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Feature Review

Targeting Mitochondrial Metabolism in Neuroinflammation: Towards a Therapy for Progressive Multiple Sclerosis

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The lack of effective treatment options for chronic neurological conditions, such as multiple sclerosis (MS), highlights the need to re-evaluate disease pathophysiology in the process of identifying novel therapeutic targets. The persistent activation of mononuclear phagocytes (MPs) is one of the major drivers of neurodegeneration and it sustains central nervous system (CNS) damage. Mitochondrial metabolism influences the activity of MPs, and the metabolites that they produce have key signalling roles in inflammation. However, how changes in immune cell metabolism sustain a chronic state of neuroinflammation is not fully understood. Novel molecular and cellular therapies for chronic neuroinflammation should be developed to target mitochondrial metabolism in innate immune cells to prevent secondary neuro-logical damage and the accumulation of irreversible disability in patients.

The Cellular Biology of the Chronically Inflamed Brain

The CNS has long been considered an immune-privileged or immunologically unique organ because of the absence of a route to the lymph nodes for the antigen-presenting cells (APCs) that reside in the parenchyma and perivascular spaces of the healthy CNS. Furthermore, the CNS generally lacks a cell-mediated response to instilled antigens, and a robust response to intraparenchymal CNS antigens can be elicited by peripheral immunisation [1]. However, the presence of a functional **meningeal lymphatic system** (see Glossary) in the CNS suggests that current dogmas regarding brain tolerance and the immune privilege of the brain should be revisited [2], and highlights a close relationship between the CNS and immune system in both health and disease [3,4].

In physiological conditions, the CNS is continuously guarded by resident microglia and bloodborne immune cells, such as macrophages, dendritic cells and T cells (including regulatory T cells), that detect damaging agents that would disrupt homeostasis and optimal functioning [5]. Among the sentinels that patrol the CNS, **MPs** [6] are in charge of mediating local immune surveillance by residing within specific anatomical niches [1]. These resident MPs include both microglia, which constitute 5–10% of total brain cells and are the only true CNS parenchymal MPs [7], and macrophages, which reside in the perivascular spaces, the leptomeninges, and the choroid plexus [8].

The activation of CNS-resident MPs, and especially that of microglial cells, is a salient feature of the neuroinflammation that is prominent in almost all neurodegenerative diseases, including Alzheimer's disease (AD) (Box 1), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) [9,10]. **MS** is classically defined as a secondary neurodegenerative disease, because

Highlights

MPs, including resident microglia and blood-borne macrophages, control local immune surveillance in the CNS.

In chronic neurological conditions, such as MS, MPs are persistently activated and responsible for several maladaptive responses that include neurotoxicity and inhibition of remyelination.

Cell metabolism guides the activation of MPs towards a proinflammatory or anti-inflammatory phenotype and function, depending on the local microenvironment.

Specific metabolites act as intracellular and extracellular signalling molecules regulating neuroimmune interactions and inflammation.

Neural stem cells interfere with MP metabolism via selective uptake of specific metabolites and secretion of metabolic enzymes packed into extra-cellular vesicles.

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Box 1. Disease-Associated Microglia in Neurodegeneration

Ex vivo single-cell RNA sequencing (scRNA-seq) analysis of AD-transgenic (Tg-AD) mouse brains discovered diseaseassociated microglia (DAM), a subset of microglia showing a unique transcriptional and functional signature [123]. DAM appear to be a common response signature to CNS disease that leads to the upregulation of specific genes, most of which are involved in lipid metabolism pathways, via a two-step activation process of microglia that is ultimately dependent on the triggering receptor expressed on myeloid cells 2 (TREM2) [123]. TREM2 is a sensor for a range of lipids and lipoproteins that activates microglial phagocytosis and negatively regulates autophagy via mTOR, a master regulator of glycolysis [124]. In TREM2-deficient mouse models or human samples of AD, microglial clustering around β -amyloid plaques is impaired and the rare human polymorphism R47H of TREM2 shows one of the highest odds ratios (i.e. 2.9-4.5) for susceptibility to late-onset AD [125]. These data suggest that the long-term protective function of microglial cells in neurodegenerative conditions is linked with TREM2 functions at different levels, including the maintenance of proper lipidic and glycolytic metabolic states.

neuronal death occurs secondarily to a type of myelin loss evolving on a background of inflammation, comprising T lymphocytes, B lymphocytes, and plasma cells [11,12]. Furthermore, in MS, the local activation of resident MPs is coupled with the recruitment of blood-circulating MPs to areas of CNS and blood-brain barrier (BBB) damage (Box 2).

Resident and CNS-infiltrating MPs have different roles and contribute to MS differently during the course of disease [13]. In relapsing remitting (RR) MS, active lesions predominate and the most common lesion types (patterns I and II) [14] show a diffuse perivascular and parenchymal T cell infiltration and a background of MPs. MPs are also present in the normal-appearing white matter (NAWM) and, in both cases, they express proinflammatory markers, including CD40, CD86, CD64, and CD32 [15], while downregulating the expression of homeostatic markers, such as P2RY12 [16]. Late in the disease, correlative evidence exists that the immune response progressively shifts from adaptive to innate, with the activation of MPs increased in patients with progressive MS and higher clinical disability [17]. In progressive MS, MPs are present in both subpial cortical lesions and smouldering plaques [18]. Subpial cortical lesions are characterised by demyelination of the superficial cortex, with sparse MPs in association with inflammation in the overlying leptomeninges. Smouldering plaques are frequently and almost exclusively found in progressive MS [18], and represent the slow expansion of pre-existing lesions now characterised by the accumulation of inflammatory MPs leading to active demyelination [16,19]. In progressive MS, the extent of MP activation is further increased by retrograde neurodegeneration after axonal damage in white matter lesions [20,21].

Box 2. Infiltrating MPs in Neuroinflammation

The contribution of blood-circulating MPs to CNS damage during EAE in mice commonly entails the local recruitment of GR1+/LY6C+/CX3CR1^{low}/CCR2+ monocytes (equivalent to human CD14⁺⁺/CD16⁻ monocytes) to sites of injury, with a CCR2-dependent mechanism [126]. Shortly after infiltration into the intact CNS parenchyma, monocytes downregulate their CCR2 and upregulate the CX3C chemokine receptor 1 (CX3CR1), becoming almost indistinguishable from activated CNS-resident MPs [127]. CNS-resident macrophages and microglia express several common markers (e. g., CX3CR1 and CD11b), and only recently have specific markers indicative of a microglial homeostatic signature, such as the transmembrane protein 119 (TMEM119), the P2Y purinoceptor 12 (P2RY12), and the Sal-like protein 1 (SALL1), been described [128,129].

