatory, with M2-like myeloid cells and very few T lymphocytes, although differences are found between different subtypes of a tumor entity. Also, within one tumor entity, such as in high-grade glioma and ependymoma, diverse immune responses can be observed in different areas of the tumor, such as in necrotic plaques. In DMG, a dampened inflammatory milieu has been described that is not fully anti-inflammatory (in contrast to other high-grade gliomas) and has therefore been denoted as non-inflammatory. Note that non-inflammatory microglia in DMG are morphologically different from ramified (resting) microglia that are present in the healthy brain, as they demonstrate an activated morphology with an enlarged cell body and fewer cell processes, as seen in other pediatric brain tumor entities. The cell types that are presented and their relative numbers are an approximation based on the studies described in this review.

THE ROLE OF MICROGLIA IN PEDIATRIC CNS MALIGNANCIES IS REGION, CONTEXT, AND TUMOR SPECIFIC

Microglial populations in the brain vary not only by developmental stage but also by location within the CNS. Different microglial subsets have been identified in different regions of the brain, with varying densities, morphologies, molecular signatures, and functions.[11,52–54] This is particularly relevant in pediatric brain tumors, which have a strong preference for certain locations in the brain and may therefore encounter specific microglia subsets that are more or less susceptible to support tumor growth. Additionally, some microglial subtypes have only been found in certain disease states, such as in neurodegenerative disorders like Alzheimer's disease.[55] The region and disease specificity of microglia in the brain highlights the relevance of considering pediatric CNS malignancies individually to understand the functions of microglia within the tumor immune microenvironment. Various studies have shown that the immune landscapes of all pediatric brain tumors combined do not correlate with tumor grade, mutational load, mesenchymal/epithelial phenotype, or patient outcome.[56,57] However, within a single diagnosis, microglia phenotypes are often associated with certain tumor hallmarks. In the following paragraphs, we will discuss in more detail the myeloid immune landscapes described for the most common brain tumors in children: medulloblastoma, AT/RTs, ependymoma, pediatric H3-wild-type HGG, DMG, and other (low-grade) pediatric gliomas (Figure 2).

Medulloblastoma

Medulloblastomas have been classified into four main subgroups: WNT, SHH, group 3, and group 4.[58] In general, medulloblastoma tumors are characterized by low numbers of infiltrating immune cells.[56,59,60] Within the immune cell fraction, the SHH subtype has the highest concentration of myeloid cells, while group 3/4 medulloblastomas have more $CD8^+$ T cells.[22,61] The SHH and WNT subgroups also have a higher number of microglia compared with groups 3 and 4, which contain more bone-marrow-derived macrophages.[22] Within the SHH and WNT subgroups, myeloid cells in WNT tumors have been found to

harbor an anti-inflammatory phenotype (as determined by an M2-like gene signature), in contrast to the pro-inflammatory phenotype (M1-like gene signature) of myeloid cells in SHH tumors. The pro-inflammatory nature of myeloid cells in SHH medulloblastoma tumors is also seen in the aforementioned NeuroD2:SmoA1 tumor model, where there is a strong presence of ameboid Iba1$^+$ cells.[45] These myeloid cells have an anti-tumor phenotype, as their co-culture with tumor cells enhanced tumor cell killing. However, another study reported that SHH medulloblastoma tumors express higher levels of anti-inflammatory markers (e.g., CD163 or transforming growth factor β [TGF-β]), which is associated with a tumor-supporting phenotype.[62] This study proposed that myeloid cells are reprogrammed by highly proliferating tumor cells. Other studies also highlight the key role of tumor-derived factors in medulloblastoma in the polarization of myeloid cells into a pro-tumor phenotype.[63] In a *Ptch1*$^{+/-}$ *Trp53*$^{-/-}$ SHH medulloblastoma mouse model, it was shown that myeloid cells only contribute to tumor growth in the presence of a reciprocal tumor-derived environmental influence.[64] Single-cell RNA sequencing performed on CD11b$^+$ myeloid cells obtained from this mouse model also confirmed that these tumor-supporting myeloid cells in SHH medulloblastoma have specific alterations in gene expression as a result of their interaction with the tumor. Furthermore, astrocytes in the tumor immune microenvironment have been reported to secrete interleukin-4 (IL-4) and to promote the microglial secretion of IGF1, which in turn is linked to tumor progression, indicating that not only tumor cells but also the astrocytes in the tumor immune microenvironment may dictate the myeloid phenotype in medulloblastoma.[44] Notably, the seemingly contradicting immune landscapes within medulloblastoma subtypes may be attributed to age-related differences, as the immunoreactivity pattern in medulloblastoma was found to be different between 0- to 3-, 4- to 10-, and 11- to 18-year-old children.[65] This study implies that differentially targeting tumor-associated myeloid cells is crucial in different age groups of patients and further points to an association between age of onset and microglial phenotype. Taken together, the myeloid phenotype and response in medulloblastoma appear to be highly heterogeneous, with each individual tumor dictating a specific gene expression pattern and actively inducing a certain shift in the immune landscape.

