Role of metabolic pathways and sensors in regulation of dendritic cell-driven T cell responses

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Metabolic control of type 2 immunity

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Review
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Abstract

Type 2 immune responses play key roles in protection against parasitic worm infections, whole-body metabolic homeostasis, wound healing, and the development of allergies. As a result, there is considerable interest in understanding the pathways that regulate type 2 immunity in order to identify strategies of targeting and controlling these responses. In recent years, it has become increasingly clear that the functional properties of immune cells, including those involved in type 2 immune responses, are dependent on the engagement of specific metabolic pathways such as aerobic glycolysis and fatty acid oxidation (FAO). We here discuss the latest insights in the metabolic regulation of immune cells that initiate type 2 immune responses, such as dendritic cells and innate lymphoid cells, as well as immune cells involved in the effector phase, like T helper 2 (Th2) cells, B cells and alternatively activated macrophages (M2 macrophages). Finally, we consider whether these findings may provide new prospects for the treatment of type 2 immune response-associated diseases.

Introduction

Type 2 immune responses are initiated by dendritic cells (DCs) that promote the differentiation of naïve $CD4^+$ T cells towards a T helper 2 (Th2) phenotype, which is characterized by the production of the prototypical type 2 cytokines: interleukin-4 (IL-4), IL-5, and IL-13 [1]. In some settings, type 2 innate lymphoid cells (ILC2s) and basophils contribute to this process by also presenting antigens to naïve T cells and by producing type 2 cytokines. IL-4, IL-5, and IL-13 play a central role in driving the humoral and cellular arms of type 2 immunity. IL-4 released in B-cell follicles by a specialized subset of Th2 cells, termed T follicular helper (Tfh) cells, promotes B cells to induce antibody class switching towards immunoglobulin E (IgE) as well as toward IgG4 in humans and IgG1 in mice [2]. Local release of IL-5 at the site of inflammation leads to the recruitment and activation of eosinophils which, similar to basophils and mast cells, release pre-formed granules with toxic proteins, histamine, and other vasoactive amines upon engagement of their high-affinity IgE receptors (FC epsilon receptor [FcεR]) by immune complexes of IgE [1]. In addition, these granulocytes produce bio-active lipids such as leukotrienes and prostaglandins, and a wide variety of cytokines. Finally, local release of both IL-4 and IL-13 induces the alternative activation of macrophages (M2 macrophages), a subset of macrophages that is known to assist in the killing of parasites, resolution of inflammation and repair of any tissue damage that has occurred [3]. However, these responses are also the main driver of allergic reactions that result in diseases such as asthma, rhinitis, and atopic dermatitis [1]. Given the vital contributions of each of these cells in the outcome of type 2 immune response-associated diseases, there is considerable interest in understanding the pathways that regulate their function in order to identify strategies of targeting and controlling type 2 immunity.

In recent years, it has become increasingly clear that immune cell differentiation, activation, proliferation, function, and longevity are underpinned by the engagement of specific core metabolic pathways. These core metabolic pathways include glycolysis, fatty acid synthesis, fatty acid oxidation (FAO), and mitochondrial oxidative phosphorylation (OXPHOS). For an introduction into these metabolic pathways we refer to a recent comprehensive review [4]. This has generated considerable enthusiasm for the rapidly expanding field of immunometabolism and has led to the emerging concept that the manipulation of immune cell metabolism could be a powerful and attractive tool to direct immune responses. In the context of type 2 immunity, the field of immunometabolism was in part ignited by a seminal study on macrophages in 2006 [5], which for the first time documented that M2 polarization of murine macrophages was supported by an increase in OXPHOS and FAO. This was

very different from what was seen in classically activated macrophages (M1 macrophages) that instead shifted toward aerobic glycolysis during their polarization [5]. Since then, and in particular very recently, considerable progress has been made in delineating the different metabolic programs shaping the function of the various immune cells involved in type 2 immune responses. In this review we will discuss the latest insights in the metabolic regulation of type 2 immune cell biology and in addition explore whether targeting metabolic pathways could hold promise as an approach to treat diseases associated with a deregulated type 2 immunity.

