Metabolic programming in dendritic cells tailors immune responses and homeostasis

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It is being increasingly acknowledged that immune cells depend on certain metabolic traits to perform their functions and that the extracellular environment can influence cell metabolism and vice versa. Dendritic cell (DC) subsets traffic through highly diverse environments from the bone marrow, where they develop, to the various peripheral tissues, where they differentiate and capture antigens, before they migrate to the lymph node to present antigens and prime T cells. It is plausible that DC subsets modulate their stimulatory abilities in response to unique metabolic programming. The metabolic requirements of DCs are just recently being discovered, and subset- and context-specific metabolic phenotypes in DCs are highly intertwined with DC functions. In this review, we present the current knowledge on the intrinsic and extrinsic determinants of DC metabolism, how they regulate DC function with examples from tumor biology and in interaction with the microbiota, and discuss how this can be applied therapeutically.

Keywords: Dendritic cells; Immunometabolism; Homeostasis; Tumor immunity; Microbiota

Cellular & Molecular Immunology (2022) 19:370–383; https://doi.org/10.1038/s41423-021-00753-1

INTRODUCTION

Dendritic cells (DCs) are critical initiators of immune responses to infections or cancer and are vital for maintaining tolerance during homeostasis. DCs are the most useful of antigen presenting cells (APCs) in terms of their ability to migrate to lymph nodes and prime naïve T cells. In addition, DCs secrete cytokines and chemokines to drive proper immune responses. Cell metabolism is being recognized as a conserved regulatory circuit that tailors immune cell function and survival. DCs likewise rely on tightly regulated metabolism to launch an immune response or promote tolerance; however, due to the limitations of in vitro models and the scarcity of DCs in accessible tissues, there are gaps in our understanding of DC metabolism that remain to be filled.

General DC biology

The most common DCs are conventional DCs (cDCs) and plasmacytoid DCs (pDCs). In addition, epidermal-resident Langerhans cells and monocyte-derived DCs (moDCs) arising from circulating monocytes in the blood are considered DC subgroups. cDCs can be subdivided into cDC1s and cDC2s, which are developed through distinct transcriptional programs [1]. Human and murine DC subsets are highly similar in transcription factor dependency and subset development but have unique surface markers. Human cDC1s and cDC2s are distinguished by their surface expression of CD141 and CD1c, respectively. Mouse cDC1s are recognized by the surface expression of XCR1, CLEC9A, and CDBox (lymphoid-resident) and/or CD103 (migratory), whereas CD11b+ SIRPa+ cDC2s only express CD103 in nonlymphoid tissues [2] (Table 1). Studies on conventional DCs are generally limited to murine DCs since access to human DCs is largely restricted to peripheral blood mononuclear cell (PBMC)-derived DCs. As such, this review is mostly based on findings from murine studies and will specify when reporting findings from humans. cDC1s and cDC2s exhibit different functional properties. cDC1s excel at activating CD8+ T cells by presenting exogenous antigen on major histocompatibility complex class I (MHC-I) in a process called cross-presentation. cDC2s, on the other hand, predominantly present antigens on MHCII, activating CD4+ T cells, and pDCs are specialized secretory cells that produce large amounts of type I interferons (IFN-I) promoting the response to viral infections (Table 1).

Metabolic changes and requirements during DC development

cDCs arise from hematopoietic stem cell-derived common myeloid progenitors (CMPs) that develop into common macrophages and DC progenitors (MDPs). MDPs give rise to restricted DC progenitors (CDPs) and common monocyte precursors (cMOPs) in the bone marrow. cMOPs differentiate into monocytes, which in tissues can generate DCs under certain conditions, including in cancer. CDPs can commit to either pre-cDC1 or pre-cDC2 that enter the circulation. Pre-DCs become immature DCs in the periphery, where they subsequently mature after activation by inflammatory stimuli (Fig. 1). pDCs can develop from both CDPs and common lymphoid progenitor cells [3]. However, by using single-cell technologies, researchers have recently revealed that lymphoid and myeloid-derived pDCs have distinct functional and transcriptional profiles despite similar phenotypic markers [4, 5]. Hence, our current categorization of DC subsets might be too...
|                      | cDC1                                | cDC2                                | pDC                                | moDC                                |
|----------------------|-------------------------------------|-------------------------------------|------------------------------------|-------------------------------------|
| **Main function**    | Antigen cross-presentation to CD8⁺ T cells, IL-12, IL-6 secretion | Direct antigen-presentation to CD4⁺ T cells, IL-6, TNF-α, IL-23 secretion | IFN-I production                     | Antigen presentation and TNF-α, IL-12, IL-23 secretion |
| **Transcription factors** | Batf3, Irf8, Id2                | Irf4, Notch2, Klf4                  | Irf8, E2-2, Irf7                    | Klf4, Irf8                            |
| **Surface markers (mouse)** | CD11c, MHCII, XCR1, CLEC9A, CD24, DEC205 CD8α (resident), CD103 (migratory) | CD11c, MHCII, SIRPs, CD11b         | CD11c, MHCI, SIRPs, PDCA1, Siglec H, B220, CD209, SIRPα | CD11c, MHCII, CD11b, Ly6C,CD14, CCR2 |
| **Surface markers (human)** | CD11c, MHCI, XCR1, CLEC9A, CD141, DEC205 | CD11c, MHCII, SIRPs, CD1c,         | MHCI⁺, CD123, CLEC4C, CD304, CCR2   | CD11c, MHCI, CD11b CD14, CD1c, CD64, CD206, CD209, SIRPs, CD1a, CCR2 |
| **Development and differentiation** | PI3K/Akt and mTOR is required for Flt3L-induced DC development abc [10] AMPK-KO or FAO-inhibition increase cDC2/cDC1 abc [11] Mst1/Mst2 deficiency impair Flt3L-expansion of splenic cDC1 abc [12] | ROS-inhibition increases cDC1/cDC2 abc [11] | PI3K/Akt and mTOR is required for Flt3L-induced DC development abc [10] | Increased mitochondrial activity and ROS is required for differentiation into moDCs abc [17] Upregulated mitochondrial biogenesis genes PGC-1α, NRF-1, TFAM abc [18] Constitutive active PI3K/mTOR signaling abc [16] |
| **Steady-state** | OXPHOS⁺ Glycolysis⁺ Glycogen storage⁺ [11, 12, 19] | OXPHOS⁻ Glycolysis⁻ Glycogen storage⁻ [11, 12, 19] | Glycogen storage⁻ [167] | Glycogen storage⁻ [167] |
| **Early Activation** | PRR stimulation induces TBK1/IKKε/Akt-dependent glycolysis and FAS ab [19, 20, 22, 23] Glycolysis and FAS are required for DC maturation c [19, 23] Glycolysis is required for DC migration abc [21] Glycogenolysis fuels glycolysis ab [167] | TLR induced glutaminolysis-driven OXPHOS required for pDC IFN-I and co-stimulatory molecules ab [36] IFN-I-induced FAO enhance autoimmune IFN-I and pDC function ab [28] Stimuli-dependent reliance on glycolysis for IFN-I production ab [35] mTOR-dependent RLR-induced IFN-I production ab [47] | PRR stimulation induces glycolysis and INOS expression bc [23, 24] Glycogenolysis fuels glycolysis ab [167] mTOR-dependent PRR activation ab [47] |
| **Mature DC** | Glucose-deprivation 8 h after LPS enhance CD8⁺ T cell priming ab [44] Mst1/2-dependent IL-12 production and CD8⁺ T cell priming ab [12] Lipid bodies promote antigen cross presentation ab [79, 80] Lipid overload diminishes antigen uptake ab [32, 33] NOX2-produced ROS required for antigen processing and CD8⁺ T cell priming ab [82, 83] | Contrasting data mTORC1 blocks MHCI expression and antigen uptake ab [42, 69, 70] mTORC1 facilitates MHCI peptide loading ab [71, 72] | Glycogenolysis and iNOS required for survival ab [24] mTOR required for antigen uptake ab [45] |