AT/RTs

AT/RTs are divided in three subgroups: MYC, SHH, and TYR. In all groups, a moderate presence of tumor-associated (CD68$^+$) myeloid cells has been observed, along with elevated CD8$^+$ T cell levels, indicating a pro-inflammatory tumor immune microenvironment.[49,66] Examination of the different subgroups revealed that the SHH subgroup has the highest concentration of CD68$^+$ myeloid cells and that the myeloid cell population in this subgroup primarily consists of microglia (as determined by the microglia-specific marker P2RY12).[67] No significant differences in the infiltration of CD8$^+$ T cells were found among the subgroups. It is also noteworthy that in AT/RTs, a high number of P2RY12$^+$ microglia is correlated with a better overall survival. Since there is a lack of extensive research on microglia and the myeloid immune response in AT/RTs, further in-depth analysis of the myeloid cell population in this type of tumor should be performed.

Ependymoma

Ependymomas are subclassified according to the location of tumor onset, leading to a range of different ependymoma malignancies. However, in many studies, no distinction is made between these subtypes, except for posterior fossa A (PFA) ependymoma. In general, ependymomas are characterized by the presence of a significant amount of tumor-associated myeloid cells, representing up to 30% of the tumor mass.[56] In a transcriptional study carried out by Griesenger et al., ependymomas were shown to have a pro-inflammatory immune microenvironment, with high levels of CD8$^+$ T cell infiltration and a high CD8:CD4 T cell ratio.[56] Furthermore, ependymomas are found to overexpress myeloid activation genes (e.g., HLA-DR and IBA1/AIF1) and underexpress myeloid dedifferentiation and suppression markers (e.g., CD50 and CD163).[56,68] Furthermore, it was found that overexpression of IBA1 is linked to better patient outcomes.[68] Overall, these studies suggest that pro-inflammatory activation of myeloid cells in ependymoma has a negative effect on tumor growth. It has been hypothesized that this also contributes to the non-recurring nature of some ependymoma tumors.[68] However, it is important to note that the pro-inflammatory activation state of myeloid cells in ependymoma may not always be associated with anti-tumoral effects. For example, in the PFA subgroup of ependymoma, overexpression of pro-inflammatory genes has been linked to pro-tumoral effects. Myeloid cells in PFA ependymomas were found to overexpress IL-8, ICAM1, PTGS2, and IL-1B, which are thought to play a role in the activation of the inflammatory IL-6/STAT3 pathway.[69] The activation of the latter is known to enhance tumor proliferation and inhibit apoptosis of tumor cells.[70] This pro-inflammatory phenotype appears to be dictated by the tumor cells, as exposure to IL-6 from PFA ependymoma tumor media increases IL-8 secretion by myeloid cells. This exemplifies the versatility of myeloid function within tumors and underlines the role of secreted tumor-derived factors in the polarization of myeloid cells. Furthermore, spatial transcriptomic studies revealed that PFA ependymomas have epithelial and mesenchymal zones, each associated with differential myeloid gene expression.[71,72] In the mesenchymal clusters, myeloid cells express genes associated with the classical M1-like pro-inflammatory polarization state, including genes related to phagocytosis, engulfment, major histocompatibility complex (MHC) class II protein complex, and complement activation. Immunohistochemical staining in these tumors has also shown a particularly high expression of the myeloid activation marker IBA1 around necrotic areas of the tumor samples. This suggests that factors such as epithelial-to-mesenchymal transition or the presence of necrotic plaques may dictate specific myeloid responses in different areas of PFA ependymoma. These findings highlight the complexity of deciphering immune function in tumors, particularly regarding myeloid cells, whose function is heavily reliant on environmental cues. Identifying the immune landscape within different regions of ependymomas is crucial for understanding myeloid cell function.