Metabolism of immune cells involved in the priming of type 2 immune responses

Th2-priming dendritic cells

It has been shown that profound changes in cellular metabolism are integral to the activation and overall T-cell priming ability of DCs [6, 7]. Specifically, triggering of toll-like receptors (TLRs) on DCs, which is generally associated with an enhanced ability to prime CD8, Th1, or Th17 responses, rapidly increases the flux of glucose through the glycolytic pathway. This leads to an increase in pentose phosphate pathway (PPP) activity and citrate metabolism, which are both necessary for fatty acid synthesis [8]. Synthesis of new fatty acids then allows for the expansion of the endoplasmic reticulum, which is likely to be required for the production of effector molecules that are central to DC activation. In addition to these short-term effects, long-term TLR signaling inhibits mitochondrial respiration in DCs [9, 10] and therefore increases their dependence on glycolysis for the production of ATP. However, to date, little is known about the role of DC metabolism in Th2 priming. Some indirect evidence that lipid metabolism plays a role in DC-driven Th2 polarization comes from a handful of studies in which peroxisome proliferator-activated receptor gamma (PPAR-γ), a master regulator of lipid metabolism, was targeted in DCs [11-13]. For example, suppression of PPAR-γ through sirtuin-1-mediated deacetylation was found to be required for the Th2-priming capacity of murine DCs [13]. Consistent with this, DCs stimulated with the PPAR-γ agonist rosiglitazone were shown to inhibit the development of eosinophilic airway inflammation in a mouse model of asthma [11]. However, this effect appears to be secondary to the induction of regulatory T cells, rather than an inability to induce Th2 responses when PPAR-γ is activated [11, 12]. More recently, adenosine monophosphate-activated protein kinase (AMPK) signaling has been linked to the ability of DCs to induce Th2 differentiation [14]. AMPK is a sensor of intracellular adenosine nucleotide levels that during conditions of decreased ATP availability promotes FAO, mitochondrial OXPHOS and other forms of catabolic metabolism to generate more ATP [15]. In a model of murine *Nippostrongylus brasiliensis*

infection, deletion of the AMPK alpha subunit in CD11c$^+$ cells resulted in the increased production of IL-12/23p40 by these cells and consequently, impaired type 2 immune responses and increased both worm burden and fecundity [14].

Finally, induction of Th2 polarization in mice was recently shown to be mediated by a specific subset of DCs [16], termed cDC2, which is dependent on the transcription factors KLF4 [16] and IRF4 [17] for its development. In myocytes, KLF4 has been implicated in mitochondrial biogenesis, mitophagy and the oxidation of both glucose and fatty acids [18], while IRF4 promotes the alternative activation of macrophages in an FAO-dependent manner [19]. Together, this makes it conceivable that Th2-priming DCs are characterized by, and rely on, a more oxidative-centered metabolism. However, evidence is still missing to support this. Future studies should focus on directly targeting the metabolic pathways involved in DC lipid and oxidative metabolism to more granularly define their importance for the Th2-priming capacity of DCs.

Type 2 innate lymphoid cells

So far, only a single study has explored the metabolic properties of ILC2s. Murine ILC2s were found to have both greater spare respiratory capacity (SRC) and glycolytic capacity than Th2 cells [20]. SRC is the extra capacity cells have to produce ATP via OXPHOS in response to stress and as such, is considered a marker for mitochondrial fitness. In addition, ILC2s, in contrast to other lymphocytes, were found to constitutively express high levels of arginase-1 (Arg1), an enzyme that converts argi- nine to urea and ornithine [20]. Importantly, Arg1 expression and enhanced bioenergetics appear to be functionally linked in ILC2s, since pharmacological inhibition of arginase with Nω-hydroxy- nor-arginine (nor-NOHA) was shown to reduce both maximal respiration and maximal glycolysis in these cells [20]. Although the mechanistic basis for this link was not explored, it is possible that ornithine derivatives could support OXPHOS. Ornithine can be converted into glutamate and subsequently into the TCA cycle-intermediate α-ketoglutarate (α-KG), which, if broken down completely in the TCA cycle, can generate 6.5 ATP molecules through OXPHOS [21] (Fig. 1A). Correspondingly, overexpression of arginase-2 in human bronchial epithelial cells increased mitochondrial oxygen consumption [22]. Inhibition of Arg1 in ILC2s also diminished their ability to produce ornithine-derived polyamines such as putrescine, spermidine, and spermine [20], which are known to be important for proliferation. In line with this, Arg1-defective ILC2s showed decreased proliferative potential [20]. Importantly, in this study, ILC-intrinsic deletion of Arg1 was found to diminish pathology in multiple murine models of lung inflammation [20], suggesting that Arg1 through regulation of cellular bioenergetics and polyamine synthesis underpins ILC2s function and thereby disease outcome. However, no role for Arg1 in ILC2 function or

outcome of lung inflammation was found in another murine study [23]. This may possibly be explained by the fact that these studies used different approaches to conditionally delete Arg1 from ILC2s. Additional studies would be needed to reconcile these contradictory results and to definitively determine the link between Arg1, cellular metabolism, and ILC2 function.

