Microglia and macrophage metabolism in CNS injury and disease: the role of immunometabolism in neurodegeneration and neurotrauma

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Abstract

Innate immune responses, particularly activation of macrophages and microglia, are increasingly implicated in CNS disorders. It is now appreciated that the heterogeneity of functions adopted by these cells dictates neuropathophysiology. Research efforts to characterize the range of pro-inflammatory and anti-inflammatory phenotypes and functions adopted by microglia and macrophages are fueled by the potential for inflammatory cells to both exacerbate neurodegeneration and promote repair/disease resolution. The stimulation-based, M1/M2 classification system has emerged over the last decade as a common language to discuss macrophage and microglia heterogeneity across different fields. However, discontinuities between phenotypic markers and function create potential hurdles for the utility of the M1/M2 system in the development of effective immunomodulatory therapeutics for neuroinflammation. A framework to approach macrophage and microglia heterogeneity from a function-based phenotypic approach comes from rapidly emerging evidence that metabolic processes regulate immune cell activation. This concept of immunometabolism, however, is only beginning to unfold in the study of neurodegeneration and has yet to receive much focus in the context of neurotrauma. In this review, we first discuss the current views of macrophage and microglia heterogeneity and limitations of the M1/M2 classification system for neuropathological studies. We then review and discuss the current literature supporting metabolism as a regulator of microglia function in vitro. Lastly, we evaluate the evidence that metabolism regulates microglia and macrophage phenotype in vivo in models of Alzheimer’s disease (AD), stroke, traumatic brain injury (TBI) and spinal cord injury (SCI).

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INTRODUCTION: CURRENT VIEWS OF MACROPHAGE AND MICROGLIA PHENOTYPE IN THE CONTEXT OF NEUROTRAUMA AND NEURODEGENERATION

Central nervous system (CNS) trauma activates resident microglia and recruits peripheral monocytes into the injured nervous system. Accumulating evidence also implicates microglial activation in the pathophysiology of neurodegenerative diseases including Alzheimer’s disease (AD). Both activated microglia and blood-derived monocytes, collectively often referred to as CNS macrophages, adopt a kaleidoscope of functional phenotypes that contribute to progressive neurodegeneration as well as CNS repair and protection. We, and others, have classified the extremes of this CNS macrophage heterogeneity using the distinction of classically-activated or “M1” pathological macrophages and alternatively-activated, anti-inflammatory, or “M2” reparative macrophages (Gensel and B. Zhang, 2015; Tang and Le, 2016).

The M1 and M2 nomenclature is derived from pro- or anti-inflammatory stimulation of macrophages in vitro. Under these controlled conditions, interferon-γ (IFNγ) + lipopolysaccharide (LPS) stimulated, M1 macrophages upregulate a unique suite of phenotypic markers distinct to the pro-inflammatory condition (Gordon, 2003). Similarly, a unique set of phenotypic markers has been identified in response to interleukin-4 (IL-4), an anti-inflammatory, M2 stimulant (Gordon, 2003). Mapping the M1 or M2 in vitro gene expression pattern (e.g. inducible nitric oxide synthase iNOS/CD86 for M1 and Arginase/CD206 for M2) on activated macrophages and microglia provides insight into how CNS macrophages function during neuropathological conditions. Further dividing M1 and M2 into subclasses based upon various in vitro stimulation protocols has expanded our ability to associate CNS macrophage marker expression profiles in vivo with different functional phenotypes (Gensel and B. Zhang, 2015). Unique subclasses of M2 cells, for example M2b and M2c, have been observed in an age-dependent manner after traumatic brain injury (TBI) and spinal cord injury (SCI), and in neurodegenerative conditions such as AD (Kumar et al., 2013; Sudduth et al., 2013; B. Zhang et al., 2015). While an M1/M2 paradigm has utility in...
describing phenotypic heterogeneity of CNS macrophages, there is growing appreciation that this classification system greatly oversimplifies the range of phenotypes, and more importantly, functions, that occur in vivo.

Traumatic brain injury researchers, for example, have identified some of the limitations of the M1 and M2 classification system (Jassam et al., 2017; Morganti et al., 2016). Single-cell transcriptional profiling of monocyte-derived macrophages reveals that individuals cells can co-express M1 and M2 markers concomitantly after TBI (Kim et al., 2016). Similar observations have been made of presumptive microglia after TBI using immunohistochemistry (Morganti et al., 2016). The dynamic nature of neurodegenerative diseases is leading the AD field to approach microglia heterogeneity in a dynamic and disease-specific manner outside the constrains of M1 and M2 polarization (Sarlus and Heneka, 2017). The M1/M2 classification system has received less scrutiny in the SCI field and is often used as a barometer for assessing the efficacy of immunomodulatory therapies or inflammatory mechanisms of injury progression (Francos-QUIJorna et al., 2016; Gensel et al., 2017; Kroner et al., 2014; S.-F. Ma et al., 2015; Shechter et al., 2013). Nonetheless, although single-cell sequencing of microglia/macrophages has not been reported after SCI, RNA-sequencing of myeloid cells indicate that CNS macrophages likely adopt mixed phenotypes in this injury condition (E Hirbec et al., 2017; Noristani et al., 2017).