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Pediatric H3-wild-type HGGs

Traditionally, adult glioblastoma tumors are classified into four molecular subtypes: neural, proneural, classical, or mesenchymal.[73] While pediatric H3-wild-type HGGs (historically known as pediatric glioblastoma) do not necessarily conform to the same molecular classification, the observed immune infiltration profiles suggest a similar immune response.[74,75] In a gene expression study that included adult and pediatric glioblastoma samples, increased expression of myeloid genes was found in the mesenchymal-like tumors, as well as a reduced expression of genes related to activated $CD8^+$ T cells and natural killer (NK) cells.[75] It is hypothesized that myeloid cells in these mesenchymal tumors are more anti-inflammatory, and another study confirms this hypothesis by finding PD1 and M2-like markers (e.g., CD163 and CD206) highly expressed in glioblastoma tumor samples.[56] It is important to note that glioblastomas, like ependymomas, contain necrotic regions, which can elicit a specific inflammatory immune response.[76,77] The changes observed in the immune landscape of glioblastomas may be related to the clearance of these necrotic regions rather than an immune response against the tumor itself. Furthermore, spatially resolved single-cell analyses demonstrated that pediatric HGGs can contain adenosine-rich regions that harbor a specific immunomodulatory function involving microglial CD39.[78] Hence, similar to ependymomas, a deeper understanding of the spatial distribution of immune cells within the tumor immune microenvironment may be crucial in understanding the immune response in pediatric HGG.

DMG

DMG is characterized by high infiltration of macrophages or microglia but limited to no T or NK cells.[79,80] DMGs do not express the necessary chemokines or cytokines to recruit these lymphocytes, nor do they express significant amounts of immunosuppressive factors, resulting in an immunologically inert microenvironment that may limit the effectiveness of immunotherapeutic compounds. This so-called non-inflammatory or dampened immune phenotypic state contrasts with glioblastomas, which are also immune cold but tend to actively express anti-inflammatory factors. Although these studies suggest complete inactivity of its microglia, tumor-associated myeloid cells in DMG do adopt a pro-tumoral phenotype through epigenetic changes.[47,48] By inducing a loss of histone 3 lysine 27 trimethylation in microglia, DMG cells have been shown to push microglia toward a stem cell-like, tumor-promoting state.[48] Aligning with the dependency that tumor-associated microglia have on this epigenetic dysregulation, it was shown that repression of the histone lysine methyltransferase EZH2 in myeloid cells results in anti-tumoral activity. Furthermore, a recent study suggested an active role for brainstem microglia in modulating the spatiotemporal patterning of DMG through IL-33 and other secreted factors.[81] A recent publication by Filbin et al. further sheds light on the spatiotemporal composition of histone 3 lysine 27 mutated DMG, demonstrating a higher number of microglia in younger patients as opposed to higher proportions of macrophages in adults.[82] Finally, it is important to note that the non-inflammatory microglia in DMG exhibit distinct morphological features from ramified (resting) microglia found in the healthy brain. These activated microglia in DMG possess enlarged cell bodies and fewer cellular processes, as is seen in other pediatric CNS malignancies.

Other pediatric gliomas

In pilocytic astrocytoma (PA), a study reported elevated levels of pro-inflammatory myeloid markers (e.g., HLA-DR and CD64) compared with non-tumorous tissue.[56] The study also found a higher infiltration of $CD8^+$ T cells in PA than in other types of brain tumors such as ependymoma, glioblastoma, and medulloblastoma. Furthermore, a high CD8:CD4 T cell ratio and low PD1 expression in PA was observed, suggesting the activation of lymphocytes in conjunction with the pro-inflammatory myeloid population. In both pediatric and adult PAs, several studies have shown that neuronal stem cells attract microglia and that this may be related to expression of the *KIAA1549-BRAF* fusion gene, although this aberration is not present in all PA tumors.[83,84] According to these studies, this fusion gene-related attraction of microglia creates a supportive environment for gliomagenesis.

Similarly, in pleomorphic xanthoastrocytoma (PXA), another type of astrocytoma found in children, a study showed that tumor-associated microglia are more abundantly present compared with other isocitrate dehydrogenase (IDH)-wild-type as trocytomas.[85] In this study, CXCL14 was identified as a modulator of the tumor immune microenvironment and a chemoattractant for microglia, particularly in mitogen-activated protein kinase (MAPK)-activated PXA.