ILC2s are considered the innate counterpart to Th2 cells as they share the ability to produce IL-5 and IL-13, and depend on the transcription factor GATA3 for their development. From a metabolic standpoint, this similarity may be extended to their shared requirement for mammalian target of rapamycin (mTOR) signaling in regulating their effector function. mTOR is a kinase that forms the core of two different complexes: mTORC1 and mTORC2, both of which promote anabolic metabolism, but also have distinct functions. mTORC1, of which Raptor is a key component, is known for its involvement in cell growth, while mTORC2, with Rictor as a key component, promotes cell survival and proliferation. The prototypical mTORC1 inhibitor rapamycin was shown to block murine ILC2 accumulation after intranasal administration of IL-33 and to diminish their production of IL-5 and IL-13 after restimulation with IL-33 ex vivo. This suggests that mTORC1 signaling is involved in murine ILC2 function [24] (Fig 1A). However, ILC2s are not unique among murine ILCs in their dependence on mTOR signaling, as ILC1s and ILC3s are almost completely absent in Nkp46-Mtor$^{-/-}$ mice [25]. Whether mTOR signaling is crucial for the development of ILCs from their progenitor, the common lymphoid progenitor (CLP), for mature ILC survival or both is still unclear. Rapamycin was found to skew the differentiation of ex vivo murine haematopoietic stem cells away from the CLP toward the common myeloid progenitor [26]. However, conditional deletion of mTOR in interferon-alpha-responsive murine cells increased the number of CLPs [27] and it was already known for a longer time that mouse models with constitutively active mTORC1 signaling have lower numbers of CLPs [28].

Altogether, these studies suggest that ILC2s, similar to other immune cells, depend on mTOR signaling for their development and function, but that they also may have a unique dependency on Arg1 to shape their metabolic profile and thereby their functional properties.

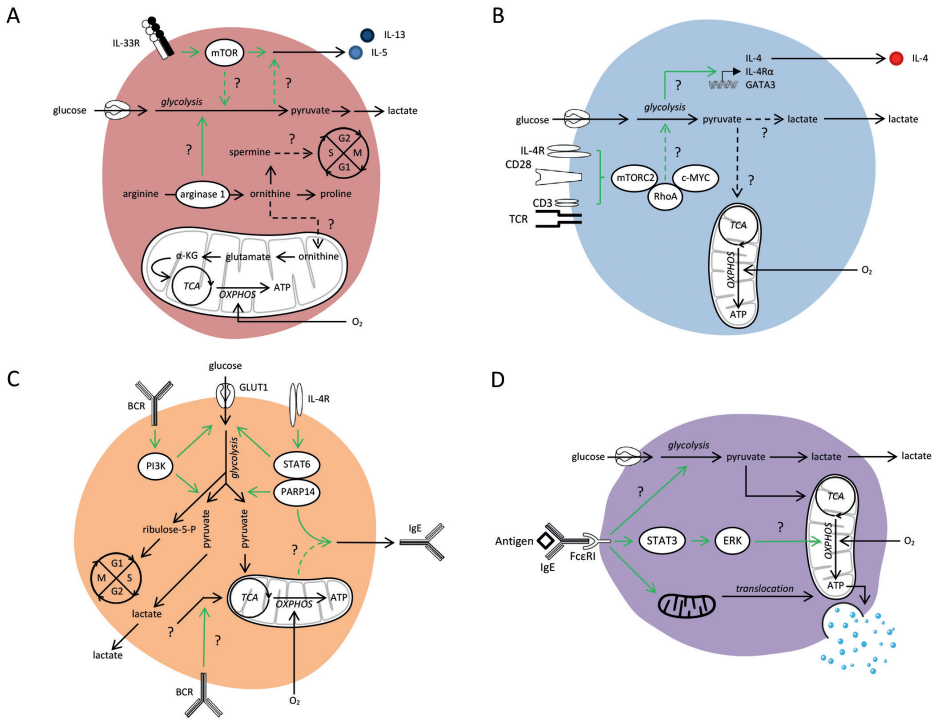

Figure 1. Metabolic characteristics of (A) ILC2s, (B) Th2 cells, (C) IgE-secreting B cells and (D) mast cells.

Proteins such as transcription factors and enzymes are circled. Green lines indicate signaling pathways, while black lines illustrate metabolic pathways. Lines with question marks represent not fully elucidated links.