The challenge of applying the M1/M2 nomenclature to in vivo conditions arises from the fact that expression profiles are derived from controlled, in vitro stimulations while stimulants in vivo are difficult to identify. For example, pro-inflammatory, M1 macrophages elicited in vitro using a combination of IFNγ+LPS or IFNγ+ tumor necrosis factor α (TNFα) have increased phagocytic capacity, release pro-inflammatory cytokines (IL-1β, IL-12), and produce high levels of reactive oxygen species (ROS). In contrast, anti-inflammatory, M2 macrophages elicited with IL-4 or IL-13 release anti-inflammatory cytokines (e.g. IL-10) and growth factors, have low levels of ROS and nitric oxide production, and promote stem cell proliferation and differentiation. There is some evidence that these same stimulants drive CNS macrophage activation after injury. For example, TNFα has been identified as a key mediator of pro-inflammatory macrophage activation in SCI (Kroner et al., 2014). Further, loss of IL-4 receptor in knock-out (KO) mice exacerbates inflammation and drives M1-like activation after SCI (Fenn et al., 2014). However, it is difficult to identify and control endogenous stimuli governing CNS macrophage activation after injury and in neurodegenerative conditions, thus complicating the association of in vivo markers with in vitro phenotypes.

In addition, not all markers specific to in vitro M1 or M2 conditions are entirely predictive of in vivo functions. This is especially evident in the context of aging and SCI. We observed previously that the IL-4 receptor (IL-4R), essential for M2 stimulation in vitro, is decreased with age in mice (Fenn et al., 2014). Expectedly, in aged animals, or animals lacking the IL-4R, the M2 hallmark, arginase, is significantly reduced in CNS macrophages after SCI (Fenn et al., 2014). Paradoxically, however, the M1-specific marker, IL-1β is also reduced in CNS macrophages from aged and IL-4R deficient mice (Fenn et al., 2014). In a separate study, we observed that arginase-positive macrophages from aged SCI mice express ROS, a feature typically associated with M1 activation (B. Zhang et al., 2016). The
production of ROS is essential for pro-inflammatory functions including the breakdown of foreign microbes and phagocytic activity (Tan et al., 2016). Therefore, the concomitant decreases in expression levels of the M2 marker-arginase along with the pro-inflammatory cytokine IL-1β, or the co-expression of arginase and M1 function-facilitating ROS, highlight the discontinuity between marker expression and function in the injured CNS.

Classifying macrophages using a functional based profiling approach, as opposed to stimulation based-classification as with M1/M2, may provide needed insights into therapeutic strategies for potentiating protective macrophage/microglia function. The burgeoning field of immunometabolism provides the framework for understanding macrophage and microglia heterogeneity from a function-based phenotypic approach.

OVERVIEW OF IMMUNOMETABOLISM AND MACROPHAGE FUNCTION

The most efficient utilization of glucose for energy is through glycolysis to generate pyruvate to then feed the tricarboxylic acid (TCA or Krebs) cycle. The resulting NADH and FADH₂ from the TCA cycle serve as proton donors for mitochondrial electron transport and aerobic/mitochondrial respiration (aka oxidative phosphorylation, OXPHOS). The collective process generates 36 ATPs per glucose molecule and is facilitated through enzymatically-regulated steps that generate intermediate metabolites (Figure 1). In addition to providing energy, metabolism is intricately linked with macrophage function. The rapidly growing immunometabolism field has identified metabolic reprogramming as a hallmark of macrophage phenotype and function (Baardman et al., 2018; Diskin and Pålsson-McDermott, 2018; Koelwyn et al., 2018; Minhas et al., 2018; Puchalska et al., 2018; Van den Bossche et al., 2016). Pro-inflammatory macrophage functions include rapid motility, ROS production to degrade bacteria, prostaglandin and cytokine production, and high membrane turnover for phagocytosis. These functions have distinct metabolic needs including quick energy utilization (glycolysis); pentose phosphate pathway (PPP) activity and subsequent NADPH production to increase ROS activity; and fatty acid synthesis. In contrast, reparative/anti-inflammatory macrophages require sustained activation to facilitate transcription of tissue repair genes and amino acid and fatty acid breakdown to produce growth factors. Therefore, anti-inflammatory macrophages require efficient energy production utilizing OXPHOS for transcription of ATP-dependent tissue repair genes; minimal ROS production and efficient ROS scavenging to support sustained activation; as well as, amino acid and fatty acid oxidation to product growth factors, including polyamines and prolines, and to support mitochondrial respiration by providing metabolites for the TCA cycle.