These studies in (low-grade) glioma suggest that non-malignant tumor-associated cells can facilitate the influx of myeloid cell and point toward a role of CXCL14, providing valuable insights into the tumor immune microenvironment in these types of brain tumors.

Taken together, pediatric CNS malignancies have developed a range of strategies to manipulate myeloid cells and alter their characteristics, resulting in the formation of a microenvironment that promotes tumor growth. This underscores the intricate and adaptable nature of interactions between myeloid cells and tumors. Ependymomas, PAs, and AT/RTs typically exhibit a more pro-inflammatory immune microenvironment characterized by M1-like myeloid cells and a substantial presence of T lymphocytes. In contrast, pediatric H3-wild-type HGG and medulloblastoma generally display a more anti-inflammatory milieu, marked by M2-like myeloid cells and a limited number of T lymphocytes. However, variations exist among different subtypes within a given tumor category. Furthermore, even within a single tumor category, such as HGG or ependymoma, heterogeneous immune responses can be observed across different regions of the tumor, such as within necrotic areas. In DMG, a dampened inflammatory environment has been described that is not pro-inflammatory but also does not entirely align with an anti-inflammatory profile as seen in other HGGs. Furthermore, although microglia within the tumor microenvironment of various pediatric CNS tumor types may exhibit similar morphological features—often characterized by enlarged cell bodies and fewer cell processes compared with the ramified microglia found in the healthy brain—their functional attributes can vary significantly. As such, drawing conclusions solely from microglia morphology, M1-/M2-like polarization states, or cell quantities within tumors is presently unfeasible. Moreover, drawing parallels to the adult

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population is difficult considering the unique nature of pediatric tumor entities, primarily confined to a very young patient demographic and consequently lacking adult counterparts. Nevertheless, with the rapid increase of studies delving into the immune microenvironment, upcoming research must stay vigilant about commonalities between tumor types. This holds particular significance in the context of designing immunotherapies.

LEVERAGING MICROGLIA PLASTICITY FOR ANTI-TUMOR THERAPY

The studies discussed in this review underline that pediatric CNS malignancies are capable of reprogramming microglia to a tumor-supporting phenotype. Consequently, these malignancies seem inclined to be strongly dependent on these reprogrammed microglia, which reveals a potential weakness that can be targeted in an immunotherapeutic approach. Here, we will highlight some examples of how tumor-associated microglia can be reprogrammed and how this may improve future therapeutic strategies.

As discussed above, DMG-associated microglia have been shown to adopt similar epigenetic aberrations as their adjacent tumor cells by losing trimethylation of histone 3 at lysine 27, despite lacking the typical H3K27M mutation that hallmarks the tumor cells. This loss of trimethylation in microglia is induced by the surrounding tumor cells and in turn leads to a change in the microglia phenotype that supports tumor growth and invasiveness. Counterintuitively, it has been shown that exacerbating the loss of H3K27 trimethylation in glioma-associated microglia by repressing the histone lysine methyltransferase EZH2 increases the pro-inflammatory, anti-tumor phenotype of these microglia.[48,86] For example, repression of EZH2 in glioma-associated microglia increased the expression of pro-inflammatory genes (e.g., NOS2 and IL-1B) and decreased the expression of genes associated with M2-like polarization of microglia. In addition, repression of EZH2 in glioma-associated microglia increased their phagocytic ability and decreased invasion and migratory capacity of tumor cells in co-cultures. It is worth noting that direct inhibition of EZH2 in tumor cells has not consistently resulted in satisfying anti-tumor activity.[87,88] Considering the abundance of microglia in DMG, targeting microglia rather than tumor cells may be a useful approach to shift the non-inflammatory tumor immune microenvironment toward a pro-inflammatory one and thereby enhance tumor cell killing.

In ependymoma, myeloid cells exposed to tumor-derived IL-6 have been found to increase the secretion of IL-8, leading to an enhanced activation of the STAT3 pathway and inhibition of apoptosis of tumor cells through the production of anti-apoptotic proteins.[69,70] The STAT3 pathway may therefore be a valuable target for myeloid-based immunotherapy in these tumors. In mouse models of medulloblastoma, disabling Stat3 has also been found to increase the secretion of pro-inflammatory cytokines (e.g., Il6 and Tnfα) by myeloid cells and reduce the number of granulocyte myeloid-derived suppressor cells as well as FoxP3$^+$ regulatory T cells within the tumor mass, although it had no effect on tumor growth.[89,90] Further research is needed to fully understand the potential of targeting STAT3 as a treatment for these tumor types.

