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Review

Microglia immunometabolism: From metabolic disorders to single cell metabolism

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ABSTRACT

Since the observation that obesity-associated low-grade chronic inflammation is a crucial driver for the onset of systemic metabolic disorders such as type 2 diabetes, a number of studies have highlighted the role of both the innate and the adaptive immune system in such pathologies. Moreover, researchers have recently demonstrated that immune cells can modulate their intracellular metabolic profile to control their activation and effector functions. These discoveries represent the foundations of a research area known as "immunometabolism", an emerging field of investigation that may lead to the development of new-generation therapies for the treatment of inflammatory and metabolic diseases. Most of the studies in the field have focused their attention on both circulating white blood cells and leukocytes residing within metabolic tissues such as adipose tissue, liver and pancreas. However, immunometabolism of immune cells in non-metabolic tissues, including central nervous system microglia, have long been neglected. In this review, we highlight the most recent findings suggesting that microglial cells play a central role in metabolic disorders and that interfering with the metabolic profile of microglia can modulate their functionality and pathogenicity in neurological diseases.

1. An introduction to immunometabolism

Immunometabolism is a ground-breaking field of investigation introduced after the observation that obesity-related comorbidities are associated with immune system activation and chronic inflammation that increase the risk of systemic metabolic dysfunctions such as insulin resistance and diabetes. The contributions of innate and adaptive immune cells to low-grade local inflammation in metabolic tissues such as adipose tissue, liver, and pancreas, are now well established and represent an active area of research [1,2].

Recently, additional aspects of complexity were introduced in the field of immunometabolism. Firstly, it is now clear that immune cells can regulate the generation, strength, and duration of immune responses by fine-tuning their intracellular metabolic profile, which is controlled by environmental cues such as infections and nutrition. These observations support the hypothesis that alterations in immune cell metabolism impact inflammatory reactions [3–5]. Secondly, recent studies demonstrated that intracellular metabolites produced by activated immune cells can act as signalling molecules to regulate immune cell activation and effector functions, and can modulate systemic inflammation [6–8]. Additionally, many cytoplasmic and mitochondrial metabolic enzymes are observed to be present in the nucleus of resting and activated immune cells, where they have functions distinct from their classical role, such as regulation of gene transcription and epigenetic DNA modification [9]. Several of such metabolic enzymes can also have secondary tasks in the cytoplasm, such as RNA binding and pathogen recognition, controlling many different aspects of immune cell functions [10,11].

From extensive studies, it is now clear that peripheral immune cells are involved in the development of metabolic disorders, and that intracellular metabolic pathways are important in peripheral immune cell activation. Even so, these two aspects have not been comprehensively investigated in central nervous system (CNS)-resident microglial cells until recently. For a long time, microglia have been considered quiescent cells, activated only in cases of infections or threats to brain homeostasis. However, over the past decade the view on microglia has radically changed, moving from the old concept of 'resting' to the new definition of 'surveying microglia', which highlights the surveillance role of these cells in the CNS [12]. Novel roles have been described in several physiological processes, ranging from neuronal apoptosis to neurogenesis to circuit remodelling [13–18]. In addition, emerging functions for microglia have been reported in the context of

Abbreviations: HFD, high fat diet; DIO, diet-induced obesity; FAs, fatty acids; SFAs, saturated fatty acids

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neurodegeneration, with genetic evidence supporting an active role for these cells in the pathogenesis of the disease, rather than being only involved as bystanders, as previously thought [19–21]. *in vivo* imaging in the healthy brain reveals that microglia are highly active, extremely motile cells, which constantly scan the surrounding parenchyma, even in the absence of injuries or toxic stimuli [22,23]. Moreover, microglia are highly phagocytic cells, and in the healthy brain they contribute to homeostatic maintenance by removal of cellular debris and clearance of apoptotic neurons and potential protein aggregates [24,25].

The constant motility of microglial processes has thus raised questions about the requirements for such an energy-demanding activity, and has prompted novel investigations to better elucidate the metabolic profile of these cells. Also, the growing interest in the relationship between the immune system and metabolic disorders has attracted attention to the possible roles of microglia, the immune cells of the CNS, in metabolic dysfunctions. Here, we review the main aspects of microglial immunometabolism, discussing its importance and implication in metabolic disease, and the potential of targeting microglia metabolism to modulate their activation and function.