- ab In vitro in Flt3L-differentiated BMDCs
- bc In vitro in GM-DCs
- c Ex vivo in vivo splenic/lymphoid DCs
- ab In vitro human GM-CSF-IL-4 differentiated moDCs
- a Ex vivo human circulating pDCs
- ab Ex vivo in vivo tumor-infiltrating DCs (TIDCs)
Commitment to CMP induces Fms-like tyrosine kinase 3 (Flt3) on the cell surface, which drives DC development. Flt3 ligand (Flt3L) is required for homeostatic DC development, evident from the profound reduction of DCs in Flt3L-deficient mice [9]. Moreover, Flt3L treatment can expand the DC population, which is exploited for in vitro studies and is proposed to have therapeutic prospects, as discussed below. Flt3L-supplemented bone marrow cultures have revealed that Flt3L drives DC development by increasing anabolism, proliferation and differentiation in a phosphoinositide 3-kinase (PI3K)- and mammalian target of rapamycin (mTOR)-dependent manner [10, 11]. Pre-cDC1s and pre-cDC2s become immature cDC1s and cDC2s with distinct transcriptional profiles, but until recently, little was known about the metabolic differences of cDC1s and cDC2s. Flt3L-derived cDC1s have higher mitochondrial content and membrane potential than cDC2s [11], and analysis of splenic CD8α+ cDC1s showed that they have higher oxidative phosphorylation (OXPHOS) than their CD8α− counterparts [12], underscoring that cDC1s and cDC2s are metabolically different even when developed in vivo. Interestingly, inhibition of AMP-activated kinase (AMPK) or fatty acid oxidation (FAO) during Flt3L-induced differentiation of bone marrow-derived DCs (BMDCs) has been shown to promote cDC2 differentiation, whereas inhibition of reactive oxygen species (ROS) skewed DC differentiation toward cDC1s [11]. In agreement with this, mice with a deficiency of the AMPK activating kinase liver kinase B1 (LKB1) in CD11c+ cells had an increased number of cDC2s versus cDC1s in the thymus [13]. Together, these results suggest that cDC1 differentiation or survival depends on LKB1/AMPK/FAO, whereas cDC2s depend on ROS signaling (Table 1) (Fig. 1). In addition to Flt3L-induced DC differentiation, multiple studies have used granulocyte macrophage-colony stimulating factor (GM-CSF) as a method to generate BMDCs (GM-DCs) or PBMC-derived monocytes cultured with IL-4 (moDCs). Given that GM-CSF promotes DC differentiation from monocytes, it is likely that GM-DCs resemble inflammatory DCs as opposed to homeostatic immature DCs [14]. Xu et al. compared these two culture models, which displayed several striking differences [15]. GM/IL-4-DC cultures do not contain any pDCs and produce more inflammatory mediators than FL-DCs. FL-DCs migrate more efficiently to lymphoid organs. Furthermore, it has been established that human GM-CSF and IL-4-differentiated
metabolism is believed to be a conserved response to PRR glycolytic increase is unaffected by NO production and fades to or zymosan [20, 21]. Thus, an immediate shift to glycolytic has been shown to increase aerobic glycolysis [19]. This glycolytic GM-DCs or Flt3L-differentiated BMDCs with different TLR agonists Glycolytic induction in early and late DC activation

Metabolic reprogramming in early and late DC activation

Immature DCs are localized in the circulation or peripheral tissue and become activated by signals elicited upon engagement of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), retinoic acid-inducible gene I-like receptors (RLRs), nucleotide-binding oligomerization domain-like receptors, and C-type lectin receptors (CLRs). These PRRs allow DCs to respond rapidly to pathogen-associated molecular patterns or danger-associated molecular patterns. DCs continuously take up antigens, which under homeostatic conditions are harmless antigens that should be tolerated. When DCs are activated through PRR stimulation, they increase the expression of chemokine receptors, such as C-C chemokine receptor type 7 (CCR7), which facilitates DC migration to the draining lymph node. Furthermore, PRR activation leads to increased expression of MHC molecules, costimulatory surface molecules (CD40, CD80, CD86), and cytokines (IL-12, IL-10, IL-23, TNF-α), which prompts the transition from resting immature DCs to functional mature DCs capable of activating T cells in the lymph node. Here, we describe the known metabolic reprogramming that occurs in response to DC activation and is required for DC maturation.