Although yet to be investigated in pediatric brain tumors, there are a few myeloid-based approaches that hold promise as a means of microglial immunomodulation. One such approach involves regulating the expression of microglial microRNAs, such as miR-155.[91] High expression of miR-155 has been shown to enhance the production of pro-inflammatory cytokines (IFN-β, IL-6, and TNF-α) and increase the phagocytic capacity of microglia by elevating production of nitric oxide (NO).[92] In contrast, other microRNAs such as miR-200b have been shown to inhibit the production of pro-inflammatory cytokines and the migratory potential of activated microglia by regulating c-Jun, the transcription factor of the cJun-N terminal kinase (JNK) pathway.[93] In this study, knockdown of miR-200b in microglia increased JNK activity along with an increase in pro-inflammatory cytokines, inducible NO synthase expression, and NO production. At present, the microRNA profile of microglia in pediatric brain tumors is unknown and warrants further investigation.

Another myeloid-based approach that is yet to be evaluated in pediatric neuro-oncology involves using chimeric antigen receptor myeloid (CAR-M) cells. Studies have shown that genetically engineering macrophages with anti-CD19 or anti-HER2 receptors can improve antigen presentation and phagocytic potential.[94–97] In these studies, the use of anti-HER2 CAR-M has also been shown to repolarize M2-like myeloid cells toward an M1-like phenotype in humanized mouse models, thereby creating a pro-inflammatory microenvironment.

Finally, microglia activity can be modulated directly through microglia-targeted monoclonal antibody therapy, for example by engaging membrane-bound receptors that may be expressed differentially in a disease setting, as described for anti-TREM2 antibody therapy in Alzheimer's disease.[98] The rapid increase of publicly available single-cell expression data in pediatric brain tumors paves the way for selection of microglia-specific monoclonal antibody therapy targets. Monoclonal antibodies can also regulate microglia activity through Fc-mediated effects such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity/phagocytosis (ADCC/P). The choice of antibody isotype and design of the Fc effector can thereby significantly influence microglia behavior and the extent of neuroinflammation, making it an important consideration for any monoclonal antibody therapy applied in pediatric neuro-oncology.[99]

In conclusion, reprogramming of the tumor-associated myeloid cell response may be a valuable addition to the growing arsenal of immunotherapy in pediatric neuro-oncology. Further research is needed to fully understand the mechanisms behind this treatment approach and to test its efficacy in a clinical setting, but the initial preclinical results in neuro-oncology and -degeneration research thus far are encouraging.

DISCUSSION

In the last decade, the diversity and adaptability of microglia in pediatric brain tumors are progressively being understood. Different subpopulations of microglia, found in different brain regions and at different stages of brain development, play distinct roles in the biology of pediatric brain tumors. In many

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cases, brain tumors are described to "hijack" the gene expression of microglia and induce a pro-tumoral state, but the functional heterogeneity of microglia in tumors makes their contribution to tumor growth difficult to predict. Identifying and understanding the cellular and molecular contexts of pediatric CNS malignancies and their associated mechanisms for microglial hijacking could lead to improved therapeutic strategies for this patient population.

Myeloid cell-based immunotherapy is gaining traction, and there are indications of its therapeutic potential, as discussed in this review. Given the central role of myeloid cells in pediatric malignancies, the application of such therapeutic advances in children seems to be an approach worth investigating. The reprogramming of myeloid cells to a pro-inflammatory phenotype may not only directly enhance tumor killing but may also improve T cell-based immunotherapies, which are more effective in tumors with a pro-inflammatory microenvironment.[100–103] Another advantage of pro-inflammatory myeloid cells is that they produce matrix metalloproteases that can break down the extracellular matrix and increase T cell infiltration into the tumor site.[104,105] This can be particularly beneficial for CAR T cell therapies in solid tumors, where a lack of inflammation and a dense fibrogenic tumor immune microenvironment can hinder their effectiveness.[106] Therefore, reprogramming microglia may not only be valuable on its own but may also lead to improved T cell therapies. However, much research is still needed to fully explore this avenue.

