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Macrophages and Microglia in Brain Malignancies

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1. Introduction

Tumour and myeloid cell interactions that occur in the brain are exposed to unique microenvironmental conditions. While tumour outgrowth ultimately has a major impact, the intrinsic properties of the brain initially impose the framework on which subsequent cellular interactions must build. The particularities of the brain are partly anatomical, with strictly controlled traffic of cells and molecules from the blood by virtue of the blood brain barrier (BBB). But they are also due to a unique cellular composition not found outside the nervous system; this includes the neurons and glial cells which have potent immunoregulatory properties. Taken together, these factors have been used to attempt to explain the "immune privilege" of the brain, which is often used to describe low level adaptive immune responses. But in addition immune privilege also impacts on, and is influenced by, the brain myeloid cells which are the subject of this chapter.

Our understanding of immune privilege must be defined and put into context, since it was originally used in the restricted field of the extended survival of allografts in the brain compared with that achieved in other sites (Barker & Billingham, 1977; Medawar, 1948). Over the years, the concept and terminology has often been used to apply to any situation of low or absent immune reactivity in the brain. Indeed, this seemed consistent with the overriding need to control inflammatory reactions in the brain because of their potentially deleterious consequences to critical neuronal functions with low regenerative capacity. Moreover, immune privilege also seemed consistent with an apparent isolation of the brain behind the BBB, and the absence of draining lymphatics and immune competent cells from much of the brain. However, this over simplistic interpretation of the brain as an immune privileged site has now been reassessed to define it as an immune specialized site, where immune cells are poised to respond to injury and infection, but in a highly regulated way (Carson et al., 2006; Galea et al., 2007). A further complexity of this updated concept of immune privilege is that this status is not uniform throughout the brain, and in addition, the myeloid cell populations are distinct in different brain regions. As argued by Perry and colleagues (Galea et al., 2007), the brain parenchyma is the brain compartment that exhibits most features associated with immune privilege. Other brain regions with distinct immune properties are the ventricles containing the choroid plexus and the cerebrospinal fluid (CSF), the meninges, and the perivascular space. Brain tumours can of course potentially invade any or all of these brain sites, but how this influences interactions with myeloid cells has not

generally been defined. However, it should be noted that most experimentally induced or grafted tumours are in the brain parenchyma, and so the initial contact will be with parenchymal myeloid cells. For the same reason, although most of the neuroimmunology described in this chapter could apply more generally to the central nervous system, we refer more specifically to the brain, to maintain a direct link to the vast majority of available information on malignancies.

2. The myeloid cells of the brain

Myeloid cells are abundantly present in the healthy brain, representing between 5% and 20% of cells, depending on the specific location. Their histological appearance, their origin, and potentially their functions are distinct from those found in other tissues. The phenotypic classification of brain myeloid cells by defined markers is challenging, which has led to imprecise or changing nomenclature over the years. This terminology arose from histological studies around a century ago, which distinguished the small, ramified microglial cell of the parenchyma from the larger "macroglial" cells such as the astrocytes, oligodendrocytes and ependymal cells, as recently reviewed (Ransohoff & Cardona, 2010). Parenchymal microglial cells are considered to be the resident macrophages of the brain. Those myeloid cells populating other brain compartments are generally referred to as macrophages, prefixed with their localization (choroid plexus, meningeal, perivascular) (Hickey et al., 1992; Matyszak et al., 1992). Confusingly, the term microglia has also sometimes been used for these cell types (or even dendritic-like cells, or perivascular cells), although as we shall discuss, recent studies indicate that the origin of these macrophages is distinct from parenchymal microglia, and so in this chapter we retain the term microglia only for parenchymal cells.

Type	Location	Phenotype	Functions
Parenchymal microglia	Within the parenchyma, not directly associated with vessels	Ramified to amoeboid, $CD11b^{+}$, $CD45^{low}$, MHC II^{low}	Ramified: parenchymal surveillance, synaptic housekeeping. Amoeboid: see text.
Perivascular macrophages	Associated with the blood vessels in the parenchyma	Amoeboid or dendritic, $CD11b^{+}$, $CD45^{high}$, MHC II^{+}	Phagocytosis, antigen presentation, blood brain barrier component.
Meningeal macrophages	Associated with the pial vasculature and the subarachnoid space	Amoeboid or dendritic, $CD11b^{+}$, $CD45^{high}$, MHC II^{+}	Phagocytosis, antigen presentation, blood brain barrier component.
Choroid plexus macrophages	Choroid plexus	Amoeboid or dendritic, $CD11b^{+}$, $CD45^{high}$, MHC II^{+}	Phagocytosis, antigen presentation.

Table 1. Principal myeloid populations in the healthy brain.

Myeloid cells of each type in the listed location are identified in all species analysed (generally humans, rat, and mouse). The phenotypic markers may be species-specific. The function is often presumed rather than experimentally demonstrated in all species.

2.1 Parenchymal microglial cells – Origins and phenotypic definition

The origins of microglial cells have been debated for many decades. The difficulties in reaching definitive conclusions are partially linked to the lack of unique markers to differentiate the microglia from other myeloid cells, and the inherent plasticity of myeloid cells to differentiate or modulate expression of many key markers (Geissmann et al., 2010; Santambrogio et al., 2001). From the 1990's accumulating evidence finally assigned microglia to the myeloid lineage (Prinz & Mildner, 2011; Ransohoff & Cardona, 2010), thus distancing them from the neuroectoderm derived macroglia. The subsequent question that has been more elusive to answer is whether microglia derive from the bone marrow. Here a distinction must be made between the principle origin of parenchymal microglial cells in embryonic development, and their potential replenishment in the adult. Compelling evidence based on in vivo lineage tracing studies in mice indicated that microglia form an ontogenically distinct population in the mononuclear phagocyte system. The microglia that populate the adult brain parenchyma derive from myeloid progenitors in the yolk sac. This first primitive hematopoiesis temporally precedes and it is distinct from the definitive hematopoietic wave which gives rise to circulating hematopoietic precursors, including monocytes (Ginhoux et al., 2010).

The mechanism by which the parenchymal microglial population is replenished during adult life or in pathological situations has been challenging to elucidate. First, as will be discussed, it is difficult to unambiguously differentiate parenchymal microglia from other macrophages. Second, the use of radiation bone marrow chimeras to conveniently replace bone marrow stem cells with labelled donor cells inevitably introduces artefacts such as vessel damage, and abnormal proportions and fluxes of donor stem cells in the circulation. With these limitations in mind, initial data suggested replenishment from bone marrow precursors (Flugel et al., 2001). However, elegant experiments based on parabiosis rather than radiation indicated that self-renewal within the brain is sufficient to maintain the adult parenchymal microglial population (Ajami et al., 2007). Nevertheless, in certain pathologies, repopulation from bone-marrow derived precursors remains a possibility (Soulet & Rivest, 2008).

The original definition of parenchymal microglia was based on morphology and localization in brain parenchyma. Specific markers are clearly essential to objectively compare findings between research groups, and to manipulate microglial cells in vitro. However, most markers are shared with other tissue macrophage populations, thus CD11b/CD18 (integrin αMβ2) is expressed, as well as F4/80 in mouse microglia. Constitutive expression of MHC class II, and costimulatory molecules is generally low on parenchymal microglia. The low expression of many myeloid markers may be reinforced by neuron-microglial-cell inhibitory signalling through cell surface and soluble ligands (Ransohoff & Cardona, 2010). The principal tool to distinguish parenchymal microglia from other brain macrophages has been the level of CD45 expression (leukocyte common antigen, Ly-5): microglia are characterized by low CD45 expression, whereas other macrophages are $CD45^{high}$ (Ford et al., 1995). However, this distinction can only be achieved by flow cytometry, not by immunohistochemistry. Parenchymal microglia of human origin are more problematic to identify, and no markers have been consistently described to discriminate among the two cell populations (Wu et al., 2010). Moreover, non-pathogical human brain tissue is infrequently studied, and so it is unclear what proportion of parenchymal microglia compared with other activated macrophage populations is to be expected. Nevertheless, at least one study has suggested that human parenchymal microglia can be identified as $CD11b^{+}$, $CD45^{low}$, and unlike other brain macrophages, negative for CD14 (Parney et al., 2009).