2. Inflammation and metabolic disease

2.1. Metaflammation in metabolic dysfunctions

Obesity and overweightness present an epidemic health issue worldwide with a high socioeconomic impact [26]. In particular, obesity is associated with many comorbidities, including obesity-related metabolic pathologies such as cardiovascular diseases and type 2 diabetes mellitus [2,27]. The presence of metabolic abnormalities in obese individuals is well established, and different causes have been identified. These include dysfunction of cells of metabolic tissues, such as adipocytes, pancreatic β-cells, and hepatocytes, mainly induced by fatty acid (FA) accumulation [2]. However, one of the major drivers of obesity-associated metabolic disorders is the so-called “metaflammation”, meaning the chronic, low-grade inflammation observed at both systemic and local levels [28]. It is important to note that while the involvement of metaflammation in obesity-related metabolic dysfunctions has been extensively investigated and consistently demonstrated in animal models, its role in obese humans is still a point of debate. Even so, both *in vitro* and *in vivo* evidence support this view [2].

Pivotal studies in animal models of obesity demonstrated that proinflammatory mediators, such as the cytokine tumor necrosis factor (TNF)- α, are up-regulated systemically and in obese adipose tissue, and TNF-α, neutralisation reversed the diabetogenic phenotype in obese rodents [29]. Following this observation, researchers introduced a new concept in the field: the connection between local metabolic tissues and the immune system in metabolic disorders. Some metabolic organs such as adipose tissue and liver are populated by subsets of immune cells that are preferentially polarised to an anti-inflammatory phenotype in a healthy, lean state. As an example, lean adipose tissue is enriched with immune cell populations including regulatory T cells (Tregs), M2-like macrophages, T helper 2 (Th2) cells, and invariant NK T cells, which help to maintain a functional, homeostatic state in adipocytes [2,30,31]. However, during obesity, metabolic tissue parenchymal cells respond to the stress induced by FA over-supplementation by releasing inflammatory mediators that, in turn, either modify the polarisation state of resident immune cells or recruit circulating immune cells from the periphery (Saltiel and Olefsky, 2018 [2],). At the same time, obesity was shown to modify the composition of the gut microbiota and to affect intestinal barrier integrity, leading to systemic elevation of proinflammatory bacterial-derived molecules such as lipopolysaccharide (LPS) [32]. The final outcome of such events is the accumulation of proinflammatory immune cells including M1-polarised macrophages, $CD8^+$ cytotoxic T cells, neutrophils, Th1 and Th17 cells in metabolic tissues. These cells severely affect the functionality of resident parenchymal cells, leading to modification of glucose and FA metabolism, and the development of hypertriglyceridemia, insulin resistance, and glucose intolerance ([1] [2,28,30,31],).

2.2. The effect of obesity-related inflammation on central nervous system functionality

As mentioned above, one intriguing observation arising from studies evaluating the role of metaflammation in metabolic disorders is that parenchymal cells in metabolic organs can release a plethora of inflammatory mediators such as cytokines, adipokines, chemokines, micro-RNAs, peptides, and lipids that act both locally on immune and parenchymal cells and systemically, affecting the homeostasis and the functionality of other organs [2,33]. Recently, much attention has been paid to the effect of weight gain, obesity, and the associated metaflammation on CNS behaviour. Initial studies demonstrated that overnutrition induced inflammation and neuronal damage in the hypothalamus, a region of the brain essential for the regulation of organismal energy homeostasis. Such a detrimental response led to insulin and leptin resistance, altered glucose metabolism, and further promoted weight gain due to unbalanced food intake ([34–37]). These works clearly show a direct link between high fat diet (HFD) and obesity development, and suggest that obesity may correlate with improper control of energy metabolism by the CNS.

Obesity can also severely affect the functionality and inflammatory milieu of other CNS regions, leading to secondary damage that goes beyond the regulation of energy homeostasis and food intake [38]. Firstly, several works demonstrated that obese rodents and humans have increased blood-brain barrier permeability in certain regions of the brain, a process that leads to accumulation of free FAs and inflammatory mediators and a reduction of insulin/leptin transport into the CNS parenchyma [38,39]. Secondly, it is now clear, particularly in animal models, that obesity-related neuroinflammation is not restricted to the hypothalamus, but also to other brain areas including the cerebral cortex and the hippocampus [38,40]. Finally, some studies have correlated obesity with alteration of brain morphology in humans [38]. Altogether, these observations suggest that obesity and malnutrition significantly impair CNS functionality and structural properties, representing a correlative risk factor for the long-term development of cognitive deficits and neurological diseases [40].