In this review, we briefly discuss the poor reliability of the markers used to distinguish microglia from macrophages in the tumor immune microenvironment. This is a more profound issue in pediatric neuro-oncology, as young children harbor microglia that do not yet express some of the classical myeloid markers. Especially in brain tumor entities that arise during fetal development, microglia do not display typical microglia markers, which might result in the faulty exclusion of tumor-associated myeloid cells in studies that investigate the immune signature of these tumors.[38–41,50,51] In adults, the distinct microglial and macrophage subpopulations and their spatial distribution have been more thoroughly studied and have been shown to predict patient outcome.[107–116] Nonetheless, with established differences between the adult and pediatric tumor immune microenvironments and the high diversity observed within pediatric tumors, the functional and spatial differences of these two myeloid cell types in children cannot be simply compared to their adult counterparts.[117,118] Future research should prioritize investigating the distribution and lineage development of microglia and macrophages in pediatric tumors and make use of the latest developments to reliably distinguish between different myeloid cell entities. Single-cell techniques such as single-cell spatial transcriptomics or cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), a sequencing-based method that simultaneously quantifies cell surface protein and transcriptomic data within a single-cell readout, could be highly beneficial in identifying the subtle intra-tumoral differences of microglia subpopulations among brain tumor entities.

Two relatively underexplored areas of research in pediatric brain tumors include the effect of conventional therapies and gender disparities on the tumor immune microenvironment. Although not yet fully understood, alterations to the myeloid compartment within the tumor have been reported following courses of chemotherapy or radiotherapy.[119–124] Moreover, biological sex is emerging as an important confounder in the immune microenvironment of brain tumors. A recent study brought to light a gender-specific, tumor-promoting function of TREM2 in gliomas, potentially entailing sex-specific variances in myeloid cells present within the tumor.[125] This investigation demonstrated that a deficiency in TREM2 leads to a reduction in glioma-associated myeloid cells within glioma tissue, along with diminished vessel growth and a smaller glioma size. Importantly, this effect was observed exclusively in male animals and not in females. Given the disparities in brain tumor occurrence between males and females,[126] taking these distinctions into account when evaluating the impact on the immune response in both preclinical and clinical studies could offer valuable insights.

The dynamic nature of microglial interactions with the tumor and the plasticity of their phenotype represent major obstacles in studying these interactions, as they are difficult to reproduce in a laboratory setting. There is increasing awareness that the behavior and the phenotype of myeloid cells are dependent on the methods used to establish *in vitro* model systems, such as the source of myeloid cells and artificial polarization. It is also important to consider the effects of culture conditions, such as extracellular matrix scaffolds and co-culture media, when designing these models. Despite progress being made, co-culture systems that accurately represent tumor-microglial communication remain scarce.[127–131]

Establishing accurate *in vivo* models has also proven to come with its limitations. A major limitation of patient-derived xenografts is that they require the use of immunocompromised mice, thereby limiting the study of microglial and macrophage functions. One solution is to use humanized xenograft hosts, in which the peripheral blood or bone marrow of the patient is co-engrafted with the tumor material into an immunodeficient mouse strain. However, thus far, no humanized xenograft models have been developed for pediatric brain tumors due to ethical, legal, financial, and procedural constraints. Alternatives include immunocompetent genetically engineered mouse models or carcinogen-induced models, but these are often poor representations of the malignancy.[40,132–137] Recently, the use of *in utero* electroporation has allowed for the generation of immunocompetent allograft models of brainstem tumors that closely mimic the immune microenvironment seen in patients, effectively addressing the limitation that has hindered replication of the complexity of the human immune response in traditional mouse models.[138]

In conclusion, the communication between tumor cells and associated myeloid cells, specifically microglia, is significant for future immunotherapeutic approaches for pediatric brain tumors. Microglia's phenotypic plasticity can be used to turn an anti-inflammatory tumor immune microenvironment into a pro-inflammatory one by repolarizing microglia into an anti-tumoral phenotype, which would also improve T cell-based therapy results. This shift to a myeloid-centered immunotherapeutic approach may be the next revolution in immunotherapy for brain tumors, especially in children.

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AUTHOR CONTRIBUTIONS

I.Q.V. performed the literature search. I.Q.V., A.d.C., and D.S.M. contributed to the design and writing of the first manuscript draft. All authors critically reviewed the manuscript. A.d.C. led the process of editing and integrated the revisions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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