Metabolism of cells involved in the effector phase of type 2 immune responses

T helper 2 cells

The relevance of cellular metabolism in T-cell activation, differentiation, and memory formation has been extensively studied in $CD8^+$ T cells (for a recent review see [29]). This significance is now also being unravelled for Th2 cells. In vitro-polarized murine Th2 cells show strongly enhanced glycolytic rates [30, 31]. Functionally, inhibition of glycolysis using 2-deoxyglucose (2-DG) diminished the expression of the transcription factor GATA3 [32], lowered the expression of the IL-4 receptor α chain (IL-4Rα) [32, 33] and blocked the production of IL-4 [32, 33] under Th2-polarizing conditions. Lowering glucose levels in the medium had a similar effect on IL-4Rα expression [33]. In contrast, inhibition of glycolysis did not affect the production of IFN-γ under Th1- polarizing conditions [32], suggesting that among Th cells, Th2 cells

have a unique dependency on glycolysis for their effector function. In line with this, glycolytic rates seem to be higher in Th2 cells than Th1 and Th17 cells [30, 31]. The fate of glucose-derived pyruvate in these settings is yet to be elucidated, but the finding that Th2 cells have a higher mitochondrial oxygen consumption rate than Th1 cells [32], may indicate that pyruvate in Th2 cells is preferentially oxidized in the mitochondria to support OXPHOS. Moreover, Th2 cells from asthmatic individuals were shown to have a higher expression of carnitine palmitoyltransferase I isoform a (CPT1a), a transporter involved in the mitochondrial import of long-chain fatty acids, compared to Th2 cells from healthy controls [34]. This suggests that FAO-driven OXPHOS may also facilitate the effector functions of Th2 cells (Fig. 1B).

When looking for upstream metabolic regulators that are potentially involved in these Th2-specific metabolic adaptations, multiple studies support a role for mTORC2 signaling in the preferential differentiation of naïve T cells toward Th2 cells [32, 35-37]. For instance, conditional deletion of the GTPase RhoA, a downstream target of mTORC2, decreased glycolysis in, and IL-4 production by Th2 cells, while sparing glycolysis in, and IFN-γ production by Th1 cells [32]. Correspondingly, mice with a T-cell-specific deletion of RhoA were protected against the development of allergic asthma [32]. How RhoA promotes glycolysis remains to be determined. However, since RhoA is known to promote expression of transcription factor c-MYC [38], a master regulator of glycolysis [39], and expression of c-MYC in human primary Th2 cells was found to be positively associated with the allergic status of the individuals these cells were isolated from [34], makes it interesting to speculate that mTORC2 through activation of RhoA and c-MYC specifically controls the glycolytic reprogramming and effector function of Th2 cells, but not that of Th1 and Th17 cells (Fig. 1B).

From these studies a picture is emerging that mTORC2-driven glycolytic reprogramming plays a central role in supporting the differentiation and function of Th2 cells. It remains to be seen whether these findings are relevant for Tfh cells in the context of type 2 immune responses, as the metabolic properties of Tfh cells have only been studied during viral infection. In this setting, Tfh cells were found to have enhanced glycolytic rates compared to naïve $CD4^+$ T cells [40], but lower overall metabolic rates than Th1 cells [41].

B cells

Currently, no studies have specifically addressed whether there are metabolic requirements for the production of IgE. However, it is known that B-cell receptor (BCR) activation by cognate antigen induces surface glucose transporter 1 (GLUT1) expression and glucose uptake in murine splenic B cells in a phosphoinositide 3-kinase (PI3K)-dependent manner [42] (Fig. 1C). In this

setting, large amounts of lactate are produced and glucose-derived carbons end up in the PPP. The PPP is an alternative glucose-fuelled metabolic pathway that branches off from glycolysis and serves to generate nicotinamide adenine dinucleotide phosphate (NAPDH) to support fatty acid synthesis and redox balance, and ribose-5-phosphate to support nucleotide synthesis. These nucleotides are needed for the DNA replication that occurs within 31–48 h after BCR activation [43]. This suggests that B-cell activation, similar to T-cell activation, results in increased aerobic glycolysis and PPP activity to support cellular proliferation. T-cell activation also increases the expression of amino acid transporters [29] and in line with this, B-cell activation also increases the expression of the small subunit 1 of the large neutral amino acids transporter (Slc7a5) and correspondingly increases leucine uptake [44]. However, in contrast to activated T cells, activated B cells maintain a balanced glycolysis/OXPHOS ratio [45]. If BCR activation is associated with aerobic glycolysis as described above, then likely other macromolecules such fatty acids or amino acids are needed to fuel the TCA cycle to accompany the observed increase in OXPHOS. However, the necessity for OXPHOS for BCR-induced B-cell activation is still uncertain and will require further research (Fig. 1C).