Glycolytic induction in early and late DC activation. Activation of GM-DCs or Flt3L-differentiated BMDCs with different TLR agonists has been shown to increase aerobic glycolysis [19]. This glycolytic burst has also been reported in response to other PRRs, including CLRs that recognize fungal-associated molecules such as β-glucan or zymosan [20, 21]. Thus, an immediate shift to glycolytic metabolism is believed to be a conserved response to PRR signaling in DCs (Fig. 2). Sustained glycolysis supports the survival of DCs under decreased OXPHOS, which was found to be suppressed due to robust nitric oxide (NO) production by inducible NO synthase (iNOS) [19, 22, 23]. Remarkably, the acute glycolytic increase is unaffected by NO production and fades to levels of unstimulated DCs after ~9 h [24]. Hence, activation of immature DCs can be subdivided into an early and a late phase with distinct metabolic regulation and requirements. NO-dependent late glycolysis was predominantly seen in GM-DCs and inflammatory moDCs and not in splenic cDCs, corresponding to iNOS expression selectively in inflammatory moDCs [24]. Despite the fact that NO derived from bystander cells such as macrophages can similarly inhibit OXPHOS in DCs [25], the iNOS-dependent glycolytic increase in vivo predominantly affects inflammatory moDCs [24]. Unlike the early glycolytic response, which is required for upregulation of costimulatory molecules, cytokine production and the T cell stimulatory capacity [19–23], late glycolysis is not required for DC maturation, as iNOS-inhibited DCs do not maintain glycolysis but retain their ability to upregulate costimulatory molecules [24]. It is thus likely that after the early glycolytic increase, individual DC subsets adopt distinct metabolic profiles depending on stimuli (Fig. 2).

Lipids in DC activation. Upon acute glycolytic activation, glucose can become a carbon source to support fatty acid synthesis (FAS). De novo FAS facilitates expansion of membranes of the endoplasmic reticulum (ER) and Golgi, which is required for the extensive increased biosynthesis of proinflammatory molecules during DC activation since inhibition of FAS diminishes DC maturation in response to lipopolysaccharides (LPS) [19] (Fig. 2). Furthermore, acetyl-CoA, the precursor for FAS, is a substrate for protein acetylation and a precursor for cholesterol synthesis. Cholesterol is an important component of cell membranes but can also become oxidized into oxysterols, which are regulators of lipid metabolism and can regulate transcription through nuclear hormone receptors such as liver X receptor. Cholesterol 25-hydroxylase (Ch25h) generates oxysterol 25-hydroxycholesterol (25-HC), and transcription of Ch25h is rapidly induced by TLR ligands poly(I:C) and LPS in CD11c+ GM-DCs in an IFN-γ-dependent manner [26]. IFN-γ-induced Ch25h was later shown to facilitate the suppression of enveloped viruses due to the direct antiviral properties of 25-HC [27]. Interestingly, the IFN-γ response potentiates the activation of DCs and T cell priming and promotes pDC function via the enhancement of FAO [28, 29]. In addition to intrinsic lipid metabolism, DCs are often exposed to extracellular lipids in the tissue. Saturated fatty acids can activate APCs and upregulate costimulatory molecules and cytokine production.
which is inhibited by polyunsaturated lipids [30]. Tumor-derived ceramide induces apoptosis in GM-DCs [31] and decreases the ability of human moDCs to take up soluble antigen [32], which agrees with the finding that lipid-overloaded DCs have a reduced ability to activate T cells [33].

Mitochondria in DC activation. To facilitate acute glycolytic activation, LPS induces the translocation of hexokinase II (HKII) to the mitochondrial membrane [19]. Moreover, the mitochondrial chaperone protein p32 interacts directly with the pyruvate dehydrogenase complex and facilitates glucose-derived citrate production required for TLR activation in GM-DCs [34]. Moreover, metabolic reprogramming is DC subset-specific and stimulation context-dependent. In support of this, it has been shown that IFN-α production by human PBMC-derived pDCs after activation of TLR9 is glycolysis-dependent, whereas IFN-α production after activation of RLR relies on OXPHOS. However, this is unique to pDCs, since human moDCs depend on glycolysis upon RLR activation [35]. Human pDCs also increase the expression of the mitochondrial biogenesis regulator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) and the mitochondrial fusion promoter Mfn2, supporting an increase in mitochondrial metabolism in response to TLR7/8 agonist pRNA. Conversely, human PBMC-derived myeloid CD1c+ DCs increase Drp1 expression to promote mitochondrial fission and reduce OXPHOS [36]. Intriguingly, a recent study identified that Mst1 and Mst2 kinases, which regulate mitochondrial trafficking next to phagosomes in macrophages, resulting in pathogen killing [37], are required for IL-12 production and antigen cross-presentation by cDC1s [12] (Fig. 3). In addition, pDCs can acquire the ability to cross-present antigens after TLR stimulation by increasing phagosomal pH to alkaline levels, resembling cDC1 cells [38]. This process required mitochondrial ROS, since reducing ROS with antioxidants or over-expressing a mitochondrial-targeted catalase diminished cross-presentation by pDCs [38]. Hence, mitochondrial integrity is vital for DC functions, particularly for antigen processing and presentation, which will be elaborated upon below.

mTORC1 and AMPK. mTORC1 is an important upstream regulator of glycolysis and FAS that can be activated by growth factor receptors or PRR signaling through the PI3K/Akt signaling pathway [39, 40]. mTORC1 further promotes protein translation, which is crucial for the survival and function of LPS-activated DCs [41]. In line with the central role of mTORC1 as a regulator of cell metabolism and translation, which is critical for DC function, it is clear that mTORC1 plays a significant role in guiding DC behavior. However, delineating the role of mTORC1 in DCs has proven complex, as studies have found mTORC1 inhibition in DCs to be both pro- and anti-inflammatory [42, 43]. Despite the fact that mTORC1 is a known glycolytic regulator, the early glycolytic switch in TLR-stimulated DCs was shown to be independent of mTORC1 and instead relied on the PI3K-mediated TBK1-IKK-α-Akt pathway [19]. Glycolytic metabolism 24 h after LPS, on the other hand, was inhibited by the mTORC1 inhibitor rapamycin by inhibiting hypoxia-inducible factor-1alpha (HIF-1α)-induced VEGF expression, [44]. Knockdown of HIF-1α, a downstream target of mTORC1, impaired the late glycolytic switch and maturation of LPS-stimulated GM-DCs, including IL-12 and TNF-α production [22]. Corresponding with a proinflammatory role for mTORC1 in DCs, rapamycin has been shown to inhibit maturation and promote a tolerogenic phenotype of human moDCs [45, 46], and a recent study showed that rapamycin impaired the production of proinflammatory cytokines in human moDCs in response to activation of RLR, a PRR that recognizes viral RNA [47]. In contrast, mTORC1 inhibition in CD11c+ GM-DCs and human PBMC-derived CD1c+ myeloid DCs potentiates the expression of the proinflammatory cytokines IL-12 and TNF-α in response to LPS. Moreover, constitutively active mTORC1 increases LPS-induced IL-10 but suppresses IL-12 [39, 40]. In agreement with this finding, rapamycin potentiates GM-DC-controlled activation of naïve CD8 T cells [48]. Furthermore, knockout of the mTORC1 inhibitor tuberous sclerosis (TSC1) in CD11c+ DCs results in decreased T cell proliferation [49] and a reduced number of activated CD8 T cells [50], suggesting an impaired ability to prime naïve T cells in DCs with constitutively active mTORC1. Inhibition of mTORC1 has even been shown to prevent the effects of glucocorticoids in human PBMC-derived CD1c+ DCs [51], emphasizing the anti-inflammatory potential of mTORC1 in myeloid DCs. The discrepancies in mTORC1-related findings can partially be attributed to the different models used in DC studies, since Haidinger et al. showed that rapamycin had inhibitory effects on human moDCs differentiated with GM-CSF and IL-4 but stimulatory effects on human myeloid DCs isolated directly from blood [16].