In mice with EAE, resident MPs appear to have a key pathogenic role, because early changes in vascular permeability are associated with **perivascular inflammatory cuffing** and parenchymal microglial activation that precede the arrival of blood-derived monocytes accompanying demyelination [130]. However, circulating MPs also have an important role in the pathophysiology of disease, because it has been shown that, in a transgenic mouse line expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) in peripheral helper T (Th) cells, GM-CSF leads to invasion of MPs into the CNS and the spontaneous development of neurological deficits [131]. A current hypothesis suggests that, in EAE, while tissue-resident MPs clear tissue debris, contribute to recovery, and protect the CNS, MPs entering through the blood vasculature are instead detrimental and instruct demyelination [132,133].

Glossary

Electron transport chain (ETC): comprises five complexes and two mobile electron carriers embedded in the mitochondrial membrane that link oxidation of carbon substrates to ATP production. The ETC couples the energy of electron transfer (from NADH and FADH₂) to the generation of a proton-motive force across the inner membrane, which is used by complex V to drive ATP synthesis. Electrophilic stress: electrophiles are ubiquitous reagents in aerobic organisms; they are attracted to electrons and arise from both xenobiotic challenge and internal metabolism. Although some electrophiles have physiological functions, many can trigger cell damage because of their reactive chemistry. Most species have evolved elaborate intrinsic strategies to cope with electrophilic stress. Genes encoding many of these defensive products contain an electrophile response element/ antioxidant response element (EpRE/ ARE) and are regulated by the transcription factor Nrf2, the activity of which is determined by the state of its redox-sensitive inhibitor Keap1. Experimental autoimmune

encephalomyelitis (EAE): a murine model of MS that is induced by either the administration of myelin protein or peptide in adjuvant or by the adoptive transfer of encephalitogenic T cell blasts into naïve recipients. Despite all the limitations of this model, it is still widely used to answer many of the immunological-oriented questions in MS research.

Fatty acid oxidation (FAO): the catabolic process by which FA molecules are broken down in the mitochondria to generate acetyl-CoA, which enters the TCA cycle, and NADH and FADH₂, which are co-enzymes used in the ETC.

Fatty acid synthesis (FAS): the creation of FAs from acetyl-CoA and NADPH through the action of enzymes, fatty acid synthases, in the cytoplasm of the cell.

Glycolysis: the process by which glucose is incompletely oxidised in the cytosol, yielding lactate as its final product. Compared with oxidative metabolism, glycolysis is fast but energy inefficient.

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Mechanistically, the release of mediators of inflammation by either resident or infiltrating proinflammatory MPs triggers the activation of additional microglial cells and causes damage to essential components of mitochondria in neurons, such as mtDNA and enzymes of the mitochondrial respiratory chain. This worsens the unsteady balance between the energy demands and supply, thus inducing a chronic state of virtual hypoxia in chronically demyelinated axons [22]. Proinflammatory MPs also impair glutamate transport in astrocytes, which in turn further increases the extent of direct neuronal and oligodendrocyte excitotoxicity [23]. Consequently, active demyelination and neurodegeneration are clearly associated with a dominant proinflammatory phenotype of MPs.

Immune processes and cell metabolism are conserved biological functions linked to diseases that span multiple areas of medicine. Recent evidence suggests that metabolic changes in innate immune responses influence both the effector phase and the resolution of inflammation by modulating immune cell fate and function [24]. As such, alterations of certain metabolic configurations of immune cells might contribute to dysfunctional immune responses, a typical feature of autoimmunity [24]. Unfortunately, how the innate immune system is organised in progressive MS and which role cell metabolism has in MP function during chronic neuro-inflammation are outstanding questions.

Here, we anticipate that the changes in the metabolism of macrophages and microglia have a major role in the maintenance of the persistent state of neuroinflammation that contributes to disease progression in MS.

Identification of the metabolic determinants of such a steady activation of CNS-compartmentalised innate immune responses might help pinpoint a range of druggable metabolic pathways through which to reduce neuroinflammation, reducing concomitant neurodegeneration and the accumulation of irreversible disabilities in patients with MS [25].

The Role of Cell Metabolism in Innate Immune Activation

In MPs, cellular polarisation has been traditionally categorised as having either toxic (M1-like, classically activated, proinflammatory) or protective (M2-like, alternatively activated, anti-inflammatory) phenotype and function [26]. This first classification was in part based on one important metabolic feature: M1 macrophages produce nitric oxide (NO), whereas M2 macrophages produce polyamines [27]. On the one hand, the NO synthase (NOS) family of enzymes catalyse the conversion of L-arginine to L-citrulline plus toxic NO in M1-like macrophages [28]. On the other hand, the arginases convert L-arginine into L-ornithine and urea in M2-like macrophages [29]. Despite the attractive simplicity of this dichotomy, accumulating evidence suggests that MPs polarisation is multidimensional, with an overlap in gene expression and metabolism after pro- or anti-inflammatory stimuli, as opposed to occurring on a simplified linear spectrum [30]. Nonetheless, these initial studies have highlighted a key concept in MP biology: the link between MP activation and metabolism [31].

Many of the current lines of evidence investigating how metabolism affects the proinflammatory status of MPs come from *in vitro* experiments on mouse bone marrow-derived macrophages (BMDM) challenged with lipopolysaccharides (LPS). These seminal observations revealed that proinflammatory MPs, differently from anti-inflammatory MPs (Box 3), are characterised by a switch to aerobic **glycolysis**, similar to the Warburg effect in cancer cells [32], upregulation of the **pentose phosphate pathway** (PPP) and increased **fatty acid synthesis** (FAS) due to the existence of enzymatic break points in the **tricarboxylic acid (TCA) cycle** (Figure 1).

Meningeal lymphatic system: a

network of lymphatic vessels located parallel to the dural sinuses and meningeal arteries in the mammalian CNS. Although its role in health and disease is yet to be fully

characterised, a dysfunction of the meningeal lymphatic system might be relevant for diseases of the CNS, such as MS.

Mononuclear phagocytes (MPs):

part of the innate immune system, which comprises phagocytic cells either resident within tissues (e.g., microglia in the CNS) or blood-borne (e.g., macrophages).

