Macrophage/Microglia Polarization in Treating Glioblastoma/Multiple Sclerosis

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Macrophages and microglia are implicated in several diseases with divergent roles in physiopathology. This discrepancy can be explained by their capacity to endorse different polarization states. Theoretical extremes of these states are called M1 and M2. M1 are pro-inflammatory, microbicidal, and cytotoxic whereas M2 are anti-inflammatory, immunoregulatory cells in favor of tumor progression. In pathological states, these polarizations are dysregulated, thus restoring phenotypes could be an interesting treatment approach against diseases.

macrophage | microglia | glioblastoma | multiple sclerosis | polarization | treatments | therapeutic

1. Macrophages/Microglia in MS

1.1. The M1-M2 Balance Consequences on Multiple Sclerosis

Microglia and macrophages are implicated in the pathophysiology of multiple sclerosis. On one hand, they lead to the destruction of the myelin sheath and the damage of axons by secreting pro-inflammatory cytokines, presenting antigens, and inducing oxidative stress; on the other hand, they promote remyelination, tissue repair, resolve inflammation, and perform phagocytosis of myelin debris. These contrasting effects are thought to correspond to the two major polarization states: M1-like and M2-like, respectively [1][2][3]. In fact, as discussed above, in vivo, the situation is much more complicated: the cells are able to express M1 markers as well as M2 markers simultaneously. The polarization state depends a lot on environmental signals, the length, and the combination of stimuli. It shows once more the plasticity of macrophages and microglial cells, changing from a pro-inflammatory polarization state during the acute phase of the disease, to an anti-inflammatory state during the recovery phase, but also the capacity to stay in an intermediary state between both phenotypes [1][4][3]. Interestingly, in chronic active lesions, M2 markers are lacking: they disappear when an active lesion evolves from an acute to a progressive one [4]. Targeting the imbalance between pro-inflammatory and anti-inflammatory functions of macrophages/microglia is a possible treatment option to restore polarization in favor of remyelination.

1.2. Treatments Influencing Polarization for MS Care Already on the Market

An important number of treatments indicated for MS care already exist and are on the market. Among these, some have been shown to indirectly target macrophage and microglia polarization. They are part of two different groups

of compounds. The first group is composed of proteins and peptides including interferon β and glatiramer acetate. The second group consists of small molecules including dimethylfumarate, fingolimod/siponimod, and glucocorticoids.

1.2.1. Proteins/Peptides

Interferon β is a first line background treatment for multiple sclerosis with diverse mechanisms of action. It is an anti-inflammatory cytokine that acts by increasing the production of other anti-inflammatory cytokines such as IL-10 and IL-4, by decreasing the production of pro-inflammatory cytokines such as IL-12 and IL-17, by limiting the leukocyte migration across the blood–brain barrier (BBB) and by promoting the production of nerve growth factor (NGF) in favor of neuronal survival and repair [5]. Concerning macrophages and microglia, the modifications of the expression of pro-inflammatory and anti-inflammatory cytokines directly affect their polarization state toward a M2-like phenotype. In MS patients treated with IFN-β, it was observed that the antigen presenting ability and the migration capacity of these cells was diminished and that inhibitory immune checkpoints (in particular B7-H1) were activated [6].

Glatiramer acetate is also a first line background treatment for relapsing-remitting multiple sclerosis. It is a random basic copolymer of four natural occurring amino acids: glutamic acid, lysine, alanine, and tyrosine. Its mechanism of action involves the generation of Th2 and Treg regulatory and anti-inflammatory lymphocytes, but also the induction of regulatory, anti-inflammatory, M2-like macrophages, and microglia [6]. These polarization shifts are accompanied by a diminished secretion of pro-inflammatory cytokines (e.g., TNFα and IL-12) and an augmented secretion of anti-inflammatory cytokines (IL-10 and TGF-β). The drug is also able to induce the phagocytic activity of macrophages and microglia in rats and humans, which allows for the clearance of myelin debris in favor of remyelination [6][7]. Through the APC role of macrophages, it is hypothesized that they are responsible for the amplification of Th2 cells, meaning that for this drug, macrophages and their anti-inflammatory polarization have a central duty [8].