The main cytokine regulating the type 2 immune response-associated isotype switching is IL-4. Similar to BCR activation, IL-4 was found to induce surface GLUT1 expression, glucose uptake and glycolytic flux in murine splenic B cells [46, 47]. However, in contrast to BCR activation, IL-4 also induced the expression of citrate synthase, the first enzyme in the TCA cycle, and correspondingly increased glucose oxidation [46]. These effects were found to be dependent on STAT6-PARP14 [46] (Fig. 1C) and deletion of either proteins decreased the IgE production by IL-4-stimulated B cells [48]. Of note, while IL-4 increased glucose oxidation, FAO was unaffected [44].

Taken together, these studies suggest that B-cell activation and subsequent proliferation initially depend on aerobic glycolysis to support the increased activity of biosynthetic pathways such as the PPP. In addition, when exposed to IL-4, B cells might divert a part of the glucose toward OXPHOS to aid in IgE class switching. A first hint that the switching toward different antibody isotypes requires the engagement of distinct metabolic programs comes from one study, which reported that the inhibition of OXPHOS using rotenone had no effect on the production of IgM by murine splenic B cells that were exposed to LPS [45]. However, this awaits to be formally tested in more detailed metabolic studies.

Mast cells, basophils and eosinophils

The majority of studies dedicated to the cellular metabolism of granulocytic type 2 immune cells have focused on mast cells. Murine bone marrow-derived mast cells (BMMCs) that were sensitized with complexed IgE and then stimulated with antigen showed increased OXPHOS and glycolytic capacity [49]. The importance of glycolysis in mast cell effector function was supported by the finding that removing glucose from the media decreased histamine release by primary rat mast cells [50]. Increasing glucose oxidation at the expense of lactate production using the pyruvate dehydrogenase kinase inhibitor dicholoroacetate (DCA) was shown to decrease degranulation and IL-6 production by BMMCs [49], making it imaginable that during mast cell activation especially the rapid production of ATP that aerobic glycolysis can provide is important. However, this finding is hard to unify with the limited effect of 2-DG on these parameters in the same study.

In contrast, there appears to be a more coherent picture regarding the role of mitochondrial metabolism in mast cell effector functions. Human mast cells require the translocation of mitochondria into exocytosis sites for degranulation [51] (Fig. 1C). Loss of IgE/Ag-induced oxygen consumption by inhibiting STAT3 or ERK was associated with an abolishment of β-hexosaminidase release and TNF-α production in both BMMCs and rat basophilic leukaemia (RBL) cells, a model for mast cells [52]. Finally, direct inhibition of OXPHOS using rotenone blocked degranulation and lowered IL-6 production in BMMCs [49]. It is likely that OXPHOS fuelled by glucose oxidation rather than FAO is required for mast cell effector functions, as inhibition of pyruvate dehydrogenase (PDH) with CPI-613 lowered β-hexosaminidase release and cytokine production in RBL cells and human cord blood-derived mast cells [53], while blockade of FAO using the CTP1a inhibitor etomoxir did not affect mast cell functions [49]. As a whole, these studies suggest that glucose-fuelled OXPHOS is important for mast cell function. However, the role of glycolysis in mast cell biology warrants further investigation due to conflicting results from different studies (Fig. 1D).

The metabolic properties and requirements for basophil function are relatively unknown. Sumbayev and colleagues found that FcεR triggering on primary human basophils resulted in stabilization of hypoxia-inducible factor 1 alpha (HIF-1α) [54], which promotes the expression of genes involved in glycolysis [55]. Although silencing of HIF-1α in these cells impaired IgE-driven IL-4 release, it remains unclear which other effector functions are HIF-1α-dependent and whether these effects are secondary to a reduced glycolytic capacity.

Finally, there is a single study published in 1977 that interrogated the role of cellular metabolism in eosinophil biology [56]. David and colleagues reported that glycolysis, but not OXPHOS, was important for antibody-dependent, eosinophil-mediated damage of *Schistosoma mansoni* schistosomula. However, through which mechanism glycolysis underpins this effector function of eosinophils was not addressed and warrants further detailed analysis by taking advantage of more modern experimental techniques.

Collectively, this may suggest that basophils and eosinophils, similar to other granule-releasing immune cells, such as $CD8^+$ cytotoxic T lymphocytes, rely on glycolytic metabolism for the effective release of cytokines and enzyme containing granules [57]. In contrast, mast cells appear to primarily depend on mitochondrial respiration for this process. Interestingly, mast cells, as opposed to basophils or eosinophils, are long-lived cells and longevity at the cellular as well at the organismal level is known to be strongly supported by mitochondrial OXPHOS [58]. In this light, it is tempting to speculate that mast cells rely on oxidative metabolism for long-term survival and have come to co-opt this longevity-associated metabolic makeup to also support their effector functions.