2.2 Non-microglial brain macrophages – Origins and phenotypic definition

The myeloid populations in non-parenchymal regions of the brain are phenotypically closer to their extracranial counterparts than parenchymal microglia (Hickey et al., 1992). Moreover, whilst cranial irradiation does not deplete parenchymal microglial cells, other myeloid cells are radiosensitive and are readily replenished from bone marrow precursors within about 4 to 8 weeks (Chinnery et al., 2010; Hickey & Kimura, 1988).

A wide diversity of phenotypes, in humans and rodents, is reported for choroid plexus myeloid cells: from classical tissue macrophages, to dendritic or "dendriform" like cells (Chinnery et al., 2010; Matyszak et al., 1992; McMenamin, 1999; Serot et al., 2000). In human tissue, there was an absence of co-stimulatory molecule expression (CD40, CD80, CD86) on these cells (Serot et al., 2000), but this was not studied in rodent origin choroid plexus derived cells. Similarly, in the meninges, pleomorphic myeloid cells with either macrophage or dendritic cell like features can be identified (Chinnery et al., 2010; McMenamin, 1999; McMenamin et al., 2003).

The non-parenchymal site that has been the most thoroughly studied is the perivascular space, with myeloid cells strategically located to engage in dialogue with both blood-born immigrants and parenchymal residents. Moreover, these perivascular myeloid cells are proposed to contribute to BBB function (Bechmann et al., 2007). Phenotypically, perivascular myeloid cells can be macrophage-like or dendriform, although often this is based only on morphology, as dendritic cell specific markers have not been used in all studies (Flaris et al., 1993; Platten & Steinman, 2005).

2.3 Functions of microglia and brain macrophages in the healthy and diseased brain

The spectrum of functional activities reported for myeloid cells in the brain is as wide as for conventional tissue macrophages, and will depend upon the stimulus or pathology that is inducing activation. Although the focus of this chapter is to understand myeloid cells and brain tumours, other pathologies (especially autoimmune and neurodegenerative diseases) have furnished a lot of basic information about myeloid cell function. These findings are therefore discussed first before considering the impact of malignancy on these complex cellular interactions.

Attributing functions specifically to "microglial cells" or "brain macrophages" necessitates clarification of certain basic assumptions about the identity of these cells. A microglial cell assumes its overall morphology, its specific phenotype and its functions largely because of its localization in the brain parenchyma. If we wish to isolate brain myeloid cells using CD45 staining intensity to differentiate microglia from other macrophages, we must accept the inherent limitations of this marker which is not necessarily expressed at a constant level in different in vivo pathologies, or in vitro. Moreover, cells cultured in vitro will be exposed to a microenvironment totally distinct from the brain parenchyma, and when cultured in serum containing medium, will be exposed to known microglial activators such as fibrinogen (Adams et al., 2007). These issues were thoroughly and quantitatively addressed using gene expression analysis of microglia activated by LPS/IFN-γ in vitro versus in vivo, with similar experiments performed for peritoneal and brain infiltrating macrophages (Schmid et al., 2009). This important study showed that brain-resident microglia are heterogeneous, with very different gene expression patterns after stimulation in vitro and in

vivo. Furthermore, the brain micro-environment was a more important factor in determining gene expression than the origin of the myeloid cells tested. The "microglia" and "brain macrophage" functions that we will now describe must therefore be interpreted with these complexities of terminology in mind, together with the limitations of in vitro tests that may not recapitulate the significantly overlapping properties of these cells in the in vivo brain microenvironment.

2.3.1 Transition of parenchymal microglia from "resting" to "activated"

In the healthy brain, parenchymal microglial cells are termed "resting" cells, morphologically identified as ramified cells with small soma and fine cellular processes. However, so called resting microglia have been proposed to be actively involved in maintaining synaptic integrity, indicating a different functional status than would be expected from a macrophage, as recently discussed (Graeber, 2010). With the advent of two-photon microscopy able to visualize microglia in situ in the living mouse, the term "resting" is even more directly seen to be a misnomer, as these cells are dynamically surveying their microenvironment (Davalos et al., 2005; 2008; Nimmerjahn et al., 2005). Time-lapse imaging experiments demonstrated that microglial processes are motile and continuously (over periods of a few minutes) extend and retract to survey neighbouring astrocytes, neuronal cell bodies, and blood vessels. Upon tissue damage or signals that might mimic infection (application of LPS), microglia were capable of targeted movement of their processes towards the injured site, leading to a barrier of intertwined processes between damaged and healthy tissue (Davalos et al., 2005). Although not directly demonstrated, it is likely that the same processes can occur in malignant pathologies. A key signal that promoted this microglial activation was ATP, binding to the purinergic $P2Y_{12}$ receptor on microglia. The source of ATP mediating these effects is proposed to be from both damaged cells and ATP-induced ATP release from astrocytes.

Microglial cell activation results in changes in morphology and function that are postulated to be directly linked to the consequences of initial chemoattraction by ATP (Orr et al., 2009). Extracellular ATP can be degraded to adenosine by microglia expressed ectonucleotidases, such as CD39 and CD73, which then leads to activation of the adenosine A2A receptor and process retraction by the microglia. Further activation of the microglial cell will occur when those elements maintaining their "resting" (but surveillant) status are removed. Since many of these inhibitory signals come from neurons, if tissue damage has perturbed neuronal function, this will remove or limit negative signalling, such as that mediated by CD200 (Chitnis et al., 2007; Hoek et al., 2000) or CX_3CL1 (fractaline) (Cardona et al., 2006). The activated microglial cell becomes amoeboid in shape, motile, and phagocytic, i.e., very similar to other macrophages.

2.3.2 Functions of activated microglia and brain macrophages

Microglia not only change in morphology upon activation, but also increase in number. Indeed, unlike most tissue macrophages, brain-resident microglia have a high potential for proliferation (Ajami et al., 2007; Graeber et al., 1998). Microglial functions can be potent, and so can either be beneficial or pathogenic according to the tissue targeted and when it is targeted. The products released by fully activated microglial cells include reactive oxygen species, nitric oxide (NO), and tumour necrosis factor (TNF), all of which can damage

neurons and glial cells (Block et al., 2007), although whether this is directly linked to neurodegenerative disease is disputed by some (Graeber & Streit, 2010). The other side of the spectrum of microglial cell function is that of neuroprotection, in which there is abundant evidence for beneficial functions (Glezer et al., 2007; Hanisch & Kettenmann, 2007). Probably most functions of an activated microglial cell could be carried out by other brain macrophages, and so unless specifically stated otherwise, the references to microglial cell function do not exclude this possibility.

Migration. Proliferation is not sufficient to explain the high density of microglia around brain lesions; migration is also necessary (Carbonell et al., 2005a; 2005b). One study, using focal laser injury in ex vivo retinal explants, suggested that transition to a migratory phenotype could occur whilst microglia remain in the "resting" ramified morphology (Lee et al., 2008). However, in most pathologies, migratory microglia will be amoeboid and "activated". Microglial migration is under control of several types of receptors: those found on other macrophages, as well as receptors such as purinergic receptors generally found on neural cells. Capacity to migrate towards certain stimuli is generally assessed using in vitro assays, and so their relative importance awaits in vivo validation. Among the key chemokine-chemokine receptor interactions, CCL21 produced by damaged neurons can attract microglia via CXCR3 (Biber et al., 2001); CX3CL1 (fractaline) produced by neurons and astrocytes signals through CX3CR1 on microglia (Cardona et al., 2006; Liang et al., 2009); stromal cell derived factor-1α (SDF-1α) binds to $CXCR4^+$ microglia (X. Wang et al., 2008); and monocyte chemotactic protein 1 (MCP-1, CCL2) produced by many brain cells, including microglia themselves, signals through CCR2 (Simpson et al., 1998). The multiple non-chemokine receptors expressed by activated microglial cells facilitate migration towards other categories of stimuli present in the brain, including neurotransmitters, cannabinoids, lysophosphatidic acid (LSA), opioids, bradykinin, and various growth factors. These have recently been comprehensively reviewed (Kettenmann et al., 2011).