Multiple sclerosis (MS): the most prevalent chronic inflammatory disease of the CNS, affecting more than 2 million people worldwide. MS is currently incurable.

Normal-appearing white matter (NAWM): macroscopically intact regions on conventional MRI that show a degree of chronic injury (e.g., axonal spheroids, microglial activation, gliosis, and increased expression of proteolytic enzymes) on deeper pathological characterisation.

Pentose phosphate pathway (PPP): a shunt of glycolysis that produces NADPH, important for maintaining cellular redox balance

and nucleotides. Perivascular inflammatory

cuffing: the dense accumulation of immune cells around blood vessels seen during inflammatory and/or immune reactions.

Progressive MS: characterised by the progressive accumulation of disability without identifiable acute relapses. Progressive MS includes secondary progressive (SP), which is the consequence of RR MS, and primary progressive (PP) MS, in which progression is present from the onset of the disease.

Relapsing remitting (RR) MS:

characterised by discrete episodes of acute neurological deficits and/or worsening of a given neurological function (i.e., relapse), followed by a complete or partial recovery (i.e., remission). RR MS is the most common phenotype of MS (~85% of total cases) and is the typical presentation of disease at onset. **Reverse electron transport (RET):**

this process consumes proton motive force to drive electrons in a TRMOME 1368 No. of Pages 18

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Box 3. Metabolism of Anti-Inflammatory MPs

Functional polarisation of MPs towards an anti-inflammatory phenotype can be achieved *in vitro* by IL-4 (or IL-13) stimulation.

Anti-inflammatory mouse macrophages are characterised by high levels of arginase-1 activity, which is needed to metabolise arginine to proline, a component of collagen that is required for tissue repair and resolution of inflammation [134]. Although arginine metabolism is one of the discriminative criteria between pro- and anti-inflammatory mouse macrophages, human data question the translational value of this dogma, because neither arginase-1 nor Ym1 are upregulated *in vitro* in alternatively activated human monocytes and monocyte-derived macrophages [135,136].

Accumulating evidence suggests that both human and murine anti-inflammatory MPs rely on glucose and mainly glutamine uptake *in vitro* [60,137]. In mouse BMDMs, IL-4 signalling co-opts the Akt-mTORC1 pathway to trigger the uptake of glucose and to regulate anti-inflammatory genes that include, for example, those encoding arginase 1 and macrophage galactose N-acetyl-galactosamine specific lectin 2, via phosphorylation of ATP-citrate lyase (or ACL) [60]. Glucose is used to support hexosamine biosynthesis and to generate uridine diphosphate (UDP)-*N*-acetylglucosamine (UDP-GlcNAc), which is necessary for the post-translational modification of lectin mannose receptors [138].

Glutamine is important for M2 polarisation, which activates glutamine catabolism and UDP-GlcNAc-associated modules in BMDMs. Correspondingly, glutamine deprivation (or inhibition of *N*-glycosylation) decreases M2 polarisation *in vitro* [38].

Anti-inflammatory macrophages downregulate flux through the PPP by inducing CARKL, which inhibits this pathway, resulting in reduced NADPH-mediated glutathione levels [36].

IL-4 stimulation of signal transducer and activator of transcription 6 (STAT6) results in PPAR γ -coactivator-1 β (PGC-1 β), leading to mitochondrial biogenesis, preservation of TCA cycle functionality, and increased FAO [139]. Short-chain fatty acids diffuse passively into the mitochondria, whereas medium- and long-chain fatty acids are imported through conjugation to carnitine palmitoyltransferase (CPT). Irreversible inhibition of the CPT1 with etomoxir is sufficient to inhibit expression of classic M2 genes [140], while CPT2 is dispensable for macrophage polarisation [141]. Anti-inflammatory MPs can then effectively use the acetyl-CoA derived from FAO to drive forward flux in the ETC.

The translation factor eIF5A, which is regulated by polyamines and undergoes a specific post-translational modification called hypusination, is a potential cellular therapeutic target to affect M2 polarisation in mouse macrophages using small molecules [142]. However, further experiments are needed to understand how to modify eIF5A activity to promote an efficient anti-inflammatory pro-reparative activity of MPs. Understanding how the metabolism of anti-inflammatory MPs is regulated may help identify novel pathways that control the activation of MPs to favour neuroprotection and promote tissue regeneration.

Switching cell metabolism towards aerobic glycolysis in MPs begins with an increased glucose uptake, mediated by the upregulation of glucose transporter type 1 (GLUT1), which enables the efficient uptake of glucose in an environment where nutrients are restricted by inflammation [33]. Glucose is used in the glycolytic pathway to produce ATP and pyruvate. Key regulators of these changes are the protein kinase mammalian target of rapamycin (mTOR) and the transcription factors Myc and hypoxia-inducible factor (HIF)-1 α , which induces a glycolytic gene expression profile [34]. Pyruvate is then used to produce lactate via lactate dehydrogenase (LDH), which is then released in high concentrations in the extracellular environment.

Along with glycolysis, the PPP is induced in proinflammatory MPs. Glucose-6-phosphate (G6P) is used to provide 3-phosphoglycerate for the serine biosynthetic pathway (required for the synthesis of amino acids for cytokines), and to generate ribose 5-phosphate for the synthesis of nucleotides and NADPH [35]. These changes are partially dependent on the inhibition of the carbohydrate kinase-like protein (CARKL), a key enzyme that bridges glycolysis and the PPP [36]. NADPH is an essential cofactor for the production of reactive oxygen species (ROS) by NADPH oxidases, for inducible NOS (iNOS), and for the production of the antioxidant glutathione, which protects the cell against ROS-mediated damage [35]. The inhibition or deletion of NADPH oxidase is sufficient to switch microglial activation from a proinflammatory to an anti-inflammatory state [37].

reverse direction through the ETC. RET is produced when electrons from ubiquinol are transferred back to respiratory complex I, reducing NAD⁺ to NADH. This process generates a significant amount of reactive oxygen species (BOS). Smouldering plaques: white matter slowly expanding smouldering plaques in MS are characterised by an inactive centre with few macrophages, surrounded by a rim of activated microglia. Smouldering plagues occur almost exclusively in patients with progressive MS. Tricarboxylic acid (TCA) cycle: a series of chemical reactions to release stored energy through the oxidation of acetyl-CoA in the form of ATP. In addition, the cycle provides precursors for amino acids as well as

the reducing agent NADH.