Alternatively activated macrophages

The role of cellular metabolism in immune cell biology has been studied most extensively in macrophages. Already more than a decade ago, it was observed that M1 and M2 macrophages have very distinct metabolic properties on which they depend for their polarization. This has been reviewed in detail elsewhere [59] and we will here highlight only the most recent insights in M2 macrophage metabolism. It is well known that M2 macrophages are metabolically characterized by an increased expression of genes involved in FAO and OXPHOS, fatty acid uptake, mitochondrial mass and FAO-dependent mitochondrial oxygen consumption [5, 60]. Moreover, inhibition of fatty acid uptake by deleting CD36 [60], inhibition of lipolysis using Orlistat [60] or anti-lysosomal acid lipase (LAL) short hairpin RNA [60], and inhibition of FAO using etomoxir [5, 60] were all shown to prevent murine M2 polarization, while having little or no effect on M1 polarization. These murine data are supported by the finding that IL-4-stimulated human monocyte-derived macrophages (MDMs) from CD36-deficient patients have lower SRC and lower expression of the mannose receptor 1 than MDMs from a healthy control [60]. Together, these observations led to the initial concept that M2 polarization is solely fuelled by FAO and mitochondrial OXPHOS, thereby contrasting with the metabolic requirements for the polarization of M1 macrophages, which depends on a strong commitment to aerobic glycolysis.

However, more recently, several studies challenged this view and provide support for the concept that in addition to FAO-fuelled OXPHOS, glycolysis has also an important role in the alternative activation of macrophages. Murine M2 macrophages have an increased expression of genes involved in glycolysis [19], enhanced glycolytic capacity [19, 61] and glucose uptake [5, 19, 62] in comparison to unpolarized macrophages. Inhibition of glycolysis using 2-DG [19, 61, 63], or specifically glucose oxidation using UK5099 [19] or anti-mitochondrial pyruvate carrier 1 short hairpin RNA [19] all suppressed the IL-4-induced increase in mitochondrial oxygen consumption and expression of M2 markers such as RELMα [19] and Arg1 [61, 63]. Surprisingly, inhibition of fatty acid synthesis using TOFA mimicked these effects [19]. Based on these data, a model has been proposed in which glucose-derived carbons enter the TCA cycle to support mitochondrial citrate synthesis that is later used, following conversion into acetyl-CoA, for cytosolic fatty acid synthesis. Subsequently, these fatty acids can then be used for FAO-driven mitochondrial OXPHOS (Fig. 2, center). A similar atypical pathway has been suggested to operate in memory $CD8^+$ T cells [64]. It is possible that de novo fatty acid synthesis is required to produce ligands for lipid-dependent nuclear receptors such as PPARs [65-68] or contribute to ER stress [69, 70], which are both known to play a role in M2 polarization. However, this still remains to be addressed. In addition, it was recently shown that for the alternative activation of macrophages glycolysis-driven synthesis of acetyl-CoA is not only important through its use as a substrate for fatty acid synthesis, but also because it is used for histone acetylation and thereby epigenetic regulation of M2 marker gene expression [62] (Fig. 2, top right). Finally, glucose tracing studies in M2 macrophages have revealed that there is an increased flux of glucose carbons into the hexosamine biosynthesis pathway that, in combination with glutamine, generates UDP-GlcNAc, a sugar-donor required for protein and lipid O-and N-glycosylation (Fig. 2, bottom). Functionally this appears to be important, as inhibiting N-linked glycosylation using tunicamycin was shown to decrease M2 polarization [71], although the exact mechanism underlying this phenotype is still unclear.

More evidence for a role of glycolysis in the alternative activation of macrophages comes from studies investigating the role of mTORC1 and mTORC2 signaling pathways during M2 polarization. Both mTORC1 and mTORC2 are known to support glycolytic reprogramming [55] and in agreement with a role of glycolysis in the alternative activation of macrophages, both Raptor [62] and Rictor [19] were found to be crucial for the expression of a subset of M2 genes. Deletion of Rictor in macrophages (LysM-Cre) inhibited the increase in glycolysis that is associated with IL-4 stimulation and inhibited the concomitant increase in FAO-dependent mitochondrial oxygen consumption [19]. These mTORC2-driven metabolic changes were shown to be dependent on PI3K, AKT and IRF4.