Phagocytosis. A major function of activated microglia is phagocytosis: it is essential during brain development, for homeostatic debris clearance in the normal brain, and can tilt the balance towards neuroprotection or neurodegeneration. It can also provide a link to adaptive immune responses if phagocytosed antigenic proteins are processed and presented to T cells. Activated microglial cells can express a wide array of phagocytic receptors, both those responsible for pathogen recognition and clearance, and those facilitating removal of apoptotic cells (Kettenmann et al., 2011; Neumann et al., 2009). Of the former category, Toll like receptors (TLRs) 1-9, Fc receptors, scavenger receptors, and complement receptors can be expressed, ligation of which will generally lead to an inflammatory response, with release of TNF, IL-1 and nitric oxide. Receptors of the latter category include TREM2 (triggering receptor expressed on myeloid cells-2), purine receptors (some of which are not found on other macrophages), and phosphatidylserine receptors. Ligation of this category of receptors can lead to anti-inflammatory signalling and release of TGF-β and IL-10.

Antigen presentation. As microglia are certainly sentinel cells of the brain parenchyma, and they are the most abundant phagocyte, are they also antigen presenting cells (APCs) for T cells? A prerequisite is expression of MHC class II (for CD4 T cells) and MHC class I (for CD8 T cells), and costimulatory molecules. The level of expression of these molecules is totally dependent upon the activating and inhibitory factors perceived by the microglial cell (Aloisi et al., 2000). The antigen presenting capacity as tested in vitro (sometimes ex vivo) on

CD4 T cells can vary from full to partial activation, to apoptosis induction (Ford et al., 1996; Frei et al., 1994; Matyszak et al., 1999; Platten & Steinman, 2005). The status of microglia as APC in vivo, in different pathologies, is therefore a key question. Accumulating evidence from rodent experimental autoimmune encephalomyelitis (EAE) models has now identified that the principal cell able to present antigen to restimulate infiltrating CD4 T cells resides in the perivascular space (Hickey & Kimura, 1988; McMahon et al., 2005), or the meninges (Bartholomaus et al., 2009). Unlike parenchymal microglia, these perivascular myeloid cells are radiosensitive, and may even have dendritic morphology and CD11c expression. The role of the parenchymal microglial cells in these EAE models was proposed to be to augment pathology by the release of T cell stimulating cytokines and toxic mediators such as IL-12, IL-23, osteopontin, and reactive oxygen species (Heppner et al., 2005; Platten & Steinman, 2005).

Antigen presentation of exogenously acquired antigens on MHC class I molecules (cross-presentation) is essential for CD8 T cell immune responses, but is an inefficient process in most cells. In vivo, certain subsets of dendritic cells (that are $CD11c^{+}CD8^{+}$ in the mouse) are considered to be the principle cross-presenting APC (Lin et al., 2008), but these have not been reported in the brain. However, one study showed that in the adult mouse, microglia can take up soluble ovalbumin and present processed antigen to CD8 T cells (Beauvillain et al., 2008), albeit with modest efficiency. These results are similar to earlier studies in the rat, although in this study the authors suggested that antigen presentation was very feeble and should be considered as compromised (Flugel et al., 1999). A further cross-presenting brain APC candidate is a ramified, radioresistant cell found in the parenchyma of mice that is $CD11b^{+}CD11c^{+}MHCII^{-}$, termed a "brain DC" by the authors, but with a very similar phenotype to microglial cells. When activated in vivo with IFN-γ, then tested in vitro for stimulation of ovalbumin specific CD8 (and CD4) T cells, this APC could present and cross-present soluble ovalbumin (Gottfried-Blackmore et al., 2009). The in vivo significance of these cells and their relation to other myeloid cells in the brain awaits clarification.

The most stringent task for an APC is to prime naïve T cells. Since naïve T cells normally recirculate between blood and secondary lymphoid tissue, this can only occur efficiently in the T cell zones of lymph node (or spleen). Therefore, an APC having captured antigen in the brain must be able to migrate to the lymph node, which is facilitated by expression of the chemokine receptor CCR7, promoting lymph node entry through the high endothelial venules in response to a CCL21 chemokine gradient. Murine microglia have been reported to express CCR7 (Dijkstra et al., 2006), but functional homing experiments were not performed. Indirect evidence points towards dendritic cells (DCs) as being the principle APC candidate to migrate from the brain to lymph nodes (Hatterer et al., 2006; Hatterer et al., 2008; Karman et al., 2004), although definitive tracking of cell migration required intracranial injection of dendritic cells that may not precisely recapitulate endogenous cell behaviour.

2.4 Brain tumours in humans and animal models

Cellular heterogeneity has long been accepted to be a hallmark of most brain tumours, and even the earliest histological studies suggested that myeloid cells might be present in the tumour stroma and peritumoural regions. Multiple histological types of primary and metastatic brain tumour are described and are expected to vary in their myeloid cell

involvement. The commonest primary brain tumours are astrocytomas (Schwartzbaum et al., 2006) and of these, the high grade malignant gliomas (WHO grade III and IV) are particularly lethal. Malignant glioma is profoundly resistant to conventional treatment modalities, and complete surgical resection is impossible because of their inaccessible location and highly infiltrative growth characteristics. Thus, even with the best resection possible and combined regimens of radiotherapy with new generation chemotherapy, the median survival of patients with glioblastoma multiforme (GBM: WHO grade IV glioma) is 14.6 months (Stupp et al., 2009). For animal studies, the use of xenografted human glioma in immunodeficient mice cannot be expected to induce normal myeloid cell responses as the whole immune network is perturbed in the host mice. Instead, animal brain tumour models have focused on either orthotopically implanted models, historically often in rats (Barth & Kaur, 2009), but increasing in mouse models such as GL261 glioma, syngeneic to C57BL/6 mice (Szatmari et al., 2006) There is also considerable interest in the use of genetically engineered mouse models that may better recapitulate the pathology and heterogeneity of human glioma (Huse & Holland, 2009). However, these models were often designed and exploited for genetic studies; they have only recently been investigated for immunological parameters.

2.4.1 Identification of microglia and macrophages in brain tumours

Although thorough characterization of brain tumour associated myeloid cells had to await the advent of monoclonal antibodies, an early pioneering study determined that glioblastomas, meningiomas, medulloblastomas, and metastatic tumours were all infiltrated by Fc receptor-positive cells, i.e., predominately myeloid cells (Wood & Morantz, 1979). These macrophages or microglia were particularly abundant in glioblastoma, representing a mean content of 41% (range 5-78%) of the tumour tissue digested to give a single-cell suspension. Subsequent studies were able to profit from monoclonal antibodies to better define myeloid cells, not only in suspension, but also in tissue sections. In histological sections, quantification is somewhat more challenging and difficult to compare between studies, but reports from several groups using panels of different antibodies described significant myeloid cell infiltration in low grade glioma (Rossi et al., 1988; Shinonaga et al., 1988), high grade glioma (Rossi et al., 1987; 1989; Shinonaga et al., 1988), as well as metastatic brain tumours (Shinonaga et al., 1988). Moreover, although flow cytometric analysis on single-cell suspensions of digested human tumour confirmed these findings, they did not allow an unequivocal distinction of microglia and other brain macrophages (Parney et al., 2009; Watters et al., 2005). Some of the histological studies on microglia and macrophage infiltration attempted to make correlations of type or intensity of myeloid cell infiltration and tumour type or grade (Nishie et al., 1999; Roggendorf et al., 1996; Rossi et al., 1988; 1987): there was a tendency for greater infiltration of high grade tumours such as glioblastoma. Higher myeloid cell infiltration was proposed to correlate with peritumoural oedema (Shinonaga et al., 1988), but not with survival in one early study (Rossi et al., 1989). Our contemporary understanding of the complexity of the myeloid cell compartment in the brain that we have discussed in section 2 predicts that correlation of survival and simple myeloid cell phenotyping and quantification is improbable. A more recent study on a larger series of patients with glioblastoma examined polymorphisms in the gene encoding the CX3CR1 chemokine receptor, expressed on microglial cells and used in their migration

(Rodero et al., 2008). Based on genetic analysis of 230 patients, and immunohistochemistry of over 100 patients, expression of one common allelic variant was significantly associated with prolonged survival after surgery (23.5 vs 14.1 months; $P < .0001$), as well as reduced microglia/macrophage infiltration.