Specific macrophage bioenergetics are likely essential for pro- and anti-inflammatory activation (summarized in Figure 1). Indeed, OXPHOS is effectively reduced in pro-inflammatory macrophages as essential substrates are hijacked into the PPP and out of the TCA cycle to support effector functions (Jha et al., 2015; Rodríguez-Prados et al., 2010). For example, pyruvate dehydrogenase (PDH) converts pyruvate into acetyl-coA which is then used as substrate for the TCA cycle and downstream OXPHOS, metabolic events that normally favor anti-inflammatory macrophage activation. However, under pro-inflammatory conditions where PDH activity is increased, the downstream metabolite of the TCA cycle,
itaconate, is syphoned off for pro-inflammatory cytokine production with corresponding decreases in OXPHOS (Meiser et al., 2016). Even under aerobic conditions favoring OXPHOS, pro-inflammatory macrophages maintain aerobic glycolysis, otherwise known as the “Warburg effect”. The Warburg effect includes increased glucose uptake that feeds glycolysis and increases lactate. Lactate is then used to support glycolysis with concurrent suppression of OXPHOS (Viola et al., 2019). In contrast, an intact TCA cycle and effective OXPHOS are necessary to sustain anti-inflammatory macrophage functions (Van den Bossche et al., 2016). Under conditions where OXPHOS is impaired, macrophages can not be repolarized to anti-inflammatory phenotypes and plasticity is limited to pro-inflammatory activation (Van den Bossche et al., 2016).

APPLYING IMMUNOMETABOLISM TO THE CNS

Similar to peripheral macrophages, microglia have the capacity to act in both damaging and restorative modalities in response to CNS injury and in neurodegenerative conditions. Consequently, the M1/M2 paradigm has been used to describe microglial phenotypes. Gene array experiments comparing human microglia with blood-derived macrophages reveal that the majority of genes differentially regulated in M1 and M2 macrophages are also differentially regulated in microglia (reviewed by (Orihuela et al., 2016). However, differences between microglia and macrophages exist. For example, in human cells exposed to the same stimuli, macrophages express higher levels of genes involved in antigen presentation than microglia; in response to M2 stimuli, microglia express only a subset of M2 macrophage surface markers (Durafourt et al., 2012). Further, both M1 and M2 microglia express CD64 while macrophages express this Fc receptor only after M1 polarization (Durafourt et al., 2012). Thus, microglia possess a similar capacity as macrophages to shift polarization between M1 and M2 states, but there are clear differences between the two cell types. These differences have called into question the applicability of the M1/M2 classification system to describe microglia phenotypes and function in vivo (Ransohoff, 2016).

While much of the groundwork in immunometabolism has been gather from experiments using peripheral macrophages, emerging evidence indicates that metabolism also plays a critical role in determining the inflammatory response of microglia. When viewed through the lens of metabolism, microglia share similar metabolic underpinnings as macrophages including the genetic infrastructure for both glycolysis and OXPHOS (Ghosh et al., 2018; Nagy et al., 2018). As discussed below, after pro-inflammatory stimulation both microglia cell lines (e.g. BV-2 cells) and primary microglia utilize glycolysis for glucose-mediated metabolism while anti-inflammatory microglial activation depends largely on efficient mitochondrial function and OXPHOS similar to macrophages.

Glycolysis and OXPHOS regulate microglia activation.

As highlighted in Figure 1, glucose-mediated glycolytic activity is associated with pro-inflammatory microglial activation. When examined in vitro, microglia exposed to high extracellular concentrations of glucose (75mmol/L) adopt a pro-inflammatory response through upregulation of CD11b, iNOS, TNF-α, and IL-6 compared to controls, while at the
same time downregulating M2 markers Arg-1 and IL-10 (Huang et al., 2019). In addition, pro-inflammatory stimuli increase microglia glucose uptake through increased expression and protein levels of glucose transporters, GLUT1 and GLUT4 (Gimeno-Bayón et al., 2014; S. Nair et al., 2019; L. Wang et al., 2019; Q. Wang et al., 2014). Inhibiting GLUT1 after pro-inflammatory stimulation suppresses microglia glycolysis as measured ex vivo through a reduced extracellular acidification rate (ECAR) (L. Wang et al., 2019). Further, in response to pro-inflammatory stimuli, microglia increase expression of enzymes that feed glycolysis including hexokinase 2 (HK2), phosphofructokinases (PFK), and lactate dehydrogenase (LDH) (Gimeno-Bayón et al., 2014; Holland et al., 2018) (Figure 1). Importantly, lactate concentrations increase in pro-inflammatory microglia compared to control cells indicating that glucose is being utilized for glycolysis but downstream metabolites are being converted to lactate, away from Acetyl-CoA and the TCA cycle thereby limiting OXPHOS (Gimeno-Bayón et al., 2014; Orihuela et al., 2016; Voloboueva et al., 2013).