2.3. Microglia in metabolic disorders

The growing interest in the field of immunometabolism has recently led to extending these investigations to microglia, revealing an important, yet underexplored, role for these cells. A major driver was the initial observation that exposure to a hypercaloric diet induces inflammatory responses not only in peripheral tissues but also in the CNS. Mouse models of diet-induced obesity (DIO) reported a rapid increase in expansion and activation of microglial populations, especially in the hypothalamus, even preceding weight gain [35,41]. As opposed to other glia cells such as astrocytes, microglia seem to be highly sensitive to saturated FAs (SFAs), which specifically build up in the hypothalamus during HFD. The microglial response resulted in the up-regulation of proinflammatory molecules, ultimately mediating neuronal stress in the mediobasal hypothalamic region [42]. SFAs, such as palmitic and stearic acids, can signal in microglia through Toll-like receptor 4 (TLR4) and its nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) downstream pathway, thus resulting in the secretion of proinflammatory cytokines, ROS and NO (Wang et al., 2011 [43],). On the other hand, omega-3 polyunsaturated fatty acids (PUFAs) act on microglia mostly through the GPR120 receptor and modulate the expression of inflammatory genes, by reducing IL1-β and increasing IL-10 [44]. In mice exposed to diet deficient in omega-3 PUFAs, microglial motility is significantly decreased, without major changes in number [45]. Importantly, approaches aimed at reducing inflammation by targeting the NF-kB pathway, TLR4 and TNF-α

signalling, and the NLRP3 inflammasome in the hypothalamus proved to be effective in attenuating the overnutrition-induced energy imbalance, and consequent metabolic alterations, by improving insulin and leptin sensitivity [34,41,46].

The central implication of microglia in the inflammatory process taking place in the hypothalamus during high fat diet (HFD) has been demonstrated by studies showing that preventing microglial proliferation in this brain structure is indeed sufficient to reduce diet-induced central and peripheral inflammation, and to restore leptin sensitivity (André et al., 2017). Consistent with these data, increased phagocytosis was reported in microglia isolated from HFD mice, when assessed for synaptosome engulfment *in vitro* [47]. Recent work has investigated this further, showing that microglia promote obesity-induced cognitive decline in mice by removing synapses in the hippocampus [48]. Obesity has been associated with cognitive dysfunction in several human studies, with evidence indicating that obesity in mid-life is a predictor of mild cognitive impairment later in life [49,50]. Rat models of DIO recapitulate the cognitive impairment and present with synapse loss and microglia morphological alterations [51]. Pharmacological blockade of microglial activation and/or phagocytosis is sufficient to restore synapse density and cognitive performance in obese mice, revealing a central role for microglia in mediating cognitive impairment associated with obesity [48]. However, other evidence indicated that the adaptive immunometabolic response of microglia induced by hypercaloric diet is essential for maintaining normal brain function [52]. Such an adaptive response includes the up-regulation of the enzyme lipoprotein lipase (LPL), which hydrolyses triglycerides in lipoproteins and free FAs. Among the different types of brain cells, microglia show the highest level of *Lpl* mRNA expression [53]. Microglia lacking LPL display impaired lipid uptake and disrupted intracellular lipid metabolism [52]. Furthermore, conditional knockout mice selectively lacking LPL in microglia, displayed a more severe loss of hypothalamic proopiomelanocortin (POMC) neurons upon HFD, which was associated with attenuated microglial activation and phagocytic activity [52]. These data point toward a complex role for microglia in controlling brain functionality during obesity and overnutrition.

The involvement of microglia in metabolic disorders has been also proposed in the context of hyperglycaemia, a condition characterised by aberrantly high levels of circulating glucose, in people who have type 2 diabetes. One of the most common complications of diabetes is diabetic retinopathy, which presents with vascular damage and neuronal death in the retina, leading to vision loss [54]. Microglia activation and morphological changes have been reported in different studies, using either induced or genetic models of diabetes, with evidence for very early implication in the pathological process [55–58]. A shift from an initial anti-inflammatory phenotype towards a more proinflammatory one occurs in the retina of diabetic rodents, with high levels of microglial proinflammatory markers also reported in *post mortem* retinas from hyperglycaemic patients [59].

On the other hand, low glucose levels observed during type 1 diabetes also have an effect on microglial behaviour, with experimental *in vivo* evidence showing prominent morphological changes and increased reactive oxygen species (ROS) production in animal models of insulin-induced hypoglycaemia [60–62]. Similar findings were reported in *post mortem* human brains [63]. Under prolonged conditions, hypoglycaemia resulted in neuronal death, mostly affecting hippocampal and cortical structures, ultimately causing cognitive impairment [64–66]. Hypoglycaemia-induced neuronal death has been recently proposed to be mediated by microglia, with data showing that inhibiting microglia activation by minocycline treatment was sufficient to protect against neuronal loss and to prevent cognitive decline [67].

Overall, a central role for microglia is emerging in distinct metabolic disorders, whether they are characterised by increased lipid accumulation or increased/decreased glucose levels. This suggests a link between microglial dysfunction and neuronal damage, yet further investigations are warranted to better understand the underlying molecular mechanisms and if microglia play a role in metabolic diseases in humans.