In common with many other domains of cancer research, relevant animal models have played a major role in advancing our understanding of brain myeloid cells. For brain tumours in particular, the inaccessibility of the tumour site in patients often restricts studies to a single tumour sample, rodent models have therefore been widely exploited. Studies of myeloid cells infiltrating rat gliomas allowed intensity of CD45 expression on $CD11b^+$ cells to be used to distinguish parenchymal microglia from other brain macrophages. The microglia and macrophage content varied somewhat according to the model (C6, 9L, RG-2), with $CD45^{low}$ microglial cells present in peritumoural areas and in the hemisphere contralateral to the tumour, and $CD45^{high}$ macrophages infiltrating the tumour bed (Badie & Schartner, 2001). In another study, orthotopically implanted Rat 9L glioma was also studied by immunohistochemistry, and compared with an intracranial carcinoma; both were comparably infiltrated by microglia or macrophages (Morioka et al., 1992). Mouse glioma models have similarly been assessed for myeloid cell infiltration. In GL261 glioma, the infiltrate was predominately $CD11b^+F4/80^+CD45^{high}$, considered as macrophages rather than microglia by the authors (Nakagawa et al., 2007). We have also analysed orthotopic GL261 glioma, a representative immune infiltrate is shown in Fig. 1. In the SMA-560 model, $CD11b^+$ cells were identified by immunohistochemistry (Uhl et al., 2004). A further study used an orthotopically implanted PDGF-driven glioma model to study myeloid cells (along with other immune cells) longitudinally (Kennedy et al., 2009). The frequency of $CD11b^+CD45^+$ cells was measured, without distinguishing the level of CD45 expression. These macrophages/microglia increased from 1.1% during the early phase, to 5.6% during the late stage. A further category of tumour model in which myeloid cells have been documented is genetically engineered "spontaneous" gliomas. The GFAP-V^{12}HA-*ras* model is of particular interest, because astrocytomas form spontaneously in these mice, without the trauma (and potential microglial cell activation) associated with intracranial implantation (Ding et al., 2001; Shannon et al., 2005). We have explored immune parameters in these mice, analyzing the immune infiltrate from earliest stages of pathology, but without appearance of symptoms in mice (4, 8, and 12 weeks), to mice bearing advanced tumours producing symptoms (Tran Thang et al., 2010). Although there was a significant lymphocyte infiltrate in asymptomatic mice (at 12 weeks), myeloid cell augmentation correlated with appearance of terminal symptoms. Microglia ($CD11b^+CD45^{low}$) approximately doubled in number at this stage, whereas the previously minor population of $CD11b^+CD45^{high}$ macrophages increased by more than 50 fold, becoming the major myeloid subset in the brain. The intriguing correlation of terminal progression of the brain tumour and the presence of these macrophages clearly raises issues about their origin and likely function; this is the subject of ongoing investigations. Myeloid cells were also investigated in another genetically engineered glioma model, in which de novo gliomas were generated by intracerebroventricuar transfection of *NRas* and a short hairpin RNA against *P53* using the Sleeping Beauty transposon system (Fujita et al., 2011; 2010). The brain tumours of these mice were infiltrated by $CD11b^+$ cells, although the authors focused particularly on the myeloid derived suppressor cell (MDSC) component, as described in section 3.2 below.

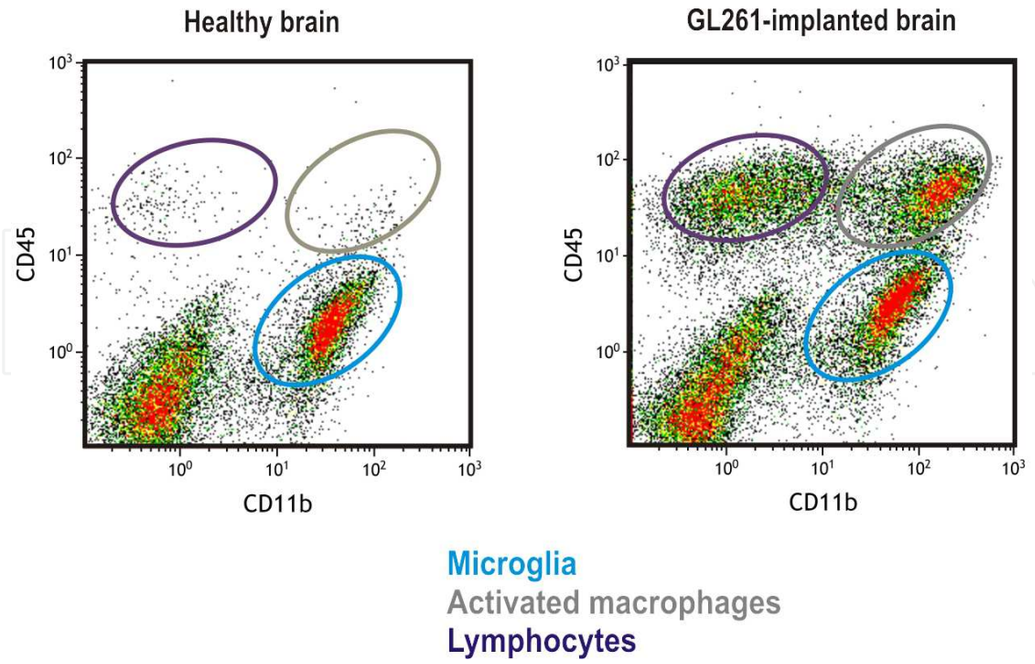

Fig. 1. Leucocyte populations in brain of healthy and tumour-bearing mice.

Leucocytes isolated from brains of healthy (left) and glioma bearing mice (right) were stained for CD45 and CD11b markers, and analysed by flow cytometry. The only highly represented leucocytes in healthy brain are $CD11b^{+}CD45^{low}$ microglia. In mice bearing advanced brain tumours, there is also an infiltrate of $CD11b^{+}CD45^{high}$ macrophages and $CD11b^{-}CD45^{high}$ lymphocytes.

2.4.2 Additional myeloid cell populations in brain tumours

Studies of various malignancies in patients and in tumour-bearing mice demonstrated that the heterogeneous cells known as MDSCs can accumulate in secondary lymphoid tissue and at the tumour site (Gabrilovich & Nagaraj, 2009). The MDSC population includes immature macrophages, granulocytes and DCs, and these cells have the potential to suppress T cell immune responses. Proposed mechanisms differ in lymphoid tissue and the tumour site, but include reactive oxygen species, arginase 1 activity and nitric oxide production. Phenotypically, they are usually defined as $CD11b^{+}GR1^{+}$ in mice (although subsets exist); in humans, phenotyping is more complex but MDSCs may include $CD14^{-}CD11b^{+}CD33^{+}$ cells.