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Figure 1. Metabolic Pathways in Mononuclear Phagocytes (MPs) during Inflammation. The metabolism of proinflammatory MPs significantly differs from that of anti-inflammatory MPs, and is characterised by increased aerobic glycolysis, upregulation of the pentose phosphate pathway (PPP) and increased fatty acid synthesis (FAS) due to the existence of enzymatic break points in the tricarboxylic acid (TCA) cycle. Anti-inflammatory MPs instead not only rely on glycolysis and glutamine uptake, but also downregulate the PPP and increase fatty acid oxidation (FAO) to feed the TCA cycle. Abbreviations: ACL, ATP-citrate lyase; C, mitochondrial complex; CAD, *cis*-aconitate decarboxylase 1; CIC, citrate carrier; ER, endoplasmic reticulum; FA, fatty acid; G6P, glucose 6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GLUT1, glucose transporter type 1; IDH, isocitrate dehydrogenase; iNOS, inducible nitric oxide; synthase; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NO, nitric oxide; OXPHOS, oxidative phosphorylation; PDH, pyruvate dehydrogenase; PGs, prostaglandins; RET, reverse electron transport; ROS, reactive oxygen species; SDH, succinate dehydrogenase; TLR4, toll like receptor 4.

In proinflammatory mouse BMDMs, TCA cycle breaks appear at two major points: one at the level of succinate dehydrogenase (SDH), which catalyses the oxidation of succinate to fumarate, and another at the level of isocitrate dehydrogenase (IDH), the enzyme that converts isocitrate to α -ketoglutarate (α KG). These breaks lead to the accumulation of specific metabolites (e.g., succinate, malate, citrate, and itaconate), and to the reduction several others (e.g., fumarate and α KG) [31,38].

The main source of succinate accumulation in proinflammatory MPs appears to be glutamine. LPS-activated MPs increase the uptake of glutamine and initiate glutaminolysis to replenish α KG in the TCA cycle and maintain global metabolic flux [32]; α KG can be then converted into succinate within the TCA cycle [39]. Succinate is also produced from succinic semialdehyde through the enzyme succinate semialdehyde dehydrogenase, an intermediate reaction of the GABA shunt [40].

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The malfunction of the SDH increases succinate concentrations by blocking its conversion to fumarate. Nonetheless, a large increase in malate is also seen, which is dependent on an enhanced arginosuccinate shunt that feeds into fumarate and malate (linking the urea and the TCA cycles) [38]. Proinflammatory macrophages use the arginosuccinate shunt because of the break after SDH, and inhibition of aspartate aminotransferase (AAT), a key enzyme in the shunt, suppresses NO and interleukin (IL)-6 production [38].

The progressive accumulation of succinate then starts driving the activity of the SDH, thus promoting mitochondrial ROS production from the NADH-ubiquinone oxidoreductase (complex I) via a process termed **'reverse electron transport'** (RET) [41]. Essentially, while in resting MPs SDH typically receives electrons from succinate and passes them on to complex III for forward **electron transport chain** (ETC) flux, in proinflammatory MP electron transfer from SDH to complex I. This depends on the presence of a high proton-motive force and increased succinate concentrations, and results in mitochondrial ROS generation. Mitochondrial ROS then stabilise HIF-1 α , thereby promoting proinflammatory cytokine IL-1 β expression.

The second break in the TCA cycle in LPS-activated MPs depends on a sevenfold relative reduction of the enzyme IDH activity compared with resting MPs, which leads to the accumulation of isocitrate and the upstream metabolites *cis*-aconitate and citrate [38]. *Cis*-aconitate is converted by the enzyme *cis*-aconitate decarboxylase 1 (CAD), coded by immunoresponsive gene 1 (*Irg1*) to itaconate [42,43], which has major antimicrobial activity (e.g.,by inhibiting the citrate-lyase expressed by different bacterial strains) and a key anti-inflammatory role (Box 4).

Another important metabolite that increases in LPS-stimulated macrophages is malonylcoenzyme A (CoA) [44]. One of its roles is to inhibit fatty acids from associating with carnitine by regulating the enzyme carnitine acyltransferase, thereby preventing them from entering the mitochondria, where **fatty acid oxidation** (FAO) occurs [45]. Preventing FAO reduces the fuel to the TCA cycle, hence contributing to the decrease in oxidative phosphorylation. By contrast, FAS is enhanced in proinflammatory MPs via different mechanisms. First, mitochondrial citrate is exported to the cytosol by the citrate carrier (CIC), where it is converted to acetyl-CoA, an important intermediate for the synthesis of fatty acids [46]. Second, mTOR activation upregulates lipid synthesis [47] and the NADPH derived from the PPP is used as a co-factor for *de novo* FAS, ultimately favouring the expansion of the endoplasmic reticulum (ER) and Golgi to support cytokine secretion [24] (Figure 1).

Box 4. Itaconate Has a Key Role in the Inflammatory Response

Itaconate has a key role in the inflammatory response of macrophages, being necessary for their early antimicrobial response. However, the observation that BMDMs from immunoresponsive gene 1 (lrg1)^{-/-} mice are deficient in itaconate production, while secreting more IL-12, NO, IL-6, IL-1 β , and IL-18, suggests that the main role of itaconate is to limit the detrimental cellular effects of prolonged inflammatory activation [143].

The major anti-inflammatory effects of itaconate during MP activation have been attributed to the inhibition of succinate oxidation by SDH [143]. Yet, itaconate is only a weak SDH inhibitor and this inhibition alone is not sufficient to account for its pronounced immunoregulatory effects. It is now well established that itaconate has other effects, including the induction of **electrophilic stress** [144] and the alkylation of Keap1 through dicarboxypropylation [78]. While in normal conditions, Keap1 binds to the transcription factor NRF2 facilitating its ubiquitination and proteolysis, itaconate blocks this process and favours the translocation of NRF2 to the nucleus and the transcription of antioxidant genes. The induction of type I interferons from activated cells via a negative feedback loop [78]. These data point at itaconate or specific derivatives [78,145] as useful metabolic therapeutics to lessen chronic MP activation in neuroinflammation.