1.2.2. Small Molecules

Dimethylfumarate is also indicated for the background treatment of relapsing-remittent multiple sclerosis. Originally a treatment for psoriasis through the induction of a shift from a Th1 response to a Th2 response, the drug also induces the expression of the anti-inflammatory cytokine IL-10 by lymphocytes, but also by macrophages and microglia. Moreover, it inhibits the expression of pro-inflammatory cytokines (e.g., TNFα, IL1-β, and IL-6) by macrophages and microglia, thus promoting a M2-like phenotype [6][7][9][10].

Fingolimod is a modulator of the sphingosine-1-phosphate receptors indicated in the second line for background treatment of very active forms of multiple sclerosis. Fingolimod treatment leads to the inhibition of the egress of lymphocytes from lymphoid tissues into the circulation, thus preventing their trafficking toward the CNS. Interestingly, macrophages and microglia also express this receptor. It has been shown in human and animal models that fingolimod is able to prevent the activation of an inflammatory phenotype of these cells [6][7][11]. Fingolimod is able to modulate macrophage and microglia activation by lowering their pro-inflammatory cytokine

production (e.g., TNFα), thus protecting oligodendrocytes from death and favoring the remyelination process. This mechanism corresponds to a changing of the polarization of macrophages and microglia toward a M2-like phenotype [11][12][13]. Another modulator of the sphingosine-1-phosphate receptors is the fingolimod-derived compound siponimod. Siponimod is selective for S1P1 and S1P5 receptors, and lowers the risk of adverse cardiac events mediated through the S1P3 receptor subtype [14]. This molecule protects against neurodegeneration by limiting inflammation and the recruitment of immune cells and by preventing neuronal loss. This neuroprotection is partly mediated through the interaction of siponimod with microglia, leading to an inhibition of the secretion of pro-inflammatory cytokines (IL-6 and CCL5), thereby re-educating them toward an anti-inflammatory phenotype [14][15][16].

Glucocorticoids are indicated for the treatment of acute relapses of MS, particularly high doses of methylprednisolone. These drugs target glucocorticoid receptors and exert their activity mainly by inhibiting T cell activation and promoting their apoptosis, but also by inhibiting the secretion of pro-inflammatory cytokines and by improving the integrity of the BBB, thus preventing the infiltration of immune cells. These effects also affect monocytes and macrophages by preventing their secretion of pro-inflammatory cytokines (like TNF-α, IL-6 and IFN-γ) and by promoting an anti-inflammatory M2-like polarization while increasing their chemotaxis. This is in favor of the resolution of the inflammation, but also for reparation and remyelination. It was noted that these effects were observed in vitro on monocytes from MS patients treated with glucocorticoids [6][17].

1.3. Compounds Influencing Polarization for MS Care: New Perspectives

All the drugs presented above are already on the market and have an authorization for the treatment of multiple sclerosis. Most of the drugs indicated for this pathology show effects on the polarization of macrophages and/or microglia. However, therapeutics specifically targeting these cells are lacking despite their important role in pathophysiology. We now present some drugs under development targeting macrophages, microglia, and their polarization. Two major groups of compounds are being investigated: small molecules with a synthetic origin and natural occurring compounds directly extracted from their natural source. All these compounds are immunomodulators without a unique molecular target.

1.3.1. Synthetic Small Molecules

Lenalidomide is an FDA approved drug derived from thalidomide for the treatment of myelodysplastic syndromes and multiple myeloma. However, it has been shown in vitro that lenalidomide possesses repolarizing effects on macrophages. In fact, the drug can skew macrophages toward a M2-like phenotype. This was observed because an upregulation of M2 markers (Arg1, Mrc1) and an increase of the secretion of anti-inflammatory cytokines (IL-4, IL10, IL-13 and TGF-β) could be detected. Interestingly, lenalidomide does not inhibit the LPS-induced M1-like polarization, meaning that the macrophages express both M1 and M2 markers. Nonetheless, lenalidomide was also tested in vivo on the mice model of MS: Experimental Autoimmune Encephalomyelitis (EAE), obtained after exposure of the animals to myelin antigens (here a MOG-EAE model). Results show that the effect on

macrophages is enough to alter their capacity to activate autoimmune CD4 T cells and to alleviate the symptoms of the disease [18].

Ethyl pyruvate is an analogue of the EMA- and FDA-approved drug dimethylfumarate. Its effect on macrophages/microglia is an inhibition of their secretion of pro-inflammatory cytokines (TNF-α, IL-6) and an inhibition of their antigen presentation capacity [19][20].