AMPK is activated in energy-depleted situations by sensing AMP. AMPK activation controls catabolism and mitochondrial activity, and can inhibit mTORC1, directed through TSC2 activation [52]. AMPK knockdown with shRNAs followed by TLR stimulation potentiates IL-12p40 and CD86 expression in GM-DCs, whereas the AMPK activating compound 5-aminoimidazole-4-carboxamide ribonucleotide impairs both the glycolytic switching process and DC maturation after LPS treatment [23]. This indicates that AMPK is a negative regulator of DC activation. DC-specific knockout of the AMPK-activating kinase LKB1 also increases susceptibility to cancer and protects mice against asthma [13, 53], arguing for an anti-inflammatory role of AMPK. On the other hand, DC-specific knockout of LKB1 leads to splenic DCs with increased glycolytic metabolism and generates GM-DCs with enhanced capacity to prime T cells. Interestingly, both studies revealed that LKB1 knockout in DCs promotes the development of regulatory T cells (Tregs) and Th17 T cells, which was driven by LKB1 deletion in cDC2s. AMPK and mTORC1 are often reciprocally regulated, and LKB1-deficient cDCs have increased phosphorylation of S6 kinase, a downstream target of mTORC1 [13]. Inhibition of mTORC1 impairs the ability of cDC2s to generate Tregs [13], implying that LKB1 deficiency may promote inflammation in an mTORC1-dependent manner. Together, these findings emphasize that AMPK and mTORC1 have central roles in balancing DC pro- and anti-inflammatory responses in a highly context-dependent manner, and the complete mechanisms remain to be determined.
Metabolic regulation of tolerogenic DCs

DCs are not only critical for initiating immune responses, but they are immensely important for maintaining tolerance. DCs also bestow peripheral tolerance by continuously presenting self-antigens as immature DCs in the absence of pro-inflammatory factors, silencing autoreactive T cell responses and promoting Tregs [19, 54]. Although immature DCs are tolerogenic, they can become activated when exposed to inflammatory stimuli. However, DC tolerance is sometimes maintained despite being in an inflammatory environment, which can be beneficial in autoinflammatory diseases but also lead to suppressed DC functions in the tumor microenvironment or during infection, facilitating immune evasion. Several factors, including IL-10, TGF-β, retinoic acids (RAs), glucocorticoids, vitamin D, and dexamethasone, can promote DC tolerance. Some of these factors are used to generate tolerogenic DCs in vitro.

In vivo, CD11c+ DC-specific β-catenin deletion substantially increases the inflammatory responses in intestinal DCs and worsens the symptoms of inflammatory bowel disease (IBD) [55]. Furthermore, tolerogenic DCs acquire the ability to produce RA, which promotes the formation of Tregs in a β-catenin-dependent manner [56]. Interestingly, tolerogenic cDC1s and cDC2s in visceral adipose tissue differ in their engagement of the canonical Wnt/β-catenin pathway [57, 58]. However, it remains elusive whether these differences are caused by subset-specific metabolic phenotypes or adaptation to the metabolic state in adipose tissues. Wnt5a activates the noncanonical Wnt pathway and has been shown to reduce IL-12 production by human moDCs [59, 60]. Vitamin D3 promotes the formation of tolerogenic DCs in vitro and in vivo [61], which may result from vitamin D, 1,25-vitamin D3 (VitD3) also promotes the formation of IDO1 expressed by splenic CD11c+ mDCs and splenic cDCs by stimulating PPARγ pathways [60]. The active form of vitamin D, 1,25-vitamin D3 (VitD3) also promotes the formation of tolerogenic DCs in vitro in vivo, which may result from VitD3-induced enhancement of both glycolysis and OXPHOS and the activation of the PI3K/Akt/mTOR pathway [62]. In addition, repetitive LPS stimulation triggers the expression of indoleamine 2,3-dioxygenase (IDO), a metabolic enzyme responsible for metabolizing tryptophan into the immunosuppressive metabolite kynurenines, in splenic CD11c+ cDCs [63]. Interestingly, intratumoral treatment with IL-12 and GM-CSF also induces slow but stable IDO expression in DCs residing in the tumor-draining lymph node, correlating with a tolerogenic phenotype [64]. Moreover, both IFN-γ and TGF-β induce IDO expression in pDCs to orchestrate an immunosuppressive phenotype [65]. IDO1 expression by splenic CD11c+ cDCs is activated in response to arginine-precipitated polyamines [66]. Therefore, it is believed thatarginase and IDO1, two immunomodulatory metabolic enzymes, can cooperate to maintain long-term immunosuppressive effects in DCs.

Metabolic regulation of antigen cross-presentation in DCs

DC maturation facilitates antigen proteolysis and efficient formation and trafficking of peptide-major histocompatibility complex (pMHC) to the plasma membrane [67]. Classical, endogenous antigens, such as proteins synthesized by virally infected cells, are presented on MHCI molecules for detection by CD8+ T cells. MHCI molecules generally bind peptides generated by lysosomal proteolysis in the endocytic and phagocytic pathways, and pMHCI is recognized by CD8+ T cells [68]. The activation of mTORC1 blocks autophagy, resulting in decreased endogenous antigen presentation of DCs [42]. In addition, IFN-γ also upregulates antigen processing and presentation as a result of suppressed mTORC1 activation and enhanced autophagy [69]. Furthermore, Pan et al. demonstrated that mTORC1 reduces MHCI mRNA transcription during DC maturation [70]. In contrast, mTOR activation positively supports lysosome tubulation, a process that facilitates peptide-loaded MHCI molecule trafficking from endolysosomes to the cell surface [71, 72]. Thus, these modulating effects controlled by mTORC1 optimize MHCI-mediated antigen presentation in DCs, and mTORC1 inhibition reduces antigen uptake at early time points while promoting antigen presentation at later time points.