In patients with malignant glioma, MDSCs were detected in peripheral blood (Raychaudhuri et al., 2011; Rodrigues et al., 2010), and the phenotype could also be induced by exposure of normal monocytes to glioma cell lines (Rodrigues et al., 2010). However, the presence of MDSCs infiltrating human brain tumours has not yet been described.

In rodents, presence of MDSCs within the brain tumour bed has been documented in several models. An early study using the T9 glioma model in Fischer 344 rats demonstrated that suppressive cells expressing both the rat granulocyte marker His48 and CD11b/c accumulated in the spleen and in the intracranial tumour bed (Prins et al., 2002). Moreover, this accumulation was enhanced by subcutaneous vaccination with irradiated glioma cells. These cells were further investigated in a recent study, in the same model, in which bone marrow replacement confirmed that the brain infiltrating MDSCs were of bone marrow

origin (Jia et al., 2010). Functionally, nitric oxide was determined to be the main suppressive molecule.

In mice, MDSCs were described in both the GL261 orthotopically implanted glioma (Umemura et al., 2008), as well in genetically engineered models (Fujita et al., 2011; 2010; Tran Thang et al., 2010). The definition of MDSCs infiltrating GL261 glioma was discussed in some detail (Umemura et al., 2008). The authors concluded that there is substantial overlap with other tumour associated macrophages, and that moreover, these cells have considerable plasticity in their function and phenotype according to the microenvironment in which they function. These issues are probably central to most myeloid cells associated with tumours. A rather simpler observation of the presence of $CD11b^{+}GR1^{+}CD45^{high}$ cells was made in the GFAP-V^{12}HA-*ras* model of spontaneous astrocytoma (Tran Thang et al., 2010), but without further phenotyping or functional tests. However, elevated proportions of these cells were present in only a minority of ill mice. This may be a reflection of the inherent heterogeneity of tumours in this model (the genetic background is outbred, and tumours appear after stochastic accumulation of genetic mutations that vary between mice), or it may signify that MDSCs are of only minor functional significance in this model. In contrast, in the genetically induced glioma models studied by the Okada group (Fujita et al., 2011; 2010), $CD11b^{+}Ly6G^{high}$ MDSCs were a major component of the brain tumour infiltrate, which was also explored functionally. Depletion of $Ly6G^{+}$ cells prolonged survival of mice developing gliomas, as did COX-2 blockade with nonsteroidal anti-inflammatory drugs. The latter approach may have acted at two levels: by inhibiting the MDSC differentiation factor prostaglandin E_2, and by limiting MDSC recruitment to the brain tumour site by reducing levels of the MDSC chemoattractant CCL2.

A final type of myeloid cell that may be specifically recruited to brain tumours is an immunosuppressive $CD11b^{+}$ DC. In the context of glioma, this cell type has been most thoroughly investigated in the GL261 mouse glioma model (Biollaz et al., 2009). The brain infiltrating dendritic cells were shown to be of bone marrow origin using bone marrow chimeric mice, and they had little or no expression of costimulatory molecules (CD40, B7.1, B7.2) and GR1. Functional tests in vitro showed that brain infiltrating DCs could induce regulatory T cells from naïve CD4 T cells, but were inefficient at inducing alloreactive or antigen specific CD4 T cell proliferation. Of particular interest was that the immunosuppressive functions of these GL261 infiltrating DCs were predominately found when the tumour was growing intracranially, but not with the same tumour implanted subcutaneously. Moreover, the overall tumour infiltrate and microenvironment was entirely different in the brain: intracranial GL261 had higher levels of DCs, regulatory T cells and TGF-β, and mice had shorter survival. Overall, this important study indicates how brain tumour-immune system interactions are a consequence of both the tumour and the specialized site of the brain.

2.4.3 Microglia and macrophage function in the contest of malignant glioma

The overall consequences of the appearance of malignant glioma in the brain have been extensively studied in patients as well as in animal models; there is progressive invasive (but non-metastatic) growth, no evidence for spontaneous immune-mediated regression, but substantial evidence for local immunosuppression (Walker et al., 2003). The local microenvironment is of course complex, and a product of multiple cell type and site-specific

factors. The role of certain cells infiltrating the tumour is unambiguous, such as $Foxp3^{+}CD4^{+}$ regulatory T cells that infiltrate both human and mouse glioma (Walker et al., 2009). Myeloid cells are less simple to define, but the unique myeloid cells constitutively present in the brain are likely to be central players in shaping the developing tumour stroma, potentially being the precursors of other brain macrophages, and able to influence the function of other immigrant myeloid cells (as described in section 2.4.2 above). Ultimately, in advanced tumours, myeloid cells of all origins will become the major non-tumoural component of the brain tumour bed (Fig. 2). Indeed, multiple chemokines secreted by glioma cells are known to attract microglia and other myeloid cells. These include CCL21 (Biber et al., 2001; Zhai et al., 2011), MCP-3 (Okada et al., 2009), stromal cell derived factor-1α (SDF-1α) (Rempel et al., 2000; X. Wang et al., 2008), and MCP-1 (Fujita et al., 2011; Platten et al., 2003). Moreover, the latter two factors may be particularly efficacious at attracting myeloid cells to hypoxic areas of the tumour. Whether myeloid cells associated with brain tumours are a cause or a consequence of tumour progression is still controversial. Indeed both pro- and anti-tumoural functions can be demonstrated on isolated cells, but in vivo there are complex mixed populations, functioning in a microenvironment totally different from that created in vitro. The conceptual framework for understanding these functions has developed both from neuroscience and macrophage biology. Myeloid cells in brain tumours may appear to display some aspects of microglial cell function, i.e., anti-inflammatory and

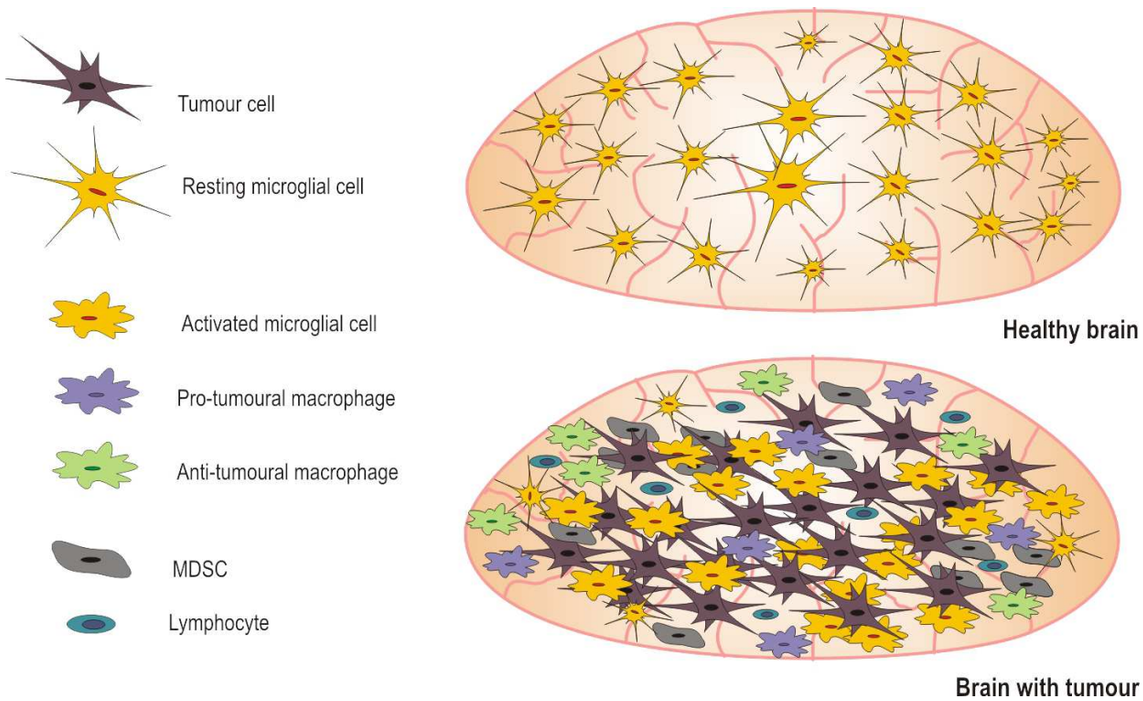

Fig. 2. Heterogenous myeloid and lymphoid cells infiltrate tumour bearing brain.