Another example is the immunomodulatory molecule laquinimod. The activity of this compound is mediated through the promotion of regulatory T cells, but also the decrease in activation of microglia and the inhibition of the recruitment of macrophages in the CNS [21][22][23]. Interestingly, laquinimod has already been tested in humans in phase II as well as phase III studies, which showed effects in reducing brain atrophy and limiting disability worsening. Moreover, the orally administered compound seems to be safe and well tolerated [24][25].

A last example is minocycline, an antibiotic of the tetracycline family, which shows neuroprotective effects in RRMS [26]. Its activity is mediated by the inhibition of T-cell migration and activation, but also by the inhibition of the proliferation and M1-like activation of macrophages and microglia by limiting the secretion of pro-inflammatory cytokines (TNF-α) and promoting the secretion of the anti-inflammatory ones (IL-10). An attenuation of the clinical course of EAE has been observed [27][28][29]. Minocycline has also been tested for its efficacy on humans and it seems to lower the risk of conversion from a clinically isolated syndrome to MS at six months but not at 24 months [30][31]. Another clinical trial assessing the effect of the combination of minocycline and interferon β-1a for RRMS showed no beneficial effect [32]. More studies are needed to better understand the mechanism of action of minocycline.

1.3.2. Natural Occurring Compounds

An important number of natural occurring molecules have been tested for their repercussions on macrophage and/or microglia polarization. These have been reviewed extensively in [33][34][35]. Most of these compounds do not target only macrophages/microglia and their polarization. However, we provide some examples of compounds that have been tested for their ability to influence polarization toward an anti-inflammatory phenotype in multiple sclerosis models.

The first example is spermidine, a polyamine found in most organisms. It is biosynthesized from putrescine, which is obtained in the body from the amino-acid ornithine. Spermidine has been shown in vitro to inhibit pro-inflammatory cytokine secretion by LPS-stimulated microglia. It inhibits the expression of iNOS and COX-2, two major pro-inflammatory enzymes and decreases the secretion of NO, PGE_2, IL-6, and TNF-α at the transcriptional level. These effects seem to be mediated through the inhibition of the NF-κB, PI3K/Akt, and MAPK signaling pathways [36]. Spermidine has also been tested for effects in vivo with the EAE model. After treatment with spermidine, the mice showed decreased EAE clinical scores and the severity of the disease was diminished in both preventive and curative treatment schemes. These repercussions are mediated through macrophages because spermidine is able to inhibit their pro-inflammatory polarization by diminishing the secretion of the pro-inflammatory cytokines IL-1β, IL-6, IL-12, and TNF-α and by decreasing their antigen-presentation to lymphocyte capacity. As a

consequence, less T-cells are activated and the EAE severity is weaker [37][38]. To our knowledge, no clinical trial has been conducted to assess the effect of spermidine intake on multiple sclerosis.

Resveratrol is a stilbene found in berries, grapes, and nuts. It is one of the most studied natural compounds in multiple sclerosis. Most of the studies agree on the protecting effect of resveratrol in the EAE model. Its administration reduces the severity of the disease in mice and favors remyelination. The activity is mediated through the inhibition of secretion of pro-inflammatory cytokines by macrophages (e.g., IL-6 and TNF-α), but also by promoting the activation of regulatory T cells (Th17) [39][40][41][42]. Nevertheless, one study by Sato et al. showed surprising results because resveratrol was responsible for an exacerbation of EAE disease in mice [43]. These counterintuitive results show that it is difficult to work with natural compounds as plants display an important variability in their composition and have a wide scope of different targets, thus the determination of the real effect is challenging.

2. Macrophages/Microglia in GBM

2.1. TAMs and M1-M2 Balance

Glioblastoma are the most frequent type of gliomas, but also one of the most aggressive. They are characterized by a very important mitotic activity, high vascular proliferation, and necrosis. Glioblastomas are highly invasive and infiltrate the surrounding tissues, although they do not metastasize [44]. These tumors are also characterized by their high capacity to evade the immune system [45]. Macrophages and microglia have a leading role in this immune evasion capacity and represent the majority of non-neoplastic cells of the tumor microenvironment. These macrophages and microglia possess a specific M2-like polarization and are called tumor associated macrophages or TAMs [46][47][48]. TAMs are in favor of GBM tumorigenesis because they do not support cytotoxic T cell activation and even inhibit their proliferation, as they secrete anti-inflammatory cytokines (e.g., IL-10 and TGF-β) and growth factors (e.g., EGF), thus promoting development and angiogenesis of the tumor [46][45][49][50]. This system is a vicious circle because TAMs are promoted by the tumor itself, which secretes various factors such as IL-6, IL-10, TGF-β, and PGE2, and in return, TAMs promote the tumor's proliferation and survival [51][45]. A solution to break this circle would be to inhibit the anti-inflammatory phenotype of TAMs and promote a repolarization toward an inflammatory phenotype [50].