In addition to conventional antigen presentation, cross-presentation is a specialized mechanism through which the antigen can be presented on MHCII, and it is critical for the induction of adaptive immunity against tumor cells and infectious pathogens [73]. The two most effective cross-presenting DCs identified are lymphoid-tissue resident cDC1s (CD11c+CD8α+XCR1+ DCs) and migratory cDC1s (CD11c+CD103+XCR1+ DCs) [74, 75]. Recent evidence indicates that DCs support their cross-presentation function through the establishment of distinct and tightly regulated metabolic processes, including glycolysis, OXPHOS and fatty acid metabolism [76, 77]. Here, we summarize the metabolic processes that have been shown to modulate cross-presentation (Fig. 3).

Lipid body. Lipid bodies (LBs), also known as lipid droplets, are neutral lipid storage organelles composed of a central core of cholesteryl esters and triglycerides surrounded by a single layer of phospholipids, which are thought to originate from ER membranes [78]. Lipid accumulation and synthesis of fatty acids have been shown to dictate DC functions, especially cross-presentation. These LBs accumulate in the cytosol and on DC phagosomes in an interferon inducible ER resident guanosine triphosphatase (GTPase) (Gtgp)-dependent manner. Bougneres et al. showed that Gtgp binds the LB coat component adipose differentiation-related protein (ADFP) to mediate the formation of LBs. Reducing LB amounts by inhibiting acyl CoA deacylacylglycerol acyltransferase blocks the cross-presentation function of GM-DCs [79]. Additionally, liver-derived cDCs that contain high concentrations of intracellular lipids are more potent activators of proinflammatory T cell, NK cell and NKT cell responses, whereas liver-derived cDCs are more potent in facilitating the establishment of regulatory T cell-mediated tolerance if they express low levels of intracellular lipids [80]. In contrast to lipid synthesis and accumulation, Everts et al. reported that treatment with TOFA, an inhibitor of acetyl-CoA carboxylase 1, abolishes FAS, enhances cytokine and chemokine production in GM-DCs. Furthermore, treatment with C75, a fatty acid synthase inhibitor, results in an increased capacity of splenic cDCs to capture antigens and upregulates DC-dependent activation of CD4+ and CD8+ T cells in vivo [19]. However, some studies argue that FAS is dispensable for the activation and function of DCs [81]. In summary, the role of FAS in DC function, especially in regulating DC cross-presentation, remains elusive, and the reported discrepancy can result from the use of experimentally different stimuli that affect DC activation.

Optimal alkaline pH and ER metabolic homeostasis. The excellent ability of DCs to cross-present is largely attributed to their antigen processing capacity. As antigens are internalized by endocytosis or phagocytosis, they undergo gradual proteolytic degradation along their journey from early endosomes and phagosomes to lysosomes. Once in lysosomes, antigens are degraded by lysosomal proteases, which could destroy potential peptide epitopes crucial for T cell activation [68]. DCs circumvent this problem by expressing low levels of lysosomal proteases. Additionally, since most of these proteases function optimally at acidic pH, maintenance of a strongly alkaline pH in the cross-presentation compartment inhibits protease activity, thus preventing overt and limiting antigen degradation. To this end, alkalinization of phagosomes in DCs was attributed to recruitment, assembly and function of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-2 (NOX2) [82]. NOX2 is recruited to DC early phagosomes and mediates the sustained production of low levels of ROS, causing active and maintained alkalinization of the phagosomal lumen. NOX2-defective DCs showed enhanced phagosomal acidification and increased antigen degradation.

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causing impaired cross-presentation [82]. Therefore, NOX2 plays a critical role in conferring DCs the ability to function as specialized phagocytes adapted to process antigens rather than kill pathogens. NOX2 activity generates superoxide anions in the phagocytic lumen, which dismutate to produce hydrogen peroxide and other ROS. Furthermore, Ding et al. found that the lectin family member siglec-G recruited the phosphatase SHP-1, which dephosphorylated the NADPH oxidase component p47phox and inhibited the activation of NOX2 on phagosomes. This resulted in excessive hydrolysis of exogenous antigens, which diminished the formation of MHC-I-peptide complexes for cross-presentation [83]. Finally, NOX2 activity was shown to be crucial for cross-presentation, as genetic deletion of the NOX2 subunit gp91phox abrogated cross-presentation. NOX2 reaches the cross-presenting compartment through the GTPases Rab27a and Rac2. In addition, VAMP-8 has also been reported to play a role in NOX2 recruitment and in mediating the cross-presentation of phagocytic antigens. To load exogenous peptides onto MHC-I molecules, antigens are transported to the cytosol for proteasomal degradation. Antigenic peptides arising from proteasomal degradation are translocated into the ER lumen for antigen processing. Once in the ER, the peptides undergo amino-terminal trimming by ER-associated amino peptidases and are loaded on neosynthesized MHC-I molecules by a complex composed of calreticulin, tapasin, and ERp57 [68]. DCs control endocytic functions that allow efficient antigen presentation, including the regulated transport of MHC molecules, the transport of antigen from endosomes and phagosomes into the cytosol, and the recruitment of ER proteins to phagosomes. All of these factors have been suspected to be controlled by metabolic processes involved in ER homeostasis in DCs.

**DCs in the tumor microenvironment.** It is now well established that tumor cells contain many antigens that can be recognized by the host immune system. Indeed, recent studies have demonstrated the important role of DCs in the generation of effective responses to immune checkpoint blockade (ICB) immunotherapy [84]. DCs are now known to contribute to various stages of the antitumor T cell response, from induction of an antigen-specific response in secondary lymphoid organs to recruitment of effector T cells into the tumor tissue and maintenance/restimulation of cytotoxic T lymphocytes within the tumor microenvironment (TME). Studies have indicated that tumor accumulation of cDC1s defined as Batf3-dependent DCs bearing the CD103 marker is associated with good prognosis and immune-mediated control across mouse and human species [85, 86]. The consequences of
the decreased number of functionally competent DCs in patients with cancer are self-evident: a decreased number of APCs renders immune stimulation less effective. However, it is possible that other manifestations driving defective DC differentiation and activation affect antitumor immune responses, and DC metabolism may also be compromised within the TME in different settings (Fig. 4).