In healthy brain (top), the principle immune cells of the parenchyma are the microglia, which are resting, but active in brain surveillance. Appearance of malignancy (bottom) promotes activation of resident microglia and infiltration of diverse myeloid and lymphoid cells with potential for pro- and anti-tumoural functions (see text).

housekeeping roles, appropriate for maintaining healthy brain function (Kettenmann et al., 2011), but not for combating a lethal tumour. On the other hand, it is tempting to model brain myeloid cells on current concepts of macrophage plasticity, in which classically activated macrophages (M1) may promote anti-tumoural responses, whereas alternatively activated macrophages (M2) are predicted to be pro-tumoural (Biswas & Mantovani, 2010). This polarization concept is certainly helpful, as long as it can be associated with the specific dialect of neuroimmunology.

Anti-tumoural functions of microglia and brain macrophages. Microglial cells and brain macrophages have the potential to exert anti-tumour effects, and this has been demonstrated in vitro (Hwang et al., 2009; Rosales, 1996). Normal rodent microglial cells can release nitric oxide and Cathepsin B, which together can promote glioma cell apoptosis, whilst sparing normal cultured astrocytes (Hwang et al., 2009). However, constitutive function of microglial cells in vivo is unlikely to achieve the same effect. Nevertheless, global depletion of $CD11b^+$ cells in mice intracranially implanted with GL261 glioma resulted in increased tumour growth (Galarneau et al., 2007). The mechanisms were not fully elucidated, but were suggested to be T-cell independent. Moreover, the immune infiltrate was characterized as a type 1 response, with $CD11b^+$ cells being the main source of TNF, with also high levels of MCP-1 and IL-1β. Levels of type 2 cytokines IL-4 and IL-10 were low. One complexity in the interpretation of this study is that myeloid cell depletion (achieved by systemic administration of ganciclovir in CD11b-HSVTK mice) was incomplete, only up to 45%. Therefore, the result may be a consequence of the preferential elimination of pro-tumoural myeloid cells. This may also account for an apparently contradictory result in a more recent study using a virtually identical model, in which CD11b+ cell depletion reduced GL261 glioma growth (Zhai et al., 2011). The principle difference was that in this latter study, ganciclovir was administered intracranially, which would be assumed to give more efficient depletion of brain myeloid cells.

Regarding brain APC, we have observed presentation of brain tumour antigens in different murine models. However, the cell type responsible for this function was not defined. In the MT539 murine glioma, brain APC were responsible for cross-presenting glioma derived antigens to CD8 T cells, and retaining them in the brain parenchyma (Calzascia et al., 2003). In a different model, tumour-specific CD8 T cell activation in the cervical lymph node was measured after intracranial tumour implantation, and in this case we determined that the responsible cells that had migrated from the brain were radiosensitive, and thus were not parenchymal microglia (Calzascia et al., 2005). In another independent study using a different mouse tumour model, presentation of tumour antigen to T cell populations including CD4 T cells was observed (Plautz et al., 2000). Overall, these observations indicate that the brain is either equipped or can recruit cells with potential to directly (cytotoxicity) or indirectly (antigen presentation) act against tumours, but in the most malignant tumours, these functions may be subverted or overwhelmed by pro-tumoural elements.

Pro-tumoural effects of brain myeloid cells. Pro-tumoural effects of microglia and brain macrophages can be indirect or direct. In the former case, brain myeloid cells can participate in creating an immunosuppressive microenvironment, thus blocking immunity that could act against the tumour. In the latter case, they can directly act on the tumour or its microenvironment to promote tumour growth and invasion. As mentioned in the above subsection, overall support for the pro-tumoural role of brain myeloid cells can be concluded from one of the two $CD11b^+$ cell depletion studies (Zhai et al., 2011), but such an

approach is arguably too extreme to either apply therapeutically, or to interpret in depth given myeloid cell heterogeneity. Based on the observations in malignant glioma that tumours progress in the presence of abundant myeloid cell infiltration (Nishie et al., 1999; Roggendorf et al., 1996; Rossi et al., 1988; Rossi et al., 1987), we will examine the evidence that this infiltrate supports tumour growth.

Many pro-tumoural effects of myeloid cells can be associated with M2 polarization. Although one group studying the GL261 model has suggested that the glioma associated myeloid cells are not readily characterized by such terminology (Umemura et al., 2008), the concept can also applied less rigidly to determine the overall balance of the infiltrate. Indeed, another study on GL261 glioma claimed that the polarization balance of the tumour associated $CD11b^+$ microglia or macrophages (mostly amoeboid) was distinctly towards a pro-tumoural M2 phenotype, with strong expression of IL-10 and arginase 1, both associated with dampening inflammation and T cell immunity. In human glioma, a histological study noted a malignancy-correlated infiltration of microglia or macrophages expressing the M2 associated markers CD163 and CD204 (Komohara et al., 2008). This infiltration was also associated with glioma production of M-CSF, and the proliferation index of the tumour. Based on additional in vitro experiments in which glioma supernatant induced CD163 and CD204 expression on macrophages, it was proposed, although not directly demonstrated, that glioma derived M-CSF is a key factor in M2 macrophage polarization.

Several studies from the Heimberger group have chronicled the in vitro functions of myeloid cells isolated from patients with malignant glioma (Hussain et al., 2006a; 2006b). These glioma associated microglia/macrophages expressed multiple toll like receptors, MHC class II, but low levels of co-stimulatory molecules. They did not support T cell proliferative responses, and they did not constitutively express significant levels of inflammatory or anti-inflammatory cytokines when tested ex vivo. Phagocytic function was detectable in these experiments, however, other studies on rat microglia indicated that continued presence of glioma cells may inhibit this process (Voisin et al., 2010).

Early demonstrations of microglia or macrophage modulation by glioma cells generally relied on co-culture with cell lines. More recent concepts in oncology have proposed that cancers, including glioma, arise from a small population of cells with stem-like properties, which can self-renew to produce tumourigenic daughter cells and more differentiated but non-tumourigenic cells (Singh et al., 2004). Glioma stem-like cells can be enriched in neurosphere cultures; these have now been assessed for their impact on human microglia and macrophage function (Wu et al., 2010). Supernatants from glioma stem-like cells were more potent at promoting monocyte recruitment than bulk glioma cells, potentially through CSF-1 and CCL2, and they inhibited macrophage phagocytosis. However, these supernatants also induced pro-tumoural characteristics, some of which may have been driven by phosphorylated *STAT3* expression in monocytes. Treated macrophages and microglia secreted immunosuppressive cytokines (IL-10, TGF-β1, IL-23) and potentiated inhibition of T cell responses. Moreover, these functions were enhanced when glioma stem-like cells were cultured under hypoxic conditions (Wei et al., 2011). Overall, these studies suggest that glioma stem-like cells acquire their central role in tumour malignancy not only through their intrinisic stem-like properties and ability to thrive in hypoxic areas of the tumour, but also by recruiting myeloid cells, recently confirmed in vivo (Yi et al., 2011), and reinforcing their pro-tumoural effects.