2.2. Treatments Influencing Polarization for GBM Care

Targeting TAMs in glioblastoma is an interesting alternative, knowing the fact that they represent up to 30% of the cells constituting the tumor bulk [46]. Contrary to that of MS, no treatment on the market for GBM is currently targeting macrophage/microglia polarization. Thus, some compounds studied for their effect in this field will be reviewed here. These compounds are either small molecules, antibodies, or nucleotides that target five major signaling pathways: CSF1 and its receptor, CD47, CD40, TLRs, and STAT.

2.2.1. Colony Stimulating Factor 1 and Its Receptor

The first and most studied target is colony stimulating factor 1 (CSF1) and its receptor (CSF1R, also called CD115). CSF1R is a tyrosine-kinase receptor activated by the binding of the ligand CSF1, followed by homodimerization of the receptor. This signaling pathway is implicated in the differentiation of monocytes into M2-like, anti-inflammatory macrophages. Thus, inhibiting the activation of the receptor by targeting the ligand or the receptor is an interesting opportunity [52][53][54]. An important number of compounds were designed, tested in vitro, in vivo, and even clinically. All of these are small molecules or antibodies [53]. The most studied among them is a small molecule inhibitor called pexidartinib (or PLX3397), which can be taken orally and is able to cross the BBB. It showed interesting results in vitro and in mice by blocking glioblastoma progression, proliferation, and evasion [55] [56]. Unfortunately, a clinical trial conducted on 37 patients with recurrent glioblastomas showed no effect of the molecule. However, pexidartinib has a good safety profile, is well tolerated by the patients, and is able to reach the tumor tissue [57]. The lack of effects of the treatment alone does not mean that it is not reaching the target or influencing it. An interesting option is to assess the effects of pexidartinib in combination with other known treatments. An enhancement of the efficacy has been shown in vivo on tumor bearing mice by combining PLX3397 and other antiangiogenic tyrosine-kinase inhibitors (dovitinib or vatalanib). An inhibition of tumor cell proliferation and an increase in tumor-cell apoptosis as well as a downregulation of M2 markers in TAMs compared to PLX3397 given alone has been observed [55]. A clinical trial has been conducted to assess the efficacy of the combination of pexidartinib, radiotherapy, and temozolomide (NCT01790503), but the results are not available at this moment [58].

Another well studied molecule is BLZ945, which is also a brain penetrating tyrosine-kinase inhibitor with a strong affinity for CSF1R. This compound is able to inhibit growth, progression, and survival of glioblastoma tumor cells in vivo by “re-educating” the TAMs and increasing phagocytosis of tumor cells. In fact, BLZ945 induces a downregulation of M2 markers in TAMs rather than depleting them [59]. However, Quail and colleagues showed that although BLZ945 strongly induces tumor regression in mice, after a few weeks of treatment, a resistance to the compound appeared and the tumor rebounded. This resistance is mediated through the TAMs. They compensate CSF1R inhibition by upregulating the IGF-1/IGF-1R axis and the PI3K signaling pathway, which are in favor of tumor growth and malignancy. A M2-like pro-tumorigenic phenotype is restored via the upregulation of M2 markers (TGF-β, IL4 and CD206). To avoid this resistance, combinational therapy is needed by combining BLZ645 with inhibitors of the resistance pathways (e.g., IGF-1R inhibitors) [60]. Further studies are needed to assess the effects of BLZ945 in association with other compounds and for the moment, no clinical trial has been conducted for its effect on GBM.