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Figure 2. Mononuclear Phagocyte (MP) Metabolites Act as Intracellular and Extracellular Signalling Molecules in Inflammation. Intracellular metabolite signalling leads to post-translational modifications of proteins, regulates the activity of metabolic enzymes capable of binding specific mRNAs (e.g., glyceraldehyde-3-phosphate dehydrogenase; GAPDH), guides mRNA expression [e.g., interleukin (IL)-1β] and ultimately affects chromatin structure and function. When released in the extracellular space, metabolites can act as 'metabokines', which bind to specific receptors to convey proinflammatory (red) or anti-inflammatory (green) signals to immune cells. Abbreviations: AHR, anyl hydrocarbon receptor; BHOB, beta-hydroxybutyrate; C, mitochondrial complex; CIC, citrate carrier; GPR, G protein-coupled receptor; HAT, histone acetyltransferases; HDACs, histone deacetylase; HIF1-α, hypoxia-inducible factor-1α; IFNs, interferons; KMD, histone demethylase; NRF2, nuclear factor erythroid 2-related factor 2; RET, reverse electron transport; ROS, reactive oxygen species; SDH, succinate dehydrogenase.

Understanding how cell metabolism is regulated in inflammatory MPs, and which metabolic pathways are responsible for the abovementioned changes, might hold the potential to reveal novel targets to interfere with the persistent activation of microglia and/or macrophages in the CNS of patients with progressive MS.

Metabolites as Signalling Molecules in Inflammation

Growing evidence suggests that metabolites are not only intermediaries for energy production, but can also shape inflammatory responses. These 'immunometabolites' function both as intracellular signalling molecules [48], as well as extracellular signals of inflammation, acting as *de facto* 'metabokines' (Figure 2).

Intracellular metabolite signalling depends on the ability to trigger post-translational modifications of proteins, including acetylation, malonylation, succinylation, succination (by fumarate),

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and glutarylation [49]. Moreover, intracellular metabolites regulate the activity of metabolic enzymes capable of binding specific mRNAs within RNA-enzyme-metabolite (REM) networks [50]. For example, the glyceraldehyde-3-phosphate (G3P) dehydrogenase (GAPDH), which normally blocks the translation of several mRNAs, including tumour necrosis factor (TNF)- α , during inflammatory activation is preferentially used as a glycolytic enzyme, thereby allowing TNF- α translation and secretion [51,52].

Intracellular metabolites can also affect chromatin structure and function. In proinflammatory MPs, glycolysis is induced and its end-product lactate inhibits class II histone deacetylase (HDAC), thus increasing acetylation and regulating gene expression [53]. Conversely, higher NAD⁺/NADH ratios influence the activities of the class III HDACs sirtuin 1 and 6, which use oxidised NAD⁺ as a substrate for not only the deacetylation process, but also the inhibition of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and protein lysine desuccinylation [54,55]. Mitochondria and mitochondrial metabolites also have a key role in the acetylation process. Cytosolic acetyl-CoA, which is partially derived from glycolysis and glutamine, is mostly regulated by the mitochondria via the CIC. Cytosolic acetyl-CoA is used for histone acetylation, thereby increasing the transcription of glycolytic genes, such as those encoding hexokinase 2, phosphofructokinase, and LDH [56].

The balance between α KG, succinate, fumarate, and 2-hydroxyglutarate (2HG) is instead critical for the activity of α KG-dependent dioxygenases involved in histone and DNA demethylation. α KG is used as a substrate for the enzymes of the ten-eleven translocation (TET) family, which catalyse DNA demethylation and the Jumonji C (JmjC)-domain-containing histone demethylase (KDMs) [57]. These enzymes use α KG and molecular oxygen to oxidise their substrate (methyl cytosine) and reduce methylation, producing succinate and carbon dioxide as by-products. Accumulation of succinate and fumarate leads to the inhibition of DNA demethylases and KDMs via product inhibition [58,59]. As consequence, a distinct DNA and histone hypermethylation profile is observed in SDH and fumarase hydratase-deficient tumours, where succinate and fumarate accumulate, respectively, and in IDH mutant tumours, where 2HG accumulates [58]. The effect of all these epigenetic changes is that, in proinflammatory BMDMs, both histone methylation and histone acetylation are present at the promoter and enhancer levels, potentiating proinflammatory gene transcription [57]. These epigenetic modifications mediated by metabolites are important for the polarisation of MPs [60], as well as for the acquisition of the specialised phenotype linked with trained immunity (Box 5).

Extracellular metabolites can also act as key signalling molecules, both at the organismal level and within specific inflammatory microenvironments [61].

At the organismal level, metabolites of dietary tryptophan produced by the microbiome (e.g., kynurenine) suppress the microglial expression of NF- κ B-dependent transcripts in an aryl hydrocarbon receptor (AHR)-dependent manner [62]. In turn, microglia-derived transforming growth factor (TGF)- α signalling limits the activity of neurotoxic A1 astrocytes in mice with **experimental autoimmune encephalomyelitis** (EAE), as a model of MS [62]. Ketone bodies, such as the beta-hydroxybutyrate (BHOB), which are produced from the liver after food deprivation [63], also have major anti-inflammatory effects via hydrocarboxylic acid receptor 2-HCAR2 (GPR109A) signalling on mouse BMDMs and human monocytes *in vitro*, and in mouse models of NLR family pyrin domain-containing 3 (NLRP3)-driven inflammation *in vivo* [64]. While short-chain fatty acids can also be sensed by both GPR43 and GPR109A to promote inflammatory resolution and mediate anti-inflammatory effects *in vivo* in models of colitis, arthritis, and asthma [65]; medium-chain fatty acids act through GPR84 to induce a

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Box 5. Metabolism and Trained Immunity in MPs

The innate immune system quickly adapts after a previous challenge through functional and epigenetic reprogramming, a process that has been termed 'trained immunity or innate immune memory' [146]. Priming human peripheral blood mononuclear cells (PBMCs) with *Candida albicans* (or beta-glucan) results in increased cytokine production upon restimulation with LPS (in contrast to the prestimulation with LPS, which induces tolerance) [147]. Induction of trained immunity by beta-glucan causes activation of the mTOR pathway and HIF-1 α activity associated with specific transcriptional and epigenetic (e.g., H3K4me3 and H3K27ac) signature [148]. In a trained cell, histone methylation tags the promoters of inflammatory genes allowing for an accelerated and stronger response upon restimulation. Several other pathways are differentially regulated in trained MPs, including increased 2HG concentrations, consumption of aspartate, accumulation of mevalonate, and increased cholesterol synthesis pathway [149]. The inhibition of histone methyltransferases or histone acetyltransferases, or the blockage of mevalonate generation all prevent the induction of trained immunity [149]. The interplay between cell metabolism and epigenetic changes is a hot topic in science that will likely unveil why maladaptive responses are maintained in the persistently inflamed tissues.

proinflammatory response in human and rat polymorphonuclear leukocytes (PMNs) and macrophages both *in vitro* and *in vivo* [66].