3. Metabolic regulation of inflammation

3.1. Metabolic pathways control immune cell activation and function

In the last two decades, a new concept has been introduced in the field of immunometabolism: the ability of immune cells to control their activation and functional properties by regulating specific intracellular metabolic pathways [3,4,68]. It has long been known that in the presence of oxygen, resting eukaryotic cells oxidise glucose, FA, and amino acids (AAs) in the mitochondrial tricarboxylic acid cycle (TCA cycle), which generates reduced nicotinamide adenine dinucleotide (NADH) used by mitochondrial oxidative phosphorylation (OXPHOS) to produce adenosine triphosphate (ATP) as an energy source, which is necessary for housekeeping functions. However, actively proliferating cells shift their metabolism to aerobic glycolysis, a process where, even in the presence of sufficient oxygen levels, the majority of glucose is fermented to pyruvate and then lactate, which is in turn secreted by the proliferating cell [69,70]. This metabolic reprogramming is known as the “Warburg effect” and was first observed in cancer cells [71]. Warburg metabolism is also characterised by a boost in the pentose phosphate pathway (PPP), and ultimately leads to the production of intermediates necessary for the synthesis of cellular building blocks such as nucleotides, proteins, and lipids, allowing the cell to increase its biomass and proliferate [69,70]. Notably, as glycolysis generates much less ATP than OXPHOS, proliferating cells must strongly increase their nutrient uptake in order to continuously fuel the anabolic metabolism and generate sufficient energy [69,70].

Recent studies demonstrated that the metabolic remodelling known as the Warburg effect also takes place in immune cell populations, which change their intracellular metabolic profile in response to activating stimuli [3,4,68]. Similar to proliferating cancer cells, upon activation, immune cells rapidly up-regulate the surface expression of transporters for nutrients such as glucose, AAs, and FAs to fuel crucial anabolic pathways. The switch to a Warburg-like metabolism is essential for both proliferation and effector functions of immune cells [3,4,68]. Interestingly, while the engagement of certain metabolic pathways (glycolysis, PPP, FA synthesis [FAS]) is usually correlated with a proinflammatory phenotype, other metabolic pathways (FA oxidation [FAO], sustained OXPHOS) are normally observed in resting or anti-inflammatory cell subsets, suggesting a complex regulation of both innate and adaptive immune cell functions by intracellular metabolism [72,73]. Overall, the discovery that immune cells can control their activation state and polarisation through the modulation of intracellular metabolism has significantly changed our view on how the immune system works and how immune responses are mounted following infections or external insults.

3.2. Metabolic reprogramming in macrophages

Among the different immune cell subsets, here we briefly discuss the metabolic reprogramming taking place during the activation and differentiation of macrophages, which represent the peripheral immune cell population most closely related to CNS microglia in terms of functional activity.

Upon activation with pathogen-associated molecular patterns (PAMPs), macrophages engage aerobic glycolysis, which is essential for expression of proinflammatory genes and polarisation toward a proinflammatory M1-like phenotype ([72], Van Den Bossche et al., 2017). PPP is also reinforced in M1-polarised macrophages, to generate NADPH necessary for nitric oxide (NO) production from arginine by inducible NO synthase (iNOS) ([72], Van Den Bossche et al., 2017). Apart from its microbial killing activity, NO nitrosylates complex II and IV of the electron transport chain (ETC), inhibiting their activity. This

process causes reduced ATP production in M1-macrophages and leads to the repurposing of the mitochondria to generate mitochondrial ROS (mtROS) by complex I, via reverse electron transport (RET) through the ETC (Van Den Bossche et al., 2018 [74,75],). mtROS are essential for microbial killing, but they also favour the stabilisation of the transcription factor hypoxia-induced factor (HIF)-1α, which drives the production of proinflammatory mediators such as interleukin (IL)-1β (Van Den Bossche et al., 2018; [74,75],). Repurposed mitochondria also have a truncated TCA cycle, due to inhibition of succinate dehydrogenase (complex II) by NO and suppression of isocitrate dehydrogenase expression (Van Den Bossche et al., 2017, Liu and Ho, 2017 [75],). These blocks in the TCA cycle lead to the accumulation of citrate, succinate, and itaconate, which represent key signalling metabolites in macrophages [6,76,77].