Increased glucose-mediated glycolytic activity appears to be not just a side effect of the pro-inflammatory state, but may actually be a core prerequisite for pro-inflammatory microglia functions. Reports from two independent laboratories demonstrate that limiting glycolysis with the glucose analog, 2-deoxyglucose (2-DG), inhibits pro-inflammatory microglial activation in vitro (Fodelianaki et al., 2019; Q. Wang et al., 2014). Specifically, blocking glycolysis with 2-DG in primary microglia significantly suppresses LPS mediated increases in IL-6, IL-1β, and Nos2 (Fodelianaki et al., 2019). Glycolysis likely signals through NF-κβ (nuclear factor kappa-light-chain-enhancer of activated B cells) pathways in primary microglia facilitating the production of the pro-inflammatory mediators TNF-α and IL–6 (Q. Wang et al., 2014). Similarly, limiting microglial glycolytic shifts in response to pro-inflammatory stimuli with nerve growth factor suppresses tropomyosin receptor kinase A (TrkA) signaling and subsequent pro-inflammatory cytokine production (Fodelianaki et al., 2019).

In contrast to pro-inflammatory stimuli increasing glucose uptake and glycolysis, reparative/anti-inflammatory microglial activation likely requires metabolic shifts toward OXPHOS (Figure 1). Indeed, treatment with the anti-inflammatory cytokine, IL-4, decreased glucose uptake and lactate production in both immortalized (BV-2) and primary microglia (Gimeno-Bayón et al., 2014). Activation of microglia with IL-4 also has no effect on glycolysis but is associated with increased OXPHOS and ATP production (Holland et al., 2018). These metabolic shifts are observable after treatment of primary microglia with a combination of IL-4 and IL-13 which does not alter oxygen consumption rate (OCR) (an ex vivo measure of OXPHOS) or ECAR as compared to untreated controls (Orihuela et al., 2016). Collectively, these results indicate that anti-inflammatory microglia continue to generate energy through OXPHOS without potentiation of glycolytic pathways.

**Mitochondrial activity dictates microglia phenotype and function.**

Metabolites produced in the cytosol can regulate glycolysis and OXPHOS (Figure 1), however, there is increasing evidence that mitochondria play an important role in driving microglia phenotype (Park et al., 2013). Indeed, the necessity for increased glycolysis with pro-inflammatory microglial activation is often associated with impaired mitochondrial
function. Primary microglia treated with the pro-inflammatory stimuli, LPS, show a decreased OCR, evidence of a shift away from OXPHOS (S. Nair et al., 2019; Orihuela et al., 2016). Pro-inflammatory stimuli also induce morphological changes at the level of the mitochondria in primary microglia (S. Nair et al., 2019). For example, pro-inflammatory activation of microglia results in mitochondrial fragmentation (Banati et al., 2004; A. B. Nair and Jacob, 2016). In response to stimulation with LPS + INFγ mitochondria adopt elongated and round phenotypes indicative of low respiratory activity (Banati et al., 2004). Further, LPS doses sufficient for pro-inflammatory activation (100ng/ml) induce mitochondrial fission, increase ECAR, and decrease OCR in mitochondria from primary microglia (S. Nair et al., 2019). Inhibition of mitochondrial fission with Mdivi-1 blocks LPS-induced mitochondrial fragmentation, restores OCR and ECAR to control levels, and prevents the production of pro-inflammatory cytokines (S. Nair et al., 2019). Thus, cellular metabolic and mitochondrial status are intricately linked, with pro-inflammatory stimuli largely suppressing mitochondrial function to facilitate pro-inflammatory cytokine production.

In contrast, efficient mitochondrial respiration and OXPHOS may be essential for microglia to adoptive reparative/anti-inflammatory phenotypes and functions. In macrophages, mitochondrial-mediated shifts from glycolysis to OXPHOS are required for anti-inflammatory stimuli to re-polarize pro-inflammatory cells (Van den Bossche et al., 2016). It is likely that similar metabolic processes regulate microglia phenotype. Indeed, suppression of mitochondrial function with toxins, 3-nitropropionic acid (3-NP) or rotenone, inhibits primary microglial arginase activity and growth factor release in response to anti-inflammatory, IL-4, stimulation (Ferger et al., 2010). Inhibiting mitochondrial function also blunts the ability of pro-inflammatory microglia to switch to an anti-inflammatory activation state. For example, primary microglia stimulated with IL-4 after LPS have reduced pro-inflammatory cytokine production including TNFα, IL-1β, and IL-6 (Ferger et al., 2010). Treatment with 3-NP or rotenone prevents the reductions in TNFα and IL-6 (Ferger et al., 2010). Interestingly, mitochondrial toxins do not effect pro-inflammatory responses to LPS (Ferger et al., 2010). Similarly, inhibiting mitochondrial function through modulation of mitochondrial uncoupling protein 2 (UCP2) potentiates pro-inflammatory responses in primary microglia while suppressing anti-inflammatory functions and gene induction (De Simone et al., 2015). These findings demonstrate that intact mitochondrial function is essential for microglia to transition to an anti-inflammatory phenotype but that pro-inflammatory activation can occur in the absence of mitochondrial OXPHOS.