Within specific microenvironments, metabolites secreted by inflammatory cells act as feedback mechanisms of local immune regulation by affecting the duration and magnitude of the inflammatory response. For example, extracellular succinate is abundantly detected in synovial fluid from patients with rheumatoid arthritis (RA) and coronary sinus blood from patients with myocardial infarction, where its concentration correlates with the extent of damage [67]. Extracellular succinate acts on the succinate receptor SUCNR1/GPR91, a G-protein-coupled cell surface sensor with pleiotropic roles in different tissues and/or organs [68] that has a key functional role in mast cells [69], dendritic cells (DCs) [70], monocytes [71], and macrophages/microglia within the immune system [70,72].

Activation of DCs via GPR91 favours their maturation and migration to lymph nodes, augments proinflammatory cytokine production in response to TLR signalling, and enhances antigen-specific activation of T helper cells [70]. GPR91 on inflammatory macrophages is a main checkpoint of a feed-forward loop of cell activation, where it senses extracellular succinate to upregulate a HIF-1 α -dependent innate pathway potentiating IL-1 β production to exacerbate experimental RA in mice [71].

By contrast, several metabolites released within the inflammatory microenvironment have antiinflammatory properties. Among these, lactate acts through the lactate receptor GPR81 to inhibit adenylyl cyclase and suppress innate immunity [73], while fumarate may influence the GPR109A/HCAR2 signalling pathway to reduce microglial activation [74] (Figure 2).

MS Therapeutics and Their Effect on Mitochondrial Metabolism

Cell metabolism is emerging as a highly relevant therapeutic target to interfere with the activation of both macrophages [75] and microglia [76] and possibly with the accumulation of irreversible disabilities in patients with chronic neuroinflammatory diseases.

Several ways to modify MP metabolism can be envisaged, including the development of specific therapeutics that target intracellular metabolic pathways. Intracellular inhibition of SDH in mouse BMDMs with dimethyl malonate (DMM), a cell permeable prodrug that generates malonate, has been proven effective in decreasing IL-1 β expression, while boosting the induction of the anti-inflammatory cytokine IL-10 in response to LPS *in vitro* [77]. Similarly, a new cell-permeable itaconate derivative, 4-octyl itaconate, decreases the production of inflammatory cytokines in LPS-stimulated mouse BMDMs and human macrophages via activation of nuclear factor erythroid 2-related factor 2 (NRF2) [78] (Box 4). Similarly, the

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metabolic modulator metformin, a frontline treatment for type 2 diabetes mellitus acting via both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms, decreases the production of the proform of the inflammatory cytokine IL-1β in response to LPS in mouse BMDMs via complex I inhibition [79].

Several disease-modifying treatments (DMTs) tested in MS are able to interfere with key metabolic mitochondrial pathways.

The methyl ester of fumaric acid dimethyl fumarate (DMF; Tecfidera, Biogen Idec.) was recommended for approval in the European Union as an orally administered DMT in adult patients with RR MS or psoriasis by the European Medicines Agency (EMA). DMF binds to Kelch-like ECH-associated protein 1 (Keap1) to enable the nuclear translocation of NRF2 [80] and mediates the transcription of antioxidative genes, such as that encoding hemoxygenase-1 (*HO1*) and the quinoline oxidoreductase-1 (*NQO1*) [81]. DMF is also an agonist of the GPR109A/HCAR2, a G-protein-coupled membrane receptor that modulates microglial activation [74]. Recent data also suggest that the main mechanism of action of DMF in RR MS depends on the succination and inactivation of the catalytic cysteine of the glycolytic enzyme GAPDH, thereby downregulating aerobic glycolysis in activated myeloid and lymphoid cells [82].

Biotin, or vitamin B7, is a co-enzyme for three carboxylases (including the pyruvate carboxylase) of the TCA cycle in the mammalian brain involved in energy production [83] and it increases the levels of cellular ATP in conditions of virtual hypoxia [84,85]. The results of a first doubleblind, controlled, randomised Phase III trial of daily oral MD1003 (Qizenday, Medday Pharmaceuticals; a highly concentrated formulation of biotin) described a significant reduction in the progression of neurological symptoms in >150 patients with progressive MS compared with placebo-treated controls [86]. A second study involved 93 patients with MS who had visual loss due to inflammation of the optic nerve. On December 2017, the EMA's Committee for Medicinal Products for Human Use (CHMP) concluded that the clinical data from these two trials enrolling >250 patients were not sufficient to assess the effectiveness or the safety of biotin. This press release was released jointly with a letter from Medday Pharmaceuticals, the biotech company developing the pharmaceutical-grade high-dose biotin, notifying the EMA of the withdrawal of the marketing authorisation application of Qizenday for the treatment of progressive MS. A further multicentre, double-blind, controlled, randomised Phase III trial of daily Qizenday is currently underway and will continue to recruit participants (>600 patients) with primary or secondary progressive MS from North America and Europe, including study centres in Edinburgh, London, and Manchester. The estimated completion date for this trial is September 2019 (ClinicalTrials.gov Identifier: NCT02936037).

Lipoic acid is an antioxidant and cofactor for at least five enzyme systems. Two of these (the pyruvate dehydrogenase and α -ketoglutarate dehydrogenase) are key enzymes of the TCA cycle. The results of a double-blind, controlled, randomised Phase II/III trial of daily oral lipoic acid described a significant reduction in the annualised percentage change in brain volume, and suggested a clinical benefit in patients with secondary progressive MS, while maintaining favourable safety, tolerability, and compliance [87].

Teriflunomide (Aubagio, Genzyme Therapeutics) is the active metabolite of the pyrimidine synthesis inhibitor leflunomide. It selectively and reversibly inhibits dihydro-orotate dehydrogenase, a key mitochondrial enzyme in the *de novo* pyrimidine synthesis pathway, leading to a reduction in proliferation of activated T and B lymphocytes [88]. The results of the Teriflunomide Multiple Sclerosis Oral (TEMSO) double-blind, controlled, randomised Phase III trial described a

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significant reduction in the progression of disability (at the higher dose) [89] in patients with RR MS.