Corresponding with a known role for M2 macrophages in the killing of parasites, tumor growth and adaptive thermogenesis, in LysM-Rictor$^{-/-}$ mice, infections with *Heligmosomoides polygyrus* [19] and *N. brasiliensis* [72] were more persistent, melanoma cells grew more slowly [19] and core body temperature was not maintained during cold challenge [72]. mTORC1 may contribute to M2 polarization through different mechanisms than mTORC2. Deletion of Raptor in macrophages inhibited the expression and IL-4-mediated activating-phosphorylation of ATP citrate lyase (Acly) [62], the cytosolic enzyme that converts citrate into acetyl-CoA. The increased availability of cytosolic acetyl-CoA during M2 polarization was found to facilitate histone acetylation of M2 genes and thereby to promote their expression [62]. This mTORC1-driven epigenetic change was shown to be dependent on AKT by operating upstream of mTORC1. Collectively, these studies suggest that both mTORC1 and mTORC2 signaling pathways are required for the alternative activation of macrophages, by each executing distinct parts of the metabolic program that supports M2 polarization. Of note, in addition to mTORC1 and mTORC2 co-operation, there is also some evidence for counter-regulation. Raptor-deficient M2 macrophages showed increased activity of N-myc downstream regulated gene 1 [19], a down-stream target of mTORC2, and conversely, macrophages with hyper-active mTORC1 signaling showed enhanced M1 polarization at the expense of M2 polarization [73]. This suggests that both mTORC1 and mTORC2 signaling pathways are active in M2 macrophages, but their activity needs to be tightly regulated for optimal M2 polarization [19].

Finally, there is a recent study that questions the importance of FAO in M2 polarization altogether. Murine macrophages that are deficient in CPT2, which in conjunction with CPT1 shuttles long-chain fatty acid into mitochondria, could still be polarized toward an M2 phenotype [74]. Moreover, the effect of the CPT1a inhibitor etomoxir on M2 polarization was still present, suggesting that etomoxir might have off-target effects [74]. This highlights the need for re-evaluation of some of the results derived from experiments in which only etomoxir was used to assess the role of FAO in M2 polarization and immune cell metabolism in general.

As a whole, these results illustrate that the role of FAO in M2 polarization is not as straightforward as thought and uncovered a previously unappreciated, but equally important role for glycolysis in the alternative activation of macrophages. Moreover, the observation that the difference between M1 and M2 macrophages in terms of metabolic phenotype is less distinct in human compared to murine macrophages [75], may point toward quantitative and possibly qualitative differences between the two species. More detailed comparative studies would be needed to carefully address these issues.

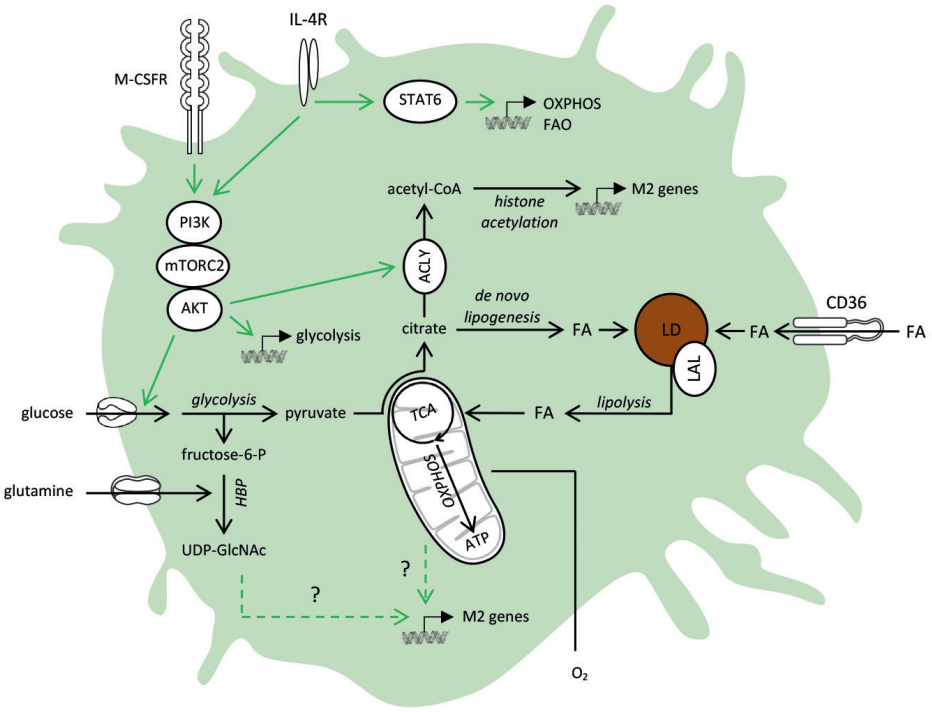

Figure 2. Metabolic pathways that support M2 macrophage polarization.

Proteins such as tran- scription factors and enzymes are circled. Green lines indicate signaling pathways, while black lines illustrate metabolic pathways. Lines with question marks represent not fully elucidated links.