Collectively, the metabolic requirements of pro- and anti-inflammatory microglia provide the framework for using metabolism to gain insight into microglia function independent of a profiling based approach as with M1/M2. Indeed, transcriptional analyses of metabolic genes map onto pro- and anti-inflammatory microglia phenotypes (Lauro et al., 2019; Viola et al., 2019) and analyses of microglial OCR and ECAR provide insight into pro- and anti-inflammatory functions (Fodelianaki et al., 2019; Gimeno-Bayón et al., 2014; Holland et al., 2018; Voloboueva et al., 2013). Viewing microglial activation through the lens of immunometabolism enables researchers to identify novel targets dictating microglial function. Below we highlight a few of these targets, for more thorough discussion see (Lynch, 2019; Orihuela et al., 2016; Paolicelli and Angiari, 2019).
Targets regulating microglia metabolism.

The differential regulation of inducible nitric oxide synthase (iNOS) vs. arginase is a defining feature of pro- and anti-inflammatory macrophages, respectively (Hesse et al., 2001). Downstream production of iNOS-induced nitric oxide (NO) or arginase-induced polyamines and prolines in large part determine the extent to which macrophage can perform pro- or anti-inflammatory functions. Interestingly, iNOS and NO are also important in the metabolic reprogramming of microglia. iNOS inhibition decreases glucose uptake and lactate production in LPS-treated BV-2 microglia (Gimeno-Bayón et al., 2014). Treatment of the N9 microglial cell line with LPS triggers a dose-dependent increase in NO production (Klimaszewska-Lata et al., 2015). The NO surge is associated with inhibition of the core TCA enzymes, α-ketoglutarate dehydrogenase and aconitase, as well as, inhibition of pyruvate dehydrogenase, the enzyme that feeds the TCA cycle, and hence cellular respiration, by generating acetyl-CoA from pyruvate (Klimaszewska-Lata et al., 2015). NO can also affect mitochondrial function. NO production from microglia cell lines in response to pro-inflammatory stimuli, is associated with irreversibly inhibition of mitochondrial complexes I and II and ATP depletion (Chénais et al., 2002; Moss and Bates, 2001). Thus, NO produced in response to pro-inflammatory stimuli is not only a means for the cell to kill potential pathogens, but also directly instructs microglia to shift metabolism from OXPHOS to glycolysis thereby sustaining pro-inflammatory activation.

The pentose phosphate pathway (PPP) also appears to be important in supporting microglial activation. LPS + IFNγ treated microglia show increased activity of glucose-6-phosphate dehydrogenase (G6PDH) (Gimeno-Bayón et al., 2014). G6PDH oxidizes glucose-6-phosphate and is therefore the first and rate-limiting enzyme of the PPP. Increased PPP activity supplies the reducing equivalents of NADPH needed for NO and ROS synthesis, and also protects the cell by fueling production of antioxidants such as glutathione (Gimeno-Bayón et al., 2014). The increase in G6PDH activity is specific to pro-inflammatory activation as treatment of BV-2 cells and primary microglia with IL-4 does not affect G6PD activity (Gimeno-Bayón et al., 2014). PPP activation, therefore, does not appear to be essential for anti-inflammatory polarization; whether the PPP is required for, or a consequence of, pro-inflammatory microglial activation is not completely understood.

EVIDENCE OF MICROGLIA METABOLIC PROGRAMMING IN VIVO

The limitation in the application of immunometabolism for neuropathologies is the large dependency to date on in vitro or ex vivo analyses. There are caveats associated extrapolating to in vivo conditions. For example, microglia isolated from adult or aged animals and tested ex vivo may be metabolically distinct from their in vivo counterparts due to manipulation during the isolation procedure. Likewise, primary microglial cultures prepared from neonatal mouse pups may not accurately reflect the properties of microglia in the mature or aging brain. Due to the emergent nature of the field, the neuroscience community has yet to overcome the challenges associated with analyzing immunometabolism in cell-specific manner in vivo. In vivo analyses are likely to be adopted by the neuroscience community as immunometabolism becomes more prevalent in the study of neuroinflammatory conditions. Below we discuss the current in vivo evidence that
macrophage and microglia metabolism play a role in the pathophysiology of neurodegenerative diseases and neurotrauma.