Finally, a periodic fasting-mimicking diet (FMD) has been proposed as an alternative approach to increase the protection of multiple systems in mice and possibly humans [90]. FMD ameliorates CNS damage and behavioural outcomes in EAE mice by increasing the corticosterone levels and regulatory T (T_{reg}) cell numbers and reducing the levels of proinflammatory cytokines, T_H1 and T_H17 cells, and APCs [91]. These effects are possibly related to an arrest of B and T cell development and concomitant selective localisation of mature B and T cell numbers in the bone marrow seen in fasting mice [92,93]. Preliminary results of the randomised, parallel-group, three-arm Pilot Trial to Test the Feasibility of Prolonged Fasting and Ketogenic Diet in Relapsing-remitting Multiple Sclerosis (IGEL) (ClinicalTrials.gov Identifier: NCT01538355) showed an increase in the β -hydroxybutyrate concentrations in plasma, a reduction in the number of autoimmune lymphocytes, and a reduction in relapses and expanded disability status scale in the FMD and ketogenic diet groups [91].

While the evidence that all the above therapeutics for MS interact with key metabolic and mitochondrial pathways is clear, whether these therapies also affect the metabolism of inflammatory MPs in progressive MS is yet to be elucidated.

Competition for Common Metabolic Substrates with Immune Cells via Exogenous Stem Cell Therapies

While the results for targeting mitochondrial metabolism in MS are exciting and metabolic modulators appear to be well tolerated and with minimal adverse effects, the delivery of these compounds to CNS-confined innate immune cells may be a challenge for the success of these therapies in progressive MS. In fact, the selective targeting of chronically activated MPs might be a crucial step to treat neuroinflammatory conditions in which inflammation is compartmentalised within the CNS and is coupled by neurodegeneration.

Researchers have attempted to solve this key issue by investigating how new experimental cellular medicines able to target the chronically inflamed brain may be beneficial for treating neuroinflammatory conditions in animal disease models [94].

Syngeneic neural stem cells (NSCs) injected through the blood stream (intravenously, IV), or locally into the CNS via the cerebrospinal fluid (CSF) circulation (intracerebroventricularly, ICV) in rodents and nonhuman primates with EAE express functional cell adhesion molecules (e.g., CD44), integrins (e.g., $\alpha 4\beta$ 1), and chemokine receptors (e.g., CCR2, CX3CR1, and CXCR4) to enter the inflamed CNS and selectively accumulate around perivascular spaces where inflammatory cells co-reside [95]. By establishing a range of cell-to-cell interactions, transplanted NSCs are able to: (i) induce the apoptosis of Th1 and Th17 T cells through Fas ligand (FasL), TNF-related apoptosis-inducing ligand (TRAIL), and Apo-3 ligand (APO3L); (ii) reduce the proliferation of T cells through NO and prostaglandin E2 (PGE2); and (iii) reduce the T cell receptor (TCR)-dependent activation of T cells through the inhibition of IL-2 and IL-6 signalling [95–104]. Thus, NSC grafts exert major immunomodulatory properties in neuroinflammation [105]. However, while much is known about the anti-inflammatory effects of grafted NSCs on the adaptive immune system, the molecular effect of NSC grafts on innate immune responses in the context of chronic neuroinflammation are not yet fully understood [106] (Figure 3).

To this aim, a multidisciplinary approach was recently developed that allowed the characterisation of the main mechanisms of chronic neuroinflammation, and the analysis of the metabolic

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Mononuclear phagocyte



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Figure 3. How Stem Cells Mediate Homeostatic Cell Competition with Mononuclear Phagocytes (MPs). The immune-modulatory properties of grafted neural stem cells (NSCs) on innate immune responses in the context of chronic neuroinflammation depend on the expression of chemokine receptors, cytokine receptors, and receptors for metabolites, which guide their pathotropism and response to inflammation. In the local inflammatory microenvironment, NSCs engage in a homeostatic cell competition with MPs by: (i) changing their own metabolism and increasing the secretion of extracellular arginase-1; (ii) releasing extracellular vesicles (EVs) that harbour functional metabolic enzymes; (iii) depleting the extracellular milieu of inflammatory immunometabolites, including succinate; and (iv) secreting a range of anti-inflammatory molecules. Abbreviations: Asrg11, asparaginase-like 1; BMP, bone morphogenetic protein; C, mitochondrial complex; CCR2, C-C chemokine receptor type 2; CX3CR1, C-X3-C motif chemokine receptor; HIF1-α, hypoxia-inducible factor-1 α ; HO-1, heme oxygenase-1; IFN, interferon; IFN-γR1, interferon γ receptor 1; IL-1 β , interleukin-1 β ; LIF, leukaemia inhibitory factor; mTOR, mammalian target of rapamycin; NF- $\kappa\beta$, nuclear factor- $\kappa\beta$; NO, nitric oxide; PGE2, prostaglandin E2; RET, reverse electron transport; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SLC, solute carrier; TGF, transforming growth factor; TN-C, tenascin-c; TNF- α , tumour necrosis factor- α ; VEGF, vascular endothelial growth factor.

determinants of the protracted and persistent activation of mouse MPs, both *in vitro* and *in vivo* in EAE [107]. Within the same study, the researchers also sought to understand to which extent experimental NSC therapeutics could interfere with the cellular metabolic processes responsible for chronic neuroinflammation. This work showed that the inflammatory metabolite succinate significantly increases in the CSF, but not in the peripheral blood, of mice with late-stage chronic EAE, indicating its compartmentalisation in the CNS. The intracerebroventricular transplantation of somatic or directly induced NSCs (iNSCs) had overlapping therapeutic and anti-inflammatory effects, which correlated with a striking reduction in the number of MPs in the CNS, and reduced the amount of succinate in the CSF only [107].

It was also demonstrated that transplanted NSCs were able to 'sense and respond to' the extracellular succinate released by MPs via GPR91. In fact, the activation of GPR91 on NSCs induced the release of anti-inflammatory PGE2 and promoted the scavenging of succinate with consequential anti-inflammatory effects. Finally, loss of *Gpr91* function in NSCs led to significantly reduced anti-inflammatory activities on proinflammatory BMDMs *in vitro* and *in vivo* after transplantation in EAE mice [107].

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NSCs are also able to secrete extracellular vesicles (EVs) that behave as fully independent metabolic units harbouring intact and functional metabolic enzymes (e.g., asparaginase-like 1 protein) capable of depleting the microenvironment (or the intracellular compartment of a target cell) of key metabolites used for the survival or proliferation of immune cells (e.g., asparagine for T cells) [108].