The lethality of malignant glioma is not due to metastasis as for most solid tumours, but due to its capacity to invade healthy brain tissue. This invasiveness is facilitated by metalloproteinases that degrade extracellular matrix. Membrane type 1 metalloprotease (MT1-MMP) is highly expressed in human glioma as well as in rodent models, and expression is concentrated within myeloid cells at the tumour borders. These cells were shown to be of amoeboid morphology and were derived from endogenous microglia (Markovic et al., 2009; Sliwa et al., 2007). In elegant in vivo and ex vivo experiments, membrane type 1 metalloprotease (MT1-MMP) was shown to be induced in microglial cells by soluble glioma-derived factors that were dependent on microglial expression of the toll-like receptor adapter protein MyD88 and the p38 MAPK pathway. Glioma growth was promoted by microglial MT1-MMP activation of glioma-derived pro-MMP-2, whereas reduction in glioma volume was achieved either in MyD88-/- mice, or after microglial cell ablation (Markovic et al., 2009). A further route by which microglia can promote invasion is through TGF-β release, which can subsequently induce metalloproteinase release from glioma cells (Wesolowska et al., 2008). Although many cells (including glioma cells) can produce TGF-β in vivo, in vitro invasion tests with microglial and C6 rat glioma co-cultures showed that microglial cells were an important source. This latter observation, although not the specific subject of this study, also confirms the likely contribution of microglial derived TGF-β to the immunosuppressive tumour microenvironment.

A further major pro-tumoural role of brain microglial cells and macrophages is achieved through their promotion of angiogenesis, a process essential for tumour progression. In an in vivo orthotopic mouse glioma model, hypoxia exposed glioma cells recruited $CD11b^+$ myeloid cells in a hypoxia-inducible factor (HIF) 1 and SDF-1α dependent manner (Du et al., 2008). Matrix metalloproteinase (MMP)-9 expression by these macrophages promoted angiogenesis by converting VEGF from a sequestered to a bioavailable state. Another central cytokine linked to angiogenesis in glioma is TNF, which can induce both VEGF (Ryuto et al., 1996) and MMP9 (Esteve et al., 2002). Although TNF is considered a product of M1 polarized cells, its expression was nevertheless described in perivascular and intratumoural macrophages in human glioma (Roessler et al., 1995) as well as in $CD11b^+CD45^{high}$ macrophages infiltrating GL261 mouse glioma (Nakagawa et al., 2007). Angiogenesis may also be driven indirectly, through microglia/macrophage stimulated release of angiogenic factors from the glioma cells. In this regard, several studies in human glioblastoma correlated microglia/macrophage macrophage infiltration of glioma with the angiogenic factor IL-8 (Hong et al., 2009; Nishie et al., 1999; X. B. Wang et al., 2011), and in some cases vessel density (Nishie et al., 1999; X. B. Wang et al., 2011).

2.4.4 Therapeutic modulation of microglia and macrophage in brain malignancy

The balance of pro- and anti-tumour functions of brain microglia and macrophages is clearly not favourable in progressive brain tumours, and so their modulation could be an attractive therapeutic strategy. In view of their essential roles of microglial cell function in the healthy brain, ablation of all brain myeloid cells would appear unwise. Multiple possibilities for modulation of cancer promoting macrophages are being explored in non-cerebral malignancies. In this section we will concentrate on approaches already developed in brain tumours: blocking the infiltration of certain myeloid cells, targeting of key transcription factors, re-polarization with strong stimuli and TGF-β inhibition (Fig.3).

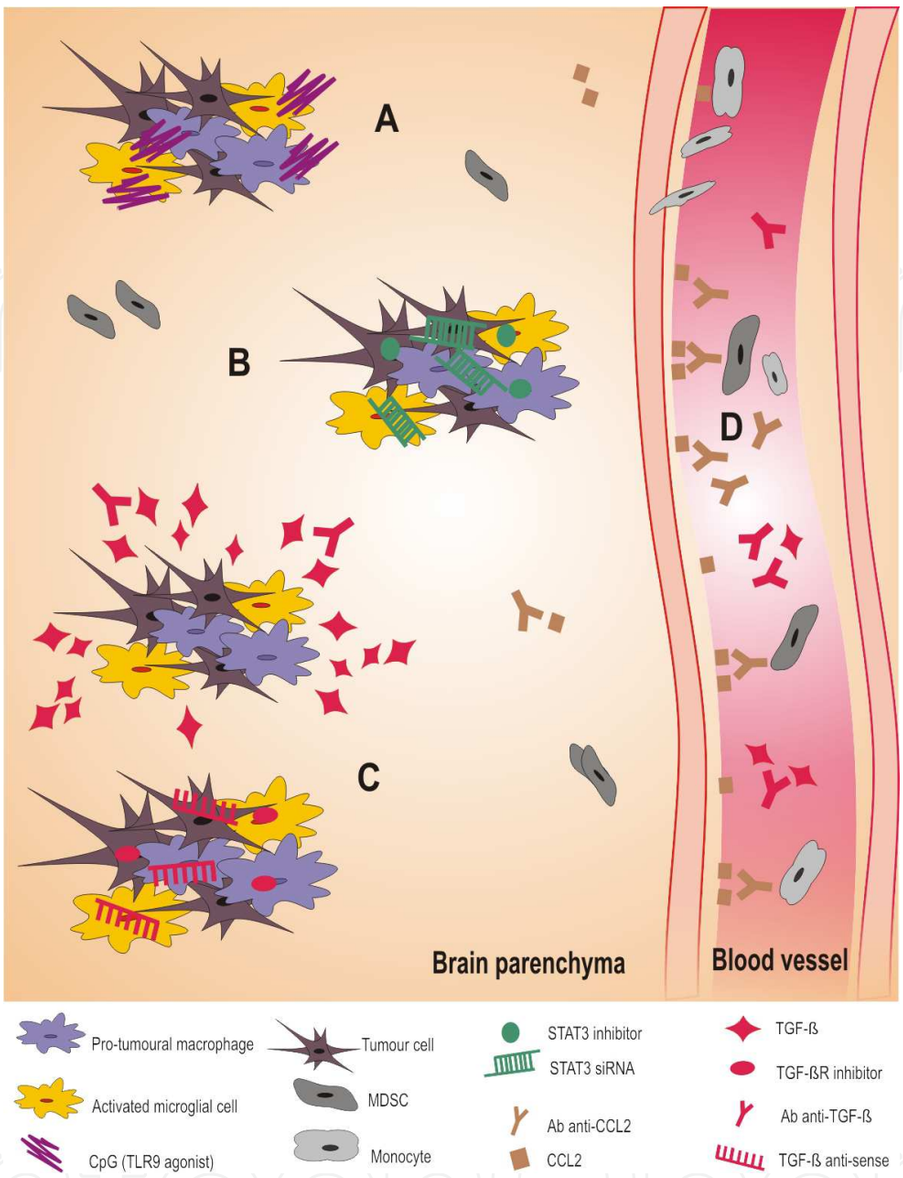

Fig. 3. Therapeutic approaches to modulate myeloid cells in brain tumours.

Examples of therapies having a major impact on myeloid cells are indicated. Promotion of M1 anti-tumoural polarization of brain myeloid cells can be achieved by use of the TLR9 agonist CpG (A) and by inhibition of STAT3 in multiple cell types (B). Inhibition of TGFβ or its signaling diminishes immunosuppression and tumour progression by acting on multiple target cells (C). Infiltration of pro-tumoural monocytes/macrophages and MDSCs can be reduced by antibody blockade of the MCP-1/CCL2 (D).

Blocking infiltration of pro-tumoural myeloid cells. Antibody inhibition of a myeloid cell chemoattractant is a straightforward and attractive approach to block immigration of deleterious cells. Based on the observations that MCP-1/CCL2 is expressed by human glioma and is associated with glioma aggressiveness and macrophage infiltration in animal models (Platten et al., 2003), antibody blockade was tested as a mono- and combination

therapy (Zhu et al., 2010). Macrophage and MDSC infiltration was decreased (but not abolished) in GL261 mouse glioma and a human xenografted model, although resident $CD11b^{+}CD45^{low}$ microglia were less affected, perhaps because of limited antibody penetration into the tumour bed. Survival was only modestly increased, but this significantly improved in combination with the alkylating chemotherapy agent temozolomide. Such approaches may have a role in modulating infiltrating macrophages, but if a high proportion of pro-tumoural macrophages are derived from brain resident macrophages, efficacy will be limited.