To support the biosynthetic and energy demands of rapid cell division, tumor cells characteristically undergo rewiring to a primarily glycolytic mode of metabolism not only in hypoxic environments where OXPHOS is naturally limited but also under normoxic conditions, a phenomenon known as Warburg metabolism [87]. Based on the aforementioned studies performed on BMDCs and monocyte-derived DCs in vitro, there are a number of potential and documented effects of glycolytically active tumor cells on DC function within the TME. Intriguingly, glucose deprivation and differential partitioning of nutrients in the TME have been suggested to modulate metabolic activities in tumor-infiltrating immune cells [88–90]. As a long-term commitment to glycosylation is needed to support ATP production in activated DCs, high AMP to ATP ratios resulting from insufficient metabolic fitness may in turn trigger AMPK-mediated downregulation of HIF-1α, a master transcriptional regulator of several enzymes essential to glycolytic metabolism, while antagonizing mTOR signaling. As described before, AMPK signaling activation possibly antagonizes anabolic metabolism, which impairs the DC effector function after stimulation with LPS [23, 91]. The PI3K/AKT/mTOR/HIF-1α signaling axis regulates the expression of enzymes necessary for glycogen synthesis. However, the functional consequences of commitment to aerobic glycolysis by DCs in the TME remain to be investigated because mTOR plays multifaceted roles in regulating DC function [43]. In murine models of melanoma as well as lung and ovarian cancers, tumor-associated DCs exhibited an increased expression of the suppressor of cytokine signaling-3 (SOCS3), which suppressed the activity of the pyruvate kinase M2 (PKM2) enzyme responsible for catalyzing the final step of glycolysis. In this study, SOCS3 was also found to limit the PKM2-driven antitumor efficacy of a DC-based vaccine against established Lewis lung carcinomas [92]. In addition to metabolic insufficiency induced by nutrient deprivation and partitioning, hypoxia is a common characteristic of the TME. Cancer cells can adapt to the diminished oxygen microenvironment, while immune cells are less capable of adapting to this microenvironment [76]. In human tumor moDCs, hypoxia-induced expression of HIF-1α activates AMP signaling by increasing A2b adenosine receptor mRNA [93]. Furthermore, HIF-1α dramatically increased the expression of programmed death-ligand 1 (PD-L1) in Gr1+ CD11b+ myeloid cells by binding directly to the hypoxia-response element (HRE) in the PD-L1 proximal promoter [94]. This finding raised the interesting possibility that this process might also take place in tumor-infiltrating DCs (TIDCs) (Fig. 4).

In addition to limiting the availability of nutrients and other key resources for use by immune cell populations infiltrating the TME, the extreme metabolic demands of rapidly growing tumor cells also result in the accumulation of toxic byproducts that are detrimental to immune functions [95]. In this regard, the accumulation of lactic acids is a common factor impairing DC function. Lactate-derived DC dysfunction was first described in moDCs differentiated in melanoma cell line-derived multicellular tumor spheroids in a lactate dehydrogenase A inhibitor-sensitive manner [96]. GPR81, a G protein-coupled receptor (GPR) for lactic acids, can be found on both immune cells and cancer cells. The activation of GPR81 in cancer cells through autocrine lactic acid signaling promotes proliferation, drug resistance, and enhanced expression of PD-L1 [97]. In this context, lactic acids alter antigen presentation and the functional activity of DCs in a paracrine manner [97]. In addition to lactic acids, tumor cells can evade immune responses by producing high levels of RA, which directs intratumoral monocytes to differentiate into immunosuppressive macrophages rather than immunostimulatory dendritic cells via suppression of the DC-promoting transcription factor IRF4 [98].

Herber et al. reported that increased lipid deposition in TIDCs can be an important driver of tumor immune evasion [33]. The increase in lipid contents in TIDCs results from the class A macrophage scavenger receptor type 1 (Msrl, CD204)-mediated uptake of extracellular fatty acids, which further impairs DC-dependent stimulation of T-cell responses [33]. In support of this, Cubillos-Ruiz et al. recently showed that TIDCs exhibit activation of the unfolded protein response (UPR), as indicated by the presence of high levels of spliced X-box-binding protein 1 and robust lipid peroxidation [33]. As a result of ER stress and lipid peroxidation, TIDCs exhibit weaker antigen presentation ability. Notably, small-molecule inhibition of Msrl restores the stimulatory capacity of these DCs. Despite these findings, how these alterations in lipid levels lead to impaired DC functionality remains uncertain. Until now, a more recent report described the molecular mechanism linking oxidized lipid bodies with the binding of the heat shock protein-70 chaperone and the defective transport of complexes of MHCI with peptide (pMHCI), which ultimately suppresses cross-presentation of antigens by DCs [99, 100]. These findings further suggest that perturbations in lipid metabolism and ER homeostasis can affect the antigen presentation ability of DCs and contribute to immune evasion.

Gangliosides are sialic acid-containing glycosphingolipids that have been implicated in the regulation of cellular proliferation and differentiation. An atypical expression pattern of gangliosides has been found in several tumor types, including neuroblastomas, medulloblastomas, melanomas, leukemias, lymphomas and breast tumors, compared with the corresponding normal tissues [101]. In addition to being expressed at the surface of tumor cell membranes, gangliosides are shed into the tumor microenvironment and eventually circulate in the peripheral blood. By using mouse and human neuroblastoma cell lines, researchers have shown that tumor-derived gangliosides inhibit the generation of DCs from mouse bone marrow cells or from human CD34+ precursors [102]. Monosialo-tetrahexosyl-ganglioside 3 (GM3) and disialoganglioside GD3 inhibit the phenotypic and functional differentiation of DCs and induce their apoptosis. In addition, these impaired DCs produces low amounts of IL-12 and large amounts of IL-10, which might hamper an efficient antitumor immune response [103]. Furthermore, ganglioside exposure alters APC function to induce Treg development or activation and may be related to ganglioside-dependent alterations in TLR signaling [104].