Along with the above intrinsic behaviours, NSCs can also change their intrinsic metabolism (i.e., arginine) in response to priming with inflammatory Th1 cytokines [interferon (IFN)- γ , TNF- α , and IL-1 β], and increase the secretion of extracellular arginase-1, which is responsible for the antiproliferative effects on lymph node cells [109]. Finally, NSC grafts also provide local paracrine support, via the *in situ* release of growth factors and cytokines [106], including TGF- β 2, inhibiting the differentiation and activation of inflammatory monocyte-derived cells (MCs) in the CNS of EAE mice, thus favouring their switch toward an anti-inflammatory phenotype [110].

Part drugs and part devices, NSCs work as naturally occurring disease-modifying agents endowed with a highly refined machinery thereby: (i) sensing diverse signals (including the extracellular metabolic and inflammatory milieu); (ii) migrating to specific sites in the body; (iii) integrating inputs to make decisions; and (iv) executing complex response behaviours (Figure 3) [111].

Altogether, these exciting findings are in line with the emerging concept of short-range intercellular communication within inflammatory microenvironments guiding cellular responses and neuroimmune interactions [112].

Most of the concepts behind this novel provocative perspective of homeostatic cell competition at the level of the stem cell graft-host environment niche are not fully unexpected. Cell competition is a well-established model in cancer, where tumour cells consume high levels of glucose and release lactate and the tryptophan-metabolising enzyme indoleamine 2,3-dioxygenase (IDO), to mediate local immunosuppression in the tumour microenvironment via the consumption of tryptophan and release of kynurenine [113,114]. Nonetheless, IFN- γ -mediated induction of IDO is recognised as the sole direct mechanism of human mesenchymal stem cell (MSC)-mediated suppression of adaptive immune responses both directly on T cells and through the differentiation of monocytes into M2-like immunosuppressive macrophages *in vitro* [115,116].

Evidence linking metabolism, mitochondrial dynamics, and proteostasis as key regulators of stem cell function is emerging [117]. Therefore, it is not completely unrealistic to speculate that a metabolic control of stemness confers grafted cells an intrinsic capacity of interfacing with the environment [118]. This also implies that biologically flexible stem cell grafts release diverse 'batches' of signalling molecules (e.g., short peptides, enzymes, nucleic acids, miRNAs, etc.) either secreted or packed into EVs with the potential of targeting different types of tissue, and largely accounts for the established therapeutic plasticity of a range of stem cell therapeutics across several tissues [106,119]. As such, the progressive exhaustion of NSCs within brain germinal niches, as a major component of brain aging and related diseases, is being proposed as a possible explanation of the emerging connection between metabolic dysfunction, anatomical and structural damage, depression, and other cognitive and mental health problems in the chronically inflamed brain [120,121].

The next challenge is to identify those metabolic processes and intracellular pathways with a role in neuroinflammation and to develop druggable therapeutic approaches that will recondition the chronically inflamed brain towards neuroprotection and tissue regeneration.

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Box 6. Clinician's Corner

Could standard protocols be produced for sampling and processing biological fluids (e.g., CSF, plasma and/or serum) to ensure that *ex vivo* metabolic studies from different cohorts of patients with MS are reproducible and unbiased?

Identification of a unique metabolic signature in biological fluids from patients with MS might help to predict disease progression and response to therapy in the future.

Therapies aimed at reducing inflammation in the CNS may have promise to block the accumulation of disability in patients with progressive MS.

Development of novel therapies that modulate mitochondrial metabolism in microglia/macrophages may also have potential for progressive MS.

Small molecules, cellular therapies, diet, or combination therapies are some of the ways to tamper with the metabolism of MPs in patients with progressive MS that should be investigated further to determine whether they have potential.

Concluding Remarks

Characterisation of the metabolic determinants of CNS-compartmentalised innate immune responses may help to identify a range of therapeutic targets to reduce the accumulation of irreversible disabilities in patients with progressive MS.

We believe that this novel wave of 'metabolic therapeutics' targeting the maladaptive metabolism of immune cells might help treat a range of neurological conditions in which the innate immune system becomes dysfunctional. However, important issues still need to be addressed (see Outstanding Questions) to ensure the successful application of these therapies in the clinic (Box 6). In addition, cell metabolism is highly fluid and adaptable, thus leading to several limitations intrinsic to the proposed hypothesis. First, it is hard to produce a snapshot of the metabolic processes of neuroinflammation in vivo without introducing experimental biases. Second, small molecules targeting cell metabolism might have ubiquitous effects, most of which are likely to be off-target effects, on both neuronal and non-neuronal cells. However, in pathological conditions in which specific cells adopt prevalent metabolic pathways (e.g., aerobic glycolysis in inflammation), the selectivity of compounds targeting these metabolic routes might be greatly increased (i.e., cellular selectivity based on metabolic demand). Lastly, it is also plausible that significant beneficial effects might be achieved only with combination therapies targeting (different and/or complementary) metabolic pathways, as well as other biological processes (e.g., neurodegeneration and failure of remyelination in the case of progressive MS). For example, potentiating oxidative phosphorylation and the TCA cycle might have dual beneficial effects in progressive MS by reverting energy deficiency in neurons [122], while counteracting the Warburg effect in MPs.

In this perspective, exogenous stem cell therapeutics may overcome most of these limitations for small molecules and biologics, being *de facto* capable of adapting their own phenotype and function, while secreting and uptaking multiple therapeutic small molecules in response to the local tissue microenvironment [111,112]. Therefore, a careful choice of the cellular sources (e.g., allogeneic versus autologous, CNS specific versus nonspecific) and delivery features (e.g., timing and route) must be made to balance the potential of cell replacement of damaged cells with the homeostatic modulation of MPs metabolism by the graft [105].

The final aim of these molecular and cellular therapies targeting mitochondrial immune cell metabolism will be to interrupt the persistent activation of innate immune responses in the CNS in a way that favours neuroprotection and ultimately promotes tissue regeneration.

Outstanding Questions

Is the persistent MP activation seen in chronic neuroinflammatory conditions a cause (outside-in model) or a result of the ongoing neurodegeneration (inside-out model)?

What is the role of MP metabolism in maintaining chronic neuroinflammation in progressive MS?

Given the sexual dimorphism in microglial function and dysfunction, and the sex bias in CNS disorders with microglial pathology, how will the development of MP-directed therapeutics differ to address pathways and targets that may differ in men and women?

What is the role of aging in the course of secondary progressive MS in the metabolism of MPs?

Is the region-dependent identity of MPs affecting their bioenergetic and immunoregulatory pathways in the brain?

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