Citrate is exported from the mitochondria to the cytosol by the cytosol citrate carrier (CIC), also known as solute carrier family 25 member 1 (SLC25A1), of which expression is induced in macrophages by stimuli such as LPS and proinflammatory cytokines [76,78,79]. CIC activity is also regulated by nutrient availability, with glucose starvation inducing CIC acetylation that increases CIC activity and citrate export to the cytosol, to fulfil the highly metabolic demand of inflammatory macrophages [80]. In the cytosol, citrate is converted into acetyl-coenzyme A (CoA) and oxaloacetate by the ATP-citrate lyase (ACLY), of which expression is also up-regulated in inflammatory macrophages [81]. Acetyl-CoA and its derivative malonyl-CoA are used by the cell for protein/histone acetylation and protein malonylation, while oxaloacetate processing leads to the generation of NAPDH required for NO and ROS production [75,76]. Malonyl-CoA also feeds into FAS, an essential step for the production of cytokines and prostaglandins and for the activation of the NLRP3 inflammasome [75,76].

Another metabolite accumulating in M1-macrophages is itaconate, which is produced from citrate-derived cis-aconitate by the mitochondrial enzyme aconitate decarboxylase 1 (ACOD1; also known as immune-responsive gene 1 [IRG1]) [82,77]. Itaconate displays anti-microbial activity by inhibition of the glyoxalate shunt in bacteria, but it also counteracts the proinflammatory potential of citrate and succinate by exerting multiple anti-inflammatory activities important to limit macrophage activation and inflammation [77].

Like citrate, succinate accumulates in M1-polarised macrophages, where it inhibits prolyl hydroxylases (PHDs), leading to stabilisation of HIF-1α [6,77]. Succinate accumulation is also important for the production of mtROS by complex I via RET ([83]; Van Den Bossche et al., 2017 [74,75]). Of note, succinate is secreted by M1-macrophages, and can be sensed by other tissue and immune cells via its cellular receptor GPR91, regulating immune responses and tissue homeostasis [6,77].

In contrast to M1-macrophages, M2-polarised macrophages induced by interleukin IL-4 display a more oxidative metabolic profile, maintaining high levels of TCA cycle and OXPHOS ([72], Van Den Bossche et al., 2017). Initial reports suggested that engagement of glycolysis and FAO are also important for M2-macrophage polarisation, but recent observations have contrasted with this hypothesis [84–86]. Overall, compared to proinflammatory M1-macrophages, metabolic reprogramming in M2-macrophages is less clear, and further studies are needed to elucidate their key metabolic features, as well as the metabolic remodelling taking place in macrophage subsets *in vivo* during inflammation (Van Den Bossche et al., 2017).

3.3. The metabolic profile of microglia

3.3.1. A general overview on the metabolic regulation of microglia

The highly dynamic nature of microglia has led to elucidating the bioenergetic sources used to adequately meet their extensive energy demand. To ensure constant surveillance, microglia rapidly scan the brain parenchyma extending and retracting their processes, in a continuous energy-dependent cytoskeleton rearrangement [22,23]. This observation raises the question, how are microglia able to meet these high-energy requirements? Transcriptomics studies reveal that, in principle, microglia express all the key enzymes implicated in the major metabolic pathways, indicative of considerable plasticity [53,87,88]. Thus, the specific metabolic profile adopted by microglia at any given moment very likely depends on the availability of the bioenergetic substrates and on the expression of the specific metabolic machinery by microglial subpopulations. This is further supported by the observation that, at least in mice, genes related to metabolic pathways are differentially expressed in microglia according to the age and the CNS structure examined [89–91]. For instance, microglia from late embryonic and early post-natal stages display a unique glycolytic profile, with similar metabolic pathways also up-regulated in injury-responsive microglial populations [91]. Functional evidence *in vivo*, however, are still lacking, as most of our current knowledge on microglial metabolism relies on transcriptomics data, or has been extrapolated from studies on murine cell lines or primary cultures.

A growing body of literature indicates that, similarly to macrophages, microglia can also switch their metabolic status to modify their function [92–94]. According to a common view, a shift to glycolysis is associated with a proinflammatory phenotype, whereas OXPHOS and mitochondrial respiration tend to be associated with a more quiescent state. However, it should be noted that the quiescent, 'resting' state of microglia does not completely equate that of macrophages, since 'resting' microglia are in fact very far from being at rest. No studies are currently available that quantify the energy expenditure in ramified-surveying versus amoeboid-activated microglia, but one can hypothesise that these microglial states may both require high levels of ATP. Recent work from Nagy et al. has compared the effects of distinct metabolites on cellular respiration and glycolytic activity in primary microglia and the BV2 murine microglia cell line. It was reported that in these systems, the addition of glucose reduces oxygen consumption by 30%, while promoting glycolysis [95]. However, comparing ATP production in response to different metabolites, it was revealed that while primary microglia rely almost indiscriminately on various bioenergetic sources to double the amount of ATP upon starvation, BV2 cells uniquely utilise glutamine [95]. These findings are another example highlighting the differences between microglia-like cell lines and primary microglia [96].

Glucose, FAs, and AAs can be efficiently utilised by microglia as a source for ATP production. The relative contribution of such metabolites on microglia polarisation and inflammatory potential are described below.