**Regulation of DCs by the gut microbiota**

The intestinal immune system carries a formidable challenge in balancing immune responses toward pathogens penetrating the gut barrier as well as tolerance toward harmless food antigens and commensal microorganisms. DCs play a fundamental role in controlling this homeostasis. Dysregulated intestinal homeostasis can lead to chronic infections or inflammatory disorders, such as IBD. In addition, the gut microbiota is being increasingly appreciated as a regulator of the systemic immune systems, for example, by providing protection against melanoma cancers and airway inflammation [105–111]. Nonetheless, the means by which the gut microbiota controls host immunity remain incompletely understood. Here, we discuss the role of DCs in maintaining gut homeostasis and how the microbiota can regulate DC function, focusing on metabolic modulations.

**Gut-associated DCs during steady-state and inflammation**

Gut-associated DCs are indispensable for maintaining gut homeostasis. Deletion of pre-DCs in Zbtb46-DTR mice has been shown to disrupt oral tolerance toward orally supplemented ovalbumin antigen [112], and ablation of CD11c+ DCs leads to spontaneous
Intestinal DCs sample luminal antigen in the form of commensal bacteria or ingested food from the gut by either penetrating epithelial cell-cell junctions and collecting antigen directly or by receiving antigen by M-cells transporting antigen to DCs in the lymphoid tissue Peyer’s patch. Peyer’s patch-localized DCs present antigens to T cells directly, directing CD4+ T cell differentiation and supporting IgA production by B cells. CD103+ cDCs are the major migratory DCs that traffic antigens to the mesenteric lymph node (mLN) to present antigens to naïve T cells. The gut microbiota produces an abundance of microbially modified metabolites that can modulate immune cells directly in the gut or enter the bloodstream and have systemic effects. The most studied immune-modulating gut-derived metabolites are short-chain fatty acids (SCFAs) that can activate G-protein coupled receptors (GPRs) or act as histone deacetylase inhibitors (HDACis). Secondary bile acids (SCFAs) that can activate G-protein coupled receptors (GPRs) or support IgA production by B cells. CD103+ cDCs were shown to promote a proinflammatory Th17 response, whereas cDC2s were shown to promote a proinflammatory Th17 response when recognizing IgA immune complexes, which are only present in LP after pathological infections [123], suggesting that environmental factors such as IgA immune complexes could be an indicator of harmful infection and thus mount an inflammatory response. Hansen et al. additionally showed that IgA immune complex-induced cytokine production required Syk-Pi3k-TBk1-Ikkε glycolytic reprogramming [123], indicating that intestinal DCs require a glycolytic switch when transitioning from tolerogenic to inflammatory and that modulation of DC metabolism may alter intestinal DC functionality.

Gut-microbiota-controlled systemic DC immunity. The microbiota influences the maturation of neonatal DC compartments and has been shown to promote the immunosuppressive functions of splenic pre-cDC1s within the first week of life [124]. Microbiota exposure of neonates from antibiotic-treated mothers kick-starts pre-cDC1 differentiation into cDC1s, at least partially facilitated by microbiota-induced TNF production from myeloid cells [125]. In addition, splenic DCs from germ-free mice fail to produce IFN-γ due to suppressive histone modifications at the cytokine promoters [126]. The gut microbiota is not only instructive in the formation of the neonatal immune system, but its immune regulatory properties can be harnessed by fecal microbiota transplants (FMTs) in adults, aiding in the treatment of antibiotic-resistant infections, type 2 diabetes, and cancer, among others [127–130]. In a very elegant study, Geya-Zatorsky et al. examined single human microbiota-derived species in monocultures in mice, discovering the extensive modulating effect the microbiota has on gut-resident immune cells. In particular, colonic pDCs were heavily regulated by different colonization, and pDC frequencies correlated with enriched transcripts of lipid and protein metabolism pathways in whole-tissue transcriptomics [131]. pDCs are the major source of IFN-γ and furthermore possess unique trafficking properties compared with cDCs. pDCs circulate the blood during steady-state conditions, indicating a possible way of maintaining systemic IFN-γ levels. However, to egress from BM, pDCs require CCL2, which is constitutively expressed in a microbiota-dependent manner [132]. Moreover, the microbiota activates TLR4-TRIF signaling in LP-localized DCs, which promotes IFN-γ production and systemic interferon-stimulated gene (ISG) responses [133]. It was recently shown that microbiota-induced steady-state IFN-γ signaling from pDCs allows cDCs to prime T cells in a metabolically dependent manner [134]. Schaupp et al. showed that Ifnar-deficient cDCs had altered histone modifications and reduced mitochondrial metabolism, similar to cDCs from germ-free mice [134]. This indicates that the commensal microbiota supports systemic IFN-γ signaling, imprinting cDCs metabolically and epigenetically, which likely facilitates the enhanced T cell priming seen in mice with full microbiota.

Two clinical trials with FMT from patients responding to ICB anti-PD-1 therapy were recently shown to enhance the response to therapy in previously nonresponding melanoma patients [127, 129]. This achievement is based on a body of work demonstrating that the response to ICB therapy is dependent on the composition of the gut microbiota and that transfer of fecal microbiota or specific commensal strains could enhance the response to ICB therapy in mice [105–108, 135]. Interestingly, DC tumor infiltration has been shown to promote the response to ICB therapy [75, 136], suggesting that the microbiota could enhance the response to ICB therapy by promoting DC tumor infiltration, cross-presentation and cytokine production. Accordingly, myeloid DCs were enriched in PBMCs of ICB responding patients [107], and Sivan et al. reported augmented DC tumor infiltration and enhanced CD8+ T cell priming in mice with a full microbiota compared with antibiotics-treated or germ-free mice [105]. Moreover, depletion of cDCs after tumor engraftment of colonized Zbtb46-DTR bone marrow chimeric mice revealed that cDCs are required for the effect of ICB-promoting bacteria [137]. Finally, a defined 11 strain consortium of commensals that promote antitumor immunity also increased MHCI expression on LP-associated DCs as well as systemically increased IFN-γ+ CD8+
T cells, indicating enhanced CD8⁺ T cell priming. This effect required both cDC1s and cDC2s, since Batf3⁻, Xcr1⁻, Ifi4-, or Notch-deficient mice all abolished 11-strain-induced IFN-γ⁻ CD8⁺ T cells [138]. Thus, the precise role of DCs in linking the microbiota to enhanced antitumor immunity remains to be uncovered.