While a paucity of in vivo analyses have been performed exploring CNS immunometabolism the results of these limited experiments provide evidence that metabolism regulates microglial activation in vivo. For example, in response to three days of systemic IL-1β administration, microglia isolated from mouse pups increase expression of inflammatory genes including the pro-inflammatory markers CD32, COX2, and iNOS and the anti-inflammatory marker, arginase 1 (S. Nair et al., 2019). Co-administration of IL-1β with Mdivi-1, which blocks mitochondrial fragmentation and thereby preserves mitochondrial function, significantly reduces microglial pro-inflammatory gene expression while retaining arginase and growth factor expression (S. Nair et al., 2019). In that study, CD11b expression was used to isolate microglia. Since this marker is expressed by peripheral macrophages and microglia, it is possible that the phenotypic shift observed were not specific to microglia. Nonetheless, these data provide evidence that preserving mitochondrial function in vivo alters the phenotypes of inflammatory cells within the CNS.

Neurodegeneration.

Accumulating evidence points to microglia as a fundamental participant in Alzheimer’s disease (AD). While the hallmarks of this disease traditionally include extracellular plaques of deposited amyloid-β (aβ) peptides and neurofibrillary tangles of intracellular tau protein, both neuroinflammation and metabolic disturbances are increasingly recognized contributors to pathology. In the hunt for genetic modifiers of AD, genome-wide association studies have uncovered that most of the genetic AD risk lies in genes that are expressed in microglia rather than neurons, such as APOE, TREM2, CD33, BIN1, INPP5D, PICALM, and others (Lambert et al., 2013) (reviewed in (Andreasson et al., 2016)). Many also modify metabolism. For instance, APOE4 carriers, of whom homozygotes are at a 15-fold increased risk for AD and heterozygotes 3-fold (Farrer et al., 1995), display a distinct pattern of glucose hypometabolism starting from a young age – as early as 20 years old (Reiman et al., 2004). This same pattern emerges in nearly all other AD patients, and is considered an early and integral feature of the disease (reviewed in (Mosconi, 2013)). Accordingly, as both metabolic deficits and inflammatory signaling appear to be key components of AD, a more complete understanding of immunometabolism will aid in unlocking the full picture of this complex disease.

Already attention has been turned toward immunometabolism by a recent finding that aβ acts through TLR4 to engage the mTOR-HIF1α pathway. Treatment of murine primary microglia with aβ induces a shift away from OXPHOS towards glycolysis, as evidenced by decreased OCR, increased ECAR, and increased lactate production (Baik et al., 2019). Aβ increases phosphorylation of mTOR and p70S6K and expression of HIF1α to similar levels as LPS treatment. The mTOR-HIF1α pathway is critical for expression of the pro-inflammatory cytokines, IL-1β and TNF-α, as inhibiting mTOR signaling with rapamycin or metformin blunts IL-1β and TNF-α expression (Baik et al., 2019). In addition, blocking glycolysis in mice with systemic administration of 2-DG significantly reduces microglia chemoattraction to intracerebroventricularly injected oligomeric aβ, as demonstrated by in
**vivo** multiphoton imaging of Cx3cr1-labeled microglia (Baik et al., 2019). These results implicate metabolic requirements for microglia in vivo to both respond to injury sites and to increase pro-inflammatory signaling.

Similar metabolic requirements may be needed to maintain microglial phagocytic activity in areas of aβ plaques. *In vivo* staining from APP/PS1 mice show that microglia in close proximity to plaques express high levels of cytosolic phosphofructokinase fructose 2,6-biphosphatase B3 (PFKFB3), an important glycolytic metabolite enzyme associated with pro-inflammatory activation (McIntosh et al., 2019; Viola et al., 2019)(Figure 1). Interestingly, primary microglia isolated from neonatal APP/PS1 mice maintain glycolytic activity ex vivo and have impaired aβ phagocytosis and decreased chemotaxis when compared to primary microglia isolated from neonatal wild type animals (Holland et al., 2018; McIntosh et al., 2019). While these results do not completely align with observations that glycolysis sustains macrophages phagocytic capabilities (Viola et al., 2019), they nonetheless provide in vivo evidence that microglia metabolism likely contributes to AD pathology.

The study of the triggering receptor expressed on myeloid cells 2 (TREM2) provides similar evidence. TREM2 is expressed by microglia and plays a crucial role in maintaining normal CNS function (Hsieh et al., 2009; Takahashi et al., 2007). Loss of function mutations in TREM2 increase AD risk (Jonsson et al., 2013). Microglia sorted from TREM2 deficient mice crossed to the 5x Familial Alzheimer’s Disease (5XFAD) mouse model express lower levels of genes associated with glycolysis and the mTOR pathway compared to TREM2 sufficient 5XFAD controls (see supplemental data of (Ulland et al., 2017)). Western blots confirm this decreased activation of the mTOR pathway in TREM2 deficient microglia (Ulland et al., 2017). TREM2−/−/5XFAD microglia sorted after incubation with MitoTracker Green show a decrease in the total number of mitochondria per cell compared to TREM2 sufficient controls (Ulland et al., 2017). These metabolic impairments may hinder the response to Alzheimer’s pathology, as brain sections from TREM2−/−/5XFAD mice show decreased numbers of activated microglia and a drastic reduction in the number of microglia surrounding amyloid plaques compared to TREM2 sufficient 5XFAD controls (Ulland et al., 2017). Interestingly, dietary supplementation with the high energy compound cyclocreatine rescued these deficits in the TREM2−/−/5XFAD mice (Ulland et al., 2017), suggesting a role from TREM2 in maintaining microglial energy homeostasis. Collectively, these emerging data implicate aβ-mediated microglial metabolism as important in the pathophysiology of AD.