3.3.2. Glucose and glycolysis

The major energy source for microglia and all other CNS cell types is glucose, and these cells express various glucose transporters [97,98]. Time-lapse imaging in hippocampal slices from neonate mice revealed that oxygen-glucose deprivation (OGD) drastically reduced microglia motility, migration, and viability [99]. Primary microglia preconditioned by OGD have shown therapeutic potential when administered to rats undergoing focal cerebral ischemia, with glucose deprivation inducing expression of anti-inflammatory molecules and promoting functional recovery [100]. On the other hand, activation of microglia with LPS induced a rapid reduction in mitochondrial ATP and increased lactate production, consistent with the inflammation-induced glycolysis reported in macrophages [92]. Importantly, treating microglia with 2-deoxy-D-glucose, a glucose analogue that blocks glycolysis by inhibiting hexokinase activity, resulted in rapid ATP depletion followed by microglia necrosis, while not having any effect on astrocytes or neurons [101]. Interestingly, under high glucose conditions, the microglial response to LPS is exacerbated, with increased secretion of TNF-α and IL-6 [102]. Notably, in starvation experiments, only cell death induced by serum- and not by glucose deprivation can be rescued by LPS in microglia, providing further *in vitro* evidence that inflammatory stimuli induce a switch to glycolysis [103]. Altogether, these data point towards an essential role for glucose and glycolysis in microglial

activation and survival. However, opposite results have been reported in other studies, with glucose deprivation enhancing phagocytic capacity and inducing release of proinflammatory cytokines in primary microglia [102,104,105], suggesting that further studies are needed to unveil the role of glucose and glycolysis in microglia function.

3.3.3. FAs and FAO

FAs are important precursors for the biosynthesis of phospholipids and sphingolipids, and also represent a source of energy in the brain, upon increase of energy demand or in fasting states when glucose is limiting. The brain is capable of synthesizing only a few FAs, while most of them enter into the brain from the blood [106]. However, the transport of FAs across the blood-brain barrier (BBB) is still controversial. One hypothesis postulates the passive diffusion of FAs through the luminal and transluminal leaflets of the endothelial cells by a `flip–flop' mechanism [107]. According to a second hypothesis, FAs cross the BBB via protein-mediated transport using a specific fatty acid transporter, such as FATP-1 fatty acid transport protein-1 [108,109] and caveolin-1 [110]. Fatty acid translocase/CD36 (FAT/CD36) and fatty acid binding proteins (FABP) might also play a role by promoting FA dissociation from albumin and facilitating the diffusion across the BBB [106].

Once internalised in the cell, FAs are transported into the mitochondrial matrix, and oxidized through FAO, which relies on the carnitine palmitoyltransferase (CPT) system [111,112]. So far, how FAs modulate microglial functions has not been clearly elucidated, but transcriptomics analyses confirmed that microglia express all the enzymes implicated in lipid metabolism [53,113].

Upon stimulation with LPS, microglia cell lines increased their content in SFAs while decreasing monounsaturated FAs. However, both lipid species exerted a similar effect in enhancing the release of IL-6, confirming previous finding that SFAs can induce inflammatory responses [114]. In contrast with this view, microglia lacking LPL, an enzyme that promotes intracellular uptake of FAs, significantly reduced the expression of anti-inflammatory markers, such as arginase-1 and chitinase-3-like protein 3 (also known as Ym1), and shifted their metabolic profile to glycolysis [115]. It should be mentioned that LPL not only favours FA uptake, but has also been proposed to facilitate the binding of and the interaction with lipid-rich structures on the cell membrane [116]. Thus, whether the functional consequences of LPL depletion in microglia depend on the defective recognition of signalling molecules at the cell surface, or on downstream metabolic dysfunction, remains to be elucidated. Additionally, as a caveat, the role of FAs and LPL in modulating the inflammatory response was only investigated in BV2 cells, and data from primary microglia are missing.

In addition to acting as substrates for energy production, lipids also work as signalling molecules in the brain. Saturated FAs such as palmitic and stearic acids, signal through TLR4 and its NF-kB downstream pathway, thus resulting in the secretion of proinflammatory cytokines, ROS and NO [43,117,118]. Omega-3 PUFAs act on microglia mostly through the GPR120 receptor and modulate the expression of inflammatory genes [44]. In mice exposed to a diet deficient in omega-3 PUFAs, microglial motility is significantly decreased, without major changes in cell number [45].