Conclusion and future perspective

The burgeoning field of immunometabolism has generated many exciting new insights into how cellular metabolic pathways shape the function of immune cells, including those that are involved in type 2 immune responses. As many of the studies discussed in this review illustrate, it becomes increasingly clear that many type 2 immune cells (e.g. cDC2s, ILC2s, Th2 cells, and M2 macrophages) are characterized by, and are depend on, different metabolic programs than their type 1 immune cell counterparts (e.g. cDC1s, ILC1s, Th1 cells, and M1 macrophages). Moreover, several immune cells within the type 2 immune response network (i.e. Th2 cells [32], M2 macrophages [19, 61, 63], ILC2s [20], mast cells [49] and IL-4-stimulated B cells [46, 47] appear to share a common metabolic signature that is characterized by increased glycolysis as well as a concomitant higher mitochondrial oxygen consumption. Exceptions to this rule seem to be basophils and eosinophils, which are characterized by a more glycolysis-centered metabolism.

It remains to be determined why several type 2 immune cells seem to have a metabolic profile consisting of both increased glycolysis and OXPHOS. It is known that IL-4, and to a lesser extent IL-13, can induce these metabolic changes in immune cells. Given that most of these immune cells are exposed to IL-4 and IL-13 during type 2 immune responses, one could speculate that this profile is in part a reflection of metabolic imprinting by these cytokines. The ability to effectively engage both metabolic pathways may endow type 2 immune cells with the metabolic flexibility to use various substrates to meet their bioenergetics needs. This might allow them to maintain their effector functions during local fluctuations in nutrient availability. However, why this would be more important for type 2 immune cells than other types of immune cells is unclear. For M2 macrophages one could envision that an increased ability to oxidize lipids may be particularly important to perform their role in maintaining adipose tissues metabolic homeostasis and the repair of damaged tissues. In both situations, fat cells and dead cells release large quantities of lipids that need to be efficiently broken down by macrophages to prevent lipotoxiticy.

The observation that type 2 immune cells have metabolic properties distinct from each other, as well as from other types of immune cells, provides a potential window of opportunity for the metabolism-based treatment of type 2 immune response-associated diseases. Consistent with an important role of glycolysis in eosinophil and Th2-cell function, inhibiting the first step in glycolysis using 2-DG reduced the number of eosinophils and the levels of Th2 cytokines in the bronchoalveolar (BAL) fluid of mice with OVA-induced AAI, without affecting the infiltration of other immune cells and the production of IFN-γ, IL-17, and TGF- β [31, 57, 76]. In the same model, blocking the entry of glycolysis-derived pyruvate into the TCA cycle using CPI-613 decreased air-way resistance, which was associated with a reduction in the release of histamine by mast cells. However, CPI-613 also promoted the infiltration of eosinophils, macrophages, neutrophils, and lymphocytes into the BAL fluid [53]. Finally, the finding that ILC2s might rely on arginine metabolism for their function sheds interesting new mechanistic light on the long-appreciated beneficial effects of the arginase inhibitor nor-NOHA on asthma pathophysiology [77]. In line with the notion that nor-NOHA specifically targets Arg1 in ILC2s to reduce asthma, macrophage-intrinsic deletion of Arg1 did not impact pathology in murine models of air- way inflammation [20, 78, 79]. These studies provide interesting proof of principle that metabolic targeting of type 2 immune cells could hold promise as strategies to manipulate type 2 immunity for therapeutic gain. However, they also illustrate that targeting specific metabolic pathways in a limited number of cell types is necessary to prevent unwanted side-effects. More translational studies will be needed to address these challenges in the near future.

Most studies thus far have focused on core metabolic pathways to address the role of metabolism in type 2 immune cells. In addition, these studies have often been based on in vitro models which use culture conditions that may not necessarily mirror the metabolic microenvironment type 2 immune cells are exposed to in vivo. For instance, a high dependence on glycolysis may reflect more the adaptation to high glucose levels in the culture media rather than representing the natural condition. The recent development of more sensitive high-throughput metabolomics, proteomics and transcriptomics techniques in conjunction with more advanced mathematical and statistical modeling, will make it possible to characterize the metabolic properties and requirements of immune cells in unprecedented detail, both in vitro as well as in situ. These kinds of approaches will help to identify new metabolic pathways in type 2 immune cells that are unique for certain cells or effector functions and that then could be considered as novel therapeutic targets for the regulation of type 2 immunity. As such, we believe that this field can provide important contributions to the development of new treatment strategies that are needed to battle the sharp rise in type 2 immune response-associated diseases throughout the world, such as allergies and type 2 diabetes that are caused by either an overzealous or defective type 2 immunity respectively.

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Conflict of interest
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