Metabolism also plays a role in the acquisition of immune tolerance in microglia chronically treated with aβ. Although acute treatment with aβ increases expression and protein levels of the pro-inflammatory markers, IL-1β and TNF-α, marker expression declines with repeated aβ treatment in murine primary microglia cultures (Baik et al., 2019). These tolerant microglia downregulate mTOR-HIF1α and decrease lactate production and ECAR with suppressed OCRs (Baik et al., 2019). Gene profiling of CD11b+ cells (presumably microglia) isolated from adult 5XFAD mice after intracerebroventricular injection of aβ indicated that metabolic processes were significantly altered in microglia relative to controls providing evidence that the metabolic changes in microglia observed in response to aβ in
*vitro likely occur in vivo* (Baik et al., 2019). Interestingly, this same pattern of simultaneous reductions in both ECAR and OCR was recently observed in non-stimulated APOE4/4 microglia generated from human induced pluripotent stem cells (Konttinen et al., 2019). It will be of great interest to further clarify the extent to which these metabolic disturbances contribute to neuroinflammation and AD pathology.

**Traumatic CNS injury.**

Neuroinflammation, and specifically microglia and macrophage activation, are hallmarks of brain and spinal cord injury. After stroke, SCI, or TBI, a surge of systemic macrophages and endogenous microglia influx into injured areas to aid in the clearance of cellular debris. CNS macrophages are capable of dichotomously facilitating repair while concurrently exacerbating injury (Gensel et al., 2009) but the overall the effects of inflammation are believed to be detrimental to CNS injury outcomes (Gensel and B. Zhang, 2015; Lourbopoulos et al., 2015; Ramlackhansingh et al., 2011). Although the concepts of immunometabolism have not yet been thoroughly investigated in neurotrauma, there is *in vivo* evidence that microglia and macrophage metabolism plays a role in the pathophysiology of injury.

The most direct evidence of microglia metabolism in neurotrauma comes from the examination of CXCL1 therapy in a mouse model of stroke. The chemokine, CXCL1 (fractalkine) is expressed on neurons and signals microglia and macrophages through its receptor, CXCR1 (Lauro et al., 2015). CXCL1 administration reduces ischemic damage *in vivo* concurrent with microglial polarization from a pro- to anti-inflammatory phenotype (Lauro et al., 2019; 2015). Based upon these observations, Lauro and colleagues examined the effect of CXCL1 administration on microglia metabolism after permanent middle cerebral artery occlusion (pMCAO) in mice (Lauro et al., 2019). Specifically, CD11b+ microglia were isolated from the injured brain and analyzed for expression of genes associated with glycolysis and OXPHOS. While genes associated with both metabolic processes increase acutely after injury (24h), genes associated with glycolysis such as lactate dehydrogenase A and pyruvate kinase M2 are maintained for 72hrs. CXCL1 treatment decreases expression of all genes associated with glycolysis at 72hrs concomitant with a microglial shift to anti-inflammatory gene expression (Lauro et al., 2019). CXCL1 treatment also increases expression of genes associated with OXPHOS such as PGC1β and SLC25A15 (Lauro et al., 2019), indicating that metabolic changes after injury may play an important role in regulating microglia phenotype in the injured CNS.

More direct evidence for glycolysis driving pathological microglia activation *in vivo* after neurotrauma comes from the investigation of hexokinase-2 (HK2) in experimental stroke. Through its phosphorylation of glucose to glucose-6-phosphate (G-6-phosphate), HK2 is a rate-limiting enzyme for glycolysis (Figure 1). Since hyperglycolysis is triggered by brain ischemia, Li and colleagues manipulated HK2 to examine the role of glycolysis in the microglial response to middle cerebral artery occlusion (MCAO) in male Sprague–Dawley rats (Li et al., 2018). Specifically, blocking HK2 activation after MCAO, pharmacologically with ionidamine or genetically, using selective rAAV delivery, reduces the infarct area and significantly limits microglial activation (Li et al., 2018). Importantly, immunoreactivity for
the pro-inflammatory cytokine, IL-1β, is also significantly reduced with HK2 inhibition indicating that glycolysis is important in pro-inflammatory microglial activation after trauma.