2.2.2. CD47

Another possibility is to target CD47 and inhibit its activation. CD47 is an integrin also called the “don’t eat me” signal found on tumor cells, which inhibits their phagocytosis by macrophages after the binding of CD47 and SIRPα (signal-regulatory protein α) found on macrophages. Inhibition of this axis leads to the induction of phagocytosis of the tumor cells by M1-like and M2-like macrophages. This induction is much more important in M1-like macrophages. Furthermore, the inhibition of the axis also leads to the re-polarization of TAMs from a M2-like to a M1-like phenotype. Inhibition of this axis is obtained by the use of anti-CD47 antibodies. Hu5F9-G4, a monoclonal humanized antibody showed anti-tumor effects on murine and human glioblastoma cell lines and in

vivo on grafted mice [61]. Another antibody targeting CD47 has been used after surgical resection of GBM in rats and extended survival of the animals and retarded relapse of the tumor has been observed [62]. A last anti-CD47 antibody has been tested on glioma stem cells: it is also effective to induce phagocytosis of these cells in vitro and in vivo, thereby limiting tumor growth [63].

2.2.3. CD40

The next target is CD40, a costimulatory molecule, member of the tumor-necrosis factor receptor family that is expressed on the surface of immune cells (B cells, dendritic cells, and macrophages), non-immune cells (endothelial and epithelial cells), and on tumor cells. Its activation by the ligand CD40L by activated T-cells, platelets, and macrophages induces the proliferation and differentiation of B cells and macrophages, which is accompanied by the promotion of antigen presentation and anti-tumor immunity [64]. Activating CD40 is a potential mechanism for targeting macrophage polarization, as shown for pancreatic adenocarcinoma. In a murine model of this cancer, a monoclonal anti-CD40 agonist antibody is able to induce tumor regression by repolarizing macrophages toward a pro-inflammatory phenotype [65]. Knowing the fact that in glioblastoma, upregulation of the CD40/CD40L axis is an indicator for better prognosis, it is interesting that in vivo, the use of this anti-CD40 agonist antibody actually shows anti-tumor effects [64][66]. This effect is mediated by pushing the macrophages toward a M1-like phenotype [67].

2.2.4. Toll-like Receptors (TLRs)

Toll-like receptors (TLRs) are also a studied target. This family of receptors is part of the pattern recognition receptors (PRRs) involved in the initiation of innate immune response by recognizing microbial-associated molecular patterns (MAMPs) and danger-associated molecular patterns (DAMPs) present on microorganisms. Their activation induces cytokine secretion, opsonization, phagocytosis, activation of the complement system, and proliferation [68]. Although there is a discrepancy regarding the role of TLRs in tumors, some agonists have been tested for their effects on glioblastoma. The most studied among them are phosphorothioate oligodeoxynucleotides (ODN) containing unmethylated cytosine-guanosine motifs (CpG) and targeting TLR9. These immunostimulatory substances are able to induce tumor death and to increase the survival of grafted rats and mice through an effect on the immune system. Indeed, in immunodeficient mice, the effect of ODN is abrogated [69][70]. CpG-ODNs were also appraised for their effects on humans. In two different phase I clinical trials, it was shown in patients with recurrent GBM that CpG-ODNs are generally well tolerated, except some cases of lymphopenia, mild fever, seizures, and transient neurological worsening. It was also observed that the survival of patients was slightly extended compared to patients treated with temozolomide [71][72]. However, in a phase II trial, CpG-ODNs injected directly in the brain tumors of patients with recurrent GBM only showed modest activity. The radiological responses were low, but the number of long-term survivors was higher than in other studies. This example shows an important limit in the use of compounds targeting macrophages and the immune system in general: the considerable patient- and tumor-dependent response to the compounds [73]. Another limitation of these compounds as well as other TLR agonists (e.g., imidazoquinolines) is the absence of specificity, which can be the source of side effects. As a matter of fact, their activity is not only mediated through macrophages, but also through NK cells, dendritic cells (DCs), and T cells [68][70][74].

2.2.5. Signal Transducers and Activators of Transcription (STATs)

Signal transducers and activators of transcription (STATs) also serve as targets for the repolarization of macrophages/microglia. STAT3 is implicated in the M2-polarization process and its inhibition is an interesting option to favor a pro-inflammatory and anti-tumoral state. Such an inhibitor is the small molecule WP1066. This compound is able to activate macrophages/microglia and to drive them toward a pro-inflammatory state. Results in vitro and in vivo showed that WP1066 is able to reduce glioblastoma viability and growth [51][75][76]. Its efficacy is mediated through the induction of the expression of costimulatory molecules (CD80 and CD86) by macrophages and microglia, the secretion of pro-inflammatory cytokines (IL-2, IL-12, and IL-15), and the induction of the proliferation of effector T-cells [77]. Two phase I clinical trials to study WP1066 and its effect on gliomas including glioblastomas are currently recruiting (NCT01904123 and NCT04334863).

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