STAT3 inhibition. Although not a myeloid cell specific approach, targeting signal transducer and activator of transcription 3 (STAT3) is predicted to influence many immunosuppressive and pro-tumoural factors in the brain tumour microenvironment. Inhibition of STAT3 can be achieved with small molecular inhibitors, and is a particularly attractive therapeutic target in cancers such as glioma, because activated STAT3 is detected both in the tumour cells and in the infiltrating immune cells. Treating human microglia/macrophages isolated from glioma with a STAT3 inhibitor induced costimulatory molecule and type 1 cytokine expression (Hussain et al., 2007). Another compound that inhibits STAT3 is the plant-derived corosolic acid (Fujiwara et al., 2010). In vitro studies on human monocyte-derived macrophages showed that this compound prevented M2 macrophage polarization, as measured by inhibition of CD163 expression and IL-10 secretion (M2 markers); it also augmented glioblastoma cell apoptosis (Fujiwara et al., 2010). In mouse models, a STAT3 inhibitor or small interfering RNA blocked "immunosuppressive polarization"(principally IL-10 and IL-6 secretion) of microglia stimulated by glioma cell supernatant (Zhang et al., 2009). Furthermore, in orthotopic GL261 murine glioma in vivo, intratumoural STAT3 siRNA injection augmented TNF mRNA expression, and prolonged survival (Zhang et al., 2009).

TLR Agonists. Pathogen stimulation of myeloid cells through pathogen recognition receptors such as toll like receptors (TLRs) can result in strong M1 polarization that would potentially be useful in anti-tumour immunity, but is not generally achieved during chronic tumour growth. However, it is predicted that use of synthetic TLR agonists can mimic such pathogen induced activation. CpG oligonucleotides as agonists of the intracellularly expressed TLR9 have been investigated in many glioma preclinical models as well as in patients. Intratumoural injection of CpG oligonucleotides in orthotopic rat glioma models reduced tumour volume and elicited therapeutic immunity in a proportion of animals. These successful preclinical experiments have led to a clinical trial, but the clinical results showed only marginal benefit (Carpentier et al., 2010). Indeed, other preclinical studies gave very variable results, ranging from tumour rejection to enhancement of tumour volume (Ginzkey et al., 2010). A recent approach has tried to improve the targeting of CpG oligonucleotides by conjugating CpG oligonucleotides to carbon nanotubes that are preferentially taken up by brain myeloid cells after intratumoural injection (Zhao et al., 2010). In vitro experiments showed that monocytes were efficiently stimulated by the conjugated CpG to express high levels of type 1 cytokines (including IL-12 and TNF). In vivo, GL261 mouse glioma was eradicated by conjugated CpG injection in half of the mice. Overall, there seems to be considerable promise in the approach of stimulating brain myeloid cells with TLR agonists to reorient their functional polarization, although optimization to improve efficacy of the next generation of clinical trials will be essential.

TGF-β inhibition. The central role of TGF-β in promoting tumour progression makes it a highly relevant therapeutic target in brain malignancy. The multiple targets and sources of this

cytokine makes interpretation of its therapeutic modulation complex, since of TGF-β acts directly on tumour cells, as well as on pro- and anti-tumoural myeloid and other immune cells. TGF-β inhibition through systemic application of neutralizing antibody (1D11) enhanced efficacy of therapeutic vaccination in mouse GL261 glioma, and promoted a type 1 immune response, although it was not effective as a single modality treatment (Ueda et al., 2009). In a further study, the same 1D11 antibody was tested on both subcutaneous and intracranial GL261; in the former site, there was total tumour regression, whereas the orthotopic glioma merely showed reduced invasion (Hulper et al., 2011) These results suggest that it will be challenging to achieve full efficacy with systemic use of blocking antibodies for tumours localised in the brain. Local TGF-β inhibition has been tested with intratumoural delivery of synthetic antisense oligonucleotides: in a rat intracranial glioma model this showed therapeutic benefit when combined with tumour vaccination (Liu et al., 2007). However, when tested clinically as a monotherapy in recurrent high grade glioma, the clinical effects were disappointing (Bogdahn et al., 2011). A potentially simpler approach is with small molecules targeting TGF-β receptor signalling pathways: these are predicted to have better penetration of the brain tumour bed. Early preclinical studies with a systemically delivered TGF-β receptor I kinase inhibitor (SD-208) indicated survival benefit on mice bearing syngeneic SMA-560 brain tumours; this also correlated with enhanced infiltration of $CD11b^+$ cells (in addition to $CD8^+$ T cells and NK cells) (Uhl et al., 2004). Similar approaches are currently being tested in clinical trials. Overall, targeting TGF-β or its effects may be a valuable way of restoring antitumoural polarization of myeloid and other elements in brain tumours, particularly in the context of multimodal therapies.

3. Conclusion

The myeloid cells populating the brain in health and in malignant disease show a heterogeneity mirroring that found in other tissues, but the microglial cells have particularities that are found in no other tissue macrophages. Their unique features are most striking in the normal brain, when "resting" but highly surveillant ramified microglia are the principle myeloid cell of the brain parenchyma. Is this only of interest for neuroscience, or does it also impact on brain cancer, in which the myeloid infiltrate of advanced cerebral malignancies may superficially resemble that of extracranial tumours? Analysis of lower grade tumours in patients, or early stages of gliomagenesis in animal models reveals that endogenous parenchymal microglia are clearly detectable, and thus shape the microenvironment that influences tumour progression and the function of immune cells immigrating from the periphery. For primary brain tumours, endogenous microglia will also be the first to detect the malignant lesion, and it is possible that their default response of dampening inflammation leaves opportunity for tumour progression.

Do these factors influence how we should treat brain tumours? We suggest that the particularities of the brain and its resident protective cells should be respected in treatment design. Pro-tumoural myeloid cells that we may wish to target will generally be of two sources: immigrating cells from the blood, and proliferating and differentiating microglia. Most systemically used drugs that aim to block myeloid cell infiltration will only efficiently target the former cells. The alternative approach of repolarizing pro-tumoural cells to release high levels of inflammatory cytokines may be promising, but it may not be advisable to convert all brain myeloid cells to highly pro-inflammatory cells, since uncontrolled brain inflammation can be as dangerous as the cancer we are aiming to treat.

Current results from treatment approaches for malignant glioma that impact on myeloid cells should offer hope, but should also impose caution. For example, the therapeutic potential of strong macrophage stimuli such as CpG is highly encouraging in certain preclinical trials. But what we now need to understand is why it is not always successful - either in different preclinical models, as well as for many patients in clinical trials. If we can better define those cells and polarizations that are deleterious, we can envisage better targeting of therapeutic agents to improve efficacy and safety of the next generation of cancer therapies.

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Tumor Microenvironment and Myelomonocytic Cells
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Tumor microenvironment represents an extremely dynamic niche shaped by the interplay of different cell types (e.g. tumor cells, stromal cells), their soluble products (e.g.cytokines, chemokines and growth factors) and varied physico-chemical conditions (e.g low oxygen concentration or hypoxia). Recent studies have identified myelomonocytic cells as key players in regulating the tumor microenvironment and hence, tumor progression in a variety of cancers. In view of these findings, the present book attemps to provide a comprehensive account of the diversity of tumor microenvironment across different cancers and how myelomonocytic cells have taken the center-stage in regulating this niche to direct cancer progression. A better understanding of the myelomonocytic cells and the mechanisms by which they regulate cancer progression will open new vistas in cancer therapeutics.

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