As mentioned above, FAO has long been considered essential for M2-like macrophage polarization, but recent work changed this view [84,86]. Similar evidence in microglia was provided by findings in the brains of mice lacking multifunctional protein 2 (MFP2), a critical enzyme in peroxisomal β-oxidation. Microglia lacking MFP2 are not polarized towards a pro- or anti-inflammatory phenotype, but are rather hyper-responsive to LPS challenge [119]. This suggests that FAO does not play an important role in microglial cell polarisation but does play a role in their activation. Translocator Protein (TSPO) has recently been implicated in modulation of FAO, with its depletion promoting a shift from glucose to FA metabolism, increasing ROS production and leaving unaltered oxygen consumption rate [120]. Interestingly, TSPO is commonly used as a marker of microglia activation in positron emission tomography (PET) studies, because of its up-regulation upon proinflammatory stimulation. However, recent human studies have failed to validate its biomarker potential, which was previously demonstrated in rodents. TSPO signal in PET human studies instead showed the opposite pattern, being reduced upon inflammation [121,122], confirming that the importance of FAO in microglia activation is far from being understood.

An alternative source of energy to glucose is represented by the ketone bodies (KBs), whose levels are elevated under caloric restriction, starvation, prolonged exercise, or the low-carbohydrate ketogenic diet. KBs are produced predominantly in the liver from FAO-derived acetyl-CoA, and then are transported to extrahepatic tissues for terminal oxidation [123]. The two main ketone bodies are Acetoacetate (AcAc) and β-hydroxybutyrate (β-HB) [124]. In the brain, βOHB is also produced by astrocytes from oxidation of FAs or catabolism of AAs [125,126]. Interestingly, recent studies have shown that microglia respond to βOHB by shifting towards an anti-inflammatory phenotype, associated with increased phagocytosis and enhanced ramified morphology [127]. Similar to previous reports in macrophages [128], βOHB treatment also blocked NLRP3 inflammasome activation in microglia in response to ATP and monosodium urate (MSU) crystals [129]. However, ketogenic diet treatment in two mouse models of Alzheimer's disease, which resulted in increased levels of peripheral βOHB, failed to show any change in Iba1 + microglia [130]. Also, βOHB did not show any effect on inflammasome activation in microglia, triggered by synuclein fibrils [129], suggesting the implication of different pathways in the modulation of NLRP3 inflammasome by βOHB. Initial findings in macrophages indicated that βOHB blocked the inflammasome activation by preventing K^+ efflux, and by inhibiting polymerization, speck formation and assembly of the inflammasome, without undergoing oxidation in the TCA cycle [128]. Whether similar mechanisms occur in microglia remains to be elucidated.

3.3.4. AAs

When carbohydrates or lipids are insufficient for adequate energy supply, proteins represent another possible metabolic substrate. In case of scarce nutrient availability, proteins can be degraded, mostly through the autophagic-lysosomal pathway, to produce free AAs [131]. Upon LPS activation, macrophage cell lines significantly up-regulated the consumption of certain AAs, such as serine and glutamine, while increasing the production of others, such as histidine, glycine and glutamic acid [132]. However, such a modulation of AA levels has never been investigated in microglia.

AAs are not only essential building blocks for *de novo* protein synthesis, but can also exert critical modulatory roles. Previous studies have provided evidence for concentration-dependent effects of serine and glycine on microglial morphology and function. Microglia cultured in the presence of physiological concentrations of serine and glycine found in the cerebrospinal fluid displayed highly ramified morphology and produce significantly less NO and superioxide anion, as compared to microglia in standard culture media, which contained concentrations approximately ten times higher or more [133]. Notably, conditioned medium from microglia in physiological serine/glycine concentrations promoted neuronal survival, whereas the medium collected from microglia cultured in high serine/glycine conditions induced toxicity [133]. Similarly, primary microglia fed a high content of the branched-chain AAs valine, leucine, and isoleucine displayed an intermediate phenotype, with enhanced IL-10 expression and phagocytic activity, but also increased free radical generation, and decreased neuroprotective functions [134], confirming that different AA compositions of the cell medium can affect microglial function.

Glutamine synthetase (GS), which converts glutamate to glutamine, and excitatory amino acids transporter-1 (EAAT-1) are up-regulated in microglia upon activation with several stimuli, i.e. viruses, bacteria, nicotine, or during ischemia, suggesting that microglia are endowed

with an inducible system for glutamate scavenging [135–138]. Interestingly, blocking GS activity impaired insulin-mediated glucose uptake and exacerbated the inflammatory response in activated microglia, with increased production of IL-6, ROS, and NO [139]. These findings support the concept that up-regulation of GS in activated microglia may counteract and repress the inflammatory response, reduce excitotoxicity, and promote neuronal protection, pointing at GS as a possible novel metabolic modulator of microglial function.