The evidence that metabolism plays a role in the inflammatory responses to traumatic brain and spinal cord injury is less direct but can be inferred from examinations of reactive oxygen species (ROS) production after injury. Typically, macrophage-derived ROS functions during innate immunity as anti-microbial defense, however, within CNS environments ROS production can permanently damage neuronal structures and is indicative of a pro-inflammatory phenotype. TBI and SCI trigger macrophage and microglia phenotypes that produces high amounts of ROS and pro-inflammatory cytokines (Kumar et al., 2015; B. Zhang et al., 2016). As mentioned above, pro-inflammatory macrophages and microglia re-direct glycolytic intermediates towards the oxidative phase of the PPP for generation of ROS through production of NADPH and NADPH oxidases (NOXs)(Figure 1)(Viola et al., 2019). This is in contrast to anti-inflammatory macrophages which utilize the non-oxidative phase of the PPP to provide redox support for cellular antioxidants such as glutathione (Blagih and Jones, 2012). After TBI and SCI, macrophage-specific NOX activity (NOX2) is increased in macrophages and microglia responding to injury (Cooney et al., 2013; 2014). Increased NOX activity is accompanied by increased macrophage/microglia ROS production and NOX inhibition improves outcomes from TBI and SCI (Ferreira et al., 2013; Khayrullina et al., 2015; Leden et al., 2016; M. W. Ma et al., 2017b; B. Zhang et al., 2016; 2019; Q.-G. Zhang et al., 2012). Importantly, in both TBI and SCI, NOX2 inhibition, whether pharmacologic or genetic, reduces pro-inflammatory macrophage/microglia activation in vivo (Ferreira et al., 2013; Kumar et al., 2016; M. W. Ma et al., 2017a; J. Wang et al., 2017). NOX2-deficiency also significantly increases anti-inflammatory macrophage/microglial activation after TBI (Barrett et al., 2017). The shifts in polarization associated with NOX2 inhibition implicate altered metabolism and the oxidative phase of the PPP in pro-inflammatory microglia/macrophage activation after neurotrauma.

In addition to the PPP, mitochondrial metabolism also likely plays a role in the inflammatory responses to neurotrauma. Abe and colleagues examined mitochondrial ROS production after brain stab wound injuries in rats treated with bromovalerylurea (BU) (Abe et al., 2018). BU limits pro-inflammatory microglial activation in part through inhibition of CCL2 expression. When delivered 1hr after stab injury, BU treatment significantly reduces lesion cavity size and improves functional recovery (Abe et al., 2018). Important here, BU treatment significantly reduces pro-inflammatory gene expression in FACS sorted microglia and macrophages isolated from the injured brain without decreasing growth factor gene expression (Abe et al., 2018). The polarization shift towards anti-inflammatory phenotypes is coincident with significantly decreased microglia/macrphages 8-OHdg labeling in vivo, indicative of reduced mitochondrial ROS production. While 8-OHdg reductions do not directly implicate altered metabolism, accompanying in vitro analyzes demonstrated that BU significantly suppresses microglial and macrophage mitochondrial and glycolytic activity (Abe et al., 2018) thus indicating that metabolism likely plays a role in TBI inflammation.
CONCLUDING REMARKS

The notion that metabolism instructs the immune response is not a novel concept. For years, immunologists have understood that engagement of specific metabolic pathways is required for immune cells to polarize to both pro- and anti-inflammatory phenotypes. Immunometabolism is well studied in peripheral macrophages, where polarization towards a pro-inflammatory phenotype is associated with enhanced glycolysis, pentose phosphate pathway activation, and fatty acid synthesis. In contrast, anti-inflammatory activation is supported by oxidative phosphorylation, efficient mitochondrial function, and fatty acid oxidation. An increased appreciation that neuroinflammation is a major driving force in CNS pathologies has led to an explosion of interest in microglia and macrophages in conditions ranging from spinal cord injury to Alzheimer’s disease. Recent insights into the mechanistic underpinnings of CNS macrophage activation states indicate that microglia engage metabolism in a similar fashion to peripheral macrophages. Metabolic reprogramming is now emerging as a core and integral feature dictating phenotypic responses. As such, immunometabolism holds promise as a functional based approach to understand microglial heterogeneity. Initial in vitro findings are now being extended to in vivo studies which implicate immunometabolic disturbances as drivers in neurodegeneration and neurotrauma. Further in vivo studies are needed to clarify the extent to which immunometabolic targets hold therapeutic potential in CNS injury and disease, and whether altering microglial metabolism can change the course of neuropathophysiology.

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Figure 1: Metabolic regulators of microglial phenotype and function.
Highlighted metabolic processes were estimated from primary research papers focused on microglia and referenced in the text. CI-CV, complexes I-V; G-6-phosphate, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; GLUT, glucose transport; HK2, hexokinase-2; LDH, lactate dehydrogenase; NADPH, Nicotinamide adenine dinucleotide phosphate; NOX, NADPH Oxidase; OXPHOS, oxidative phosphorylation; PDH, pyruvate dehydrogenase; PFK, phosphofructokinases; PPP, pentose phosphate pathway; ROS, reactive oxygen species; TCA, tricarboxylic acid.