Microglia also express all the enzymes to metabolize tryptophan in the kynurenine pathway, and in particular they are the major source of kynurenine 3-monoxygenase (KMO) in the brain [140]. Activated BV2 microglia cells were shown to secrete free radical-producing metabolites 3-hydroxykynurenine and 3-hydroxyanthranillic acid and the excitotoxin quinolinic acid, all intermediates of this pathway, with detrimental consequences on neuronal viability [141]. In accordance, recent work in the same cell line provided evidence that KMO inhibition attenuates the proinflammatory profile of microglia [142]. However, another study reported that 3-hydroxyanthranilic acid may also exert neuroprotective functions [143], suggesting a complex role for the kynurenine pathway in microglia.

All these findings support the idea that microglia are capable of rapid metabolic adaptation, possibly due to effective metabolic switches. To date, however, very little is known about how different metabolic profiles modulate microglial functions, and further investigations are certainly required to provide mechanistic insights.

4. Concluding remarks and feature perspectives

In recent years, the emerging field of immunometabolism has paved the way for the discovery of new-generation therapies able to target immune cell activation and function in inflammatory pathologies and metabolic disorders. As described above, reducing systemic and local inflammation limits obesity-related metabolic disorders in animal models, confirming its role in such pathologies. However, the overall effect of anti-inflammatory drugs in human patients is generally modest, and more studies and clinical trials are needed to clarify if metaflammation influences the development of metabolic diseases in humans, and whether it may represent a valuable therapeutic target for these pathologies [144]. On the other hand, a number of compounds able to impact specific metabolic pathways and to limit immune cell pathogenicity have been developed, and these metabolic regulators and inhibitors have successfully been used to treat autoimmunity and to favour transplant tolerance in animal models [145]. Importantly, recent reports suggest that targeting immune cell metabolism may represent a novel approach for the treatment of inflammatory and autoimmune disease in humans, confirming the potential translational impact of research in this field [146,147]. However, there are currently no indications that such approaches could interfere with microglia function, and future studies are necessary to determine if they can control microglia-induced detrimental responses in metabolic diseases, neuroinflammation, neurodegeneration and ageing. Also, the ability of inflammation- and metabolism-targeting therapeutics to specifically act on microglial cells without affecting the functionality of other immune cell subsets is currently unknown.

Another bias to make note of is that most of the knowledge in the field of cellular immunometabolism arises from *in vitro* studies, analysing the importance of specific metabolites or metabolic pathways on *in vitro* generated and polarised immune cells. Results obtained this setting only partially mirror what actually happens *in vivo*, where immune cell subsets such as macrophages or microglia are highly plastic and can vary their phenotype depending on tissue microenvironment and local nutrient availability [148]. As previously mentioned, this view can also be applied to microglia, as no studies have evaluated their metabolic profile and remodelling in response to different activation cues in the CNS *in vivo*.

An intriguing open question is whether microglial cells can actively release intracellular metabolites that may impact CNS functions. A recent study demonstrated that macrophage-derived succinate accumulated in the CNS during the chronic phase of experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis [149]. The authors reported that transplanted neural stem cells (NSCs) sensed succinate through GPR91, secreting prostaglandin E2 as a response. Together with the direct scavenging of succinate from the CNS microenvironment, NSCs showed a potent anti-inflammatory potential and ameliorated the disease course [149]. Interestingly, GPR91 was previously detected on both neurons and astrocytes, and a role for the succinate/GPR91 axis on CNS inflammation and remodelling has been reported in models of CNS ischemia [150–153]. Considering that, upon activation, mononuclear phagocytes can release several metabolites in the extracellular milieu (e.g.: succinate, itaconate, lactate), microglia-derived metabolites may significantly modulate local inflammation in the CNS and affect neuronal functionality and survival.

Another process that was recently shown to depend on intracellular metabolism is the so-called innate immune memory, also known as trained immunity. Trained immunity is the ability of innate immune cells to “remember” previous activation stimuli such as infections and to heighten their response upon a second exposure to the same or to different triggers [154]. Recent studies demonstrated that innate immune memory is controlled by specific metabolic pathways engaged upon the first activation, which cause epigenetic modifications of essential immune genes, boosting the host response [155]. Immune memory in microglia has just started to be investigated, with recent studies pointing toward mechanisms of immune training and tolerance in the brain, mediated by epigenetic reprogramming (Walden et al., 2018). However, the role of intracellular microglia metabolism in this process remains largely unknown. Considering the key role of microglia in CNS homeostasis in health and disease, and the importance of “microglia memory” upon infections or other triggers, understanding the modulation of intracellular metabolism will open new avenues for potential therapeutic intervention.

Overall, we have just begun to dissect the role of microglia in obesity-related metabolic disorders and the importance of intracellular metabolic pathways on the functions of such a dynamic cell population, and many opportunities for investigation are still open in these research areas.

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