The TME comprises bacteria as well, and characterizations of the tumor microbiome have emerged in recent years, revealing intracellular bacteria in both tumor and immune cells [139, 140]. The presence of bacteria within the tumor suggests that these can influence tumor immunity directly [141, 142]. Indeed, it has been shown that gut microbial bacteria can accumulate in subcutaneous tumors and regulate the response to anti-CD47 therapy by controlling the IFN-I response by CD11c⁺ DCs [143]. Hence, the gut microbiota can control tumors at distal sites by regulating circulating immune cells, modulating the tumor microbiome, or producing compounds that enter the circulation.

**Gut microbiota-derived metabolites.** The gut microbiota comprises trillions of microorganisms that possess unique genes, some of which encode proteins that metabolize food intake undigestible by the host and others that modify host-derived metabolites in a distinct manner. Certain microbiota-derived metabolites have immune modulating functions that directly affect intestinal immune cells, and some enter the circulation, presenting a potential way for the microbiota to modulate systemic immunity. DC infiltration and antigen uptake in the lung are, for example, reduced by microbiota-sulfated p-cresol, resulting in reduced airway inflammation [111]. Conversely, the fecal levels of the gut-derived lipid 12,13-diHOME have been shown to be associated with the risk of developing allergic inflammation and were recently found to increase pulmonary inflammation and reduce Treg development in the lungs [144], emphasizing that microbiota-derived metabolites can have both pro- and anti-inflammatory effects. Human DCs responded to 12,13-diHOME by altering PPARY-regulated genes and reducing anti-inflammatory cytokine production and Treg activation in vitro [144], suggesting that 12,13-diHOME-induced inflammation could be facilitated by breaking DC-controlled tolerance. Short-chain fatty acids (SCFAs) are also highly abundant in the gut and are produced from gut microbial fermentation of fibers undigestible by the human digestive system. SCFAs acetate, propionate, and butyrate, with two, three, and four carbon atoms, respectively, are known to have immune modulating properties. Butyrate has anti-inflammatory effects [145] and promotes Tregs in the gut, maintaining gut homeostasis [145]. Several studies have uncovered the SCFA-mediated regulation of T cells, but less is known about the mechanisms behind SCFA-mediated regulation of DCs. Most evidence points to an anti-inflammatory effect of SCFAs on DCs. Dietary fibers and SCFAs protect against airway inflammation by poorly increasing antigen-presenting DCs in airways, dampening the Th2 response [109]. Butyrate and propionate have been shown to prevent LPS-induced maturation markers and the production of proinflammatory chemokines and cytokines by human moDCs [146, 147]. High levels of butyrate and propionate are further associated with resistance to CTLA4 blockade in metastatic melanoma patients, and butyrate supplementation reduced anti-CTLA4-induced DC maturation and CD8⁺ T cell priming in a syngeneic tumor mouse model [148]. In line with this, depletion of gram-positive bacteria using vancomycin increased antigen presentation by CD103⁺ DCs and potentiated the antitumor response in a melanoma model, which was reversed by supplementation with butyrate [149]. In contrast, previous studies have reported that dietary fiber inulin, which is fermented to SCFAs, enhances DC number and proportion in PPs of rats [150, 151]. Inulin further enhanced cDC1 tumor infiltration and CD8⁺ T cell antitumor response [152], as well as provided protection against influenza infections [153], supporting a DC-activating effect of SCFAs. These inconsistencies may be explained by the different models used but could also be attributed to the multiple mechanisms of SCFAs. Mechanistically, SCFAs can regulate host cells in three ways. First, they can bind and activate GPRs. Second, they can enter host cell metabolism as metabolites. Third, butyrate and propionate have histone deacetylase inhibitor (HDACi) effects, modifying the epigenetic landscape of the host cells. Butyrate has been shown to induce an epigenetic program in macrophages, which reduces glycolysis and mTOR and promotes autophagosome-lysosome maturation [154], potentially promoting antigen presentation. However, it is not known whether butyrate has a similar effect on DCs. Interestingly, whether butyrate acts on the host cell as a metabolite or as an HDACi depends on host cell metabolism in cancerous colonocytes, resulting in highly distinctive outcomes and epigenetic changes [155]. If the same applies to DCs, butyrate can have distinct effects on individual DC subsets depending on their maturation stage.

The gut microbiota also communicates with the liver through modification of liver-synthesized bile acids. Primary bile acids produced by the host in the liver are modified by the gut microbiota to become secondary bile acids. A fraction of the secondary bile acids re-enters the circulation and can thus regulate host cells systemically. Microbiota-derived secondary bile acids were recently discovered to have substantial influence on the intestinal Treg/Th17 balance [156–158]. The most common receptors with the highest affinity for bile acids are the surface receptor Takeda G-protein receptor 5, the nuclear receptor Farnesoid-x-receptor (FXR) and the vitamin D receptor (VDR) [159]. DCs express high levels of VDR, and VDR agonists have previously been shown to target myeloid DCs in particular [160], while VDR-deficient DCs in VDR-flox CD11c-cre mice had no effect on bile acid-induced Tregs [158]. FXR agonists in DSS-induced colitis increase splenic DCs, promote Tregs, and protect mice from IBD [161, 162]. Intriguingly, ablation of FXR in DCs enhanced Tregs in a manner comparable to the secondary bile acid isoDCA [156], suggesting that isoDCA may enhance Tregs by modulating DCs, perhaps in an FXR antagonistic manner. The secondary bile acid DCA was recently shown to restore tonic pDC production of IFN-I, maintain the ISG signature in circulating monocytes of germ-free or antibiotic-treated mice, and rescue the response to Chikungunya virus [163]. Hence, bile acids and their microbiota-modified derivatives control gut homeostasis as well as systemic immunity. The gut microbiota influences immune cell epigenetics, for example, through the production of SCFAs, as mentioned above. Moreover, the gut microbiome can regulate the epigenome of systemic DCs, through regulation of tonic IFN-I production, instructing cDCs for future challenges [134]. Cell metabolism and epigenetics are tightly regulated; hence, future studies on how the gut microbiota influences DC metabolism and epigenetics may lead to an advanced understanding of DC biology.

**CONCLUSION**

The field of immunometabolism is just emerging and has become one of the most exciting research areas. Our review focuses on studies uncovering new molecular processes and metabolic pathways that control the development, activation, and function of dendritic cells. With DCs playing a central role in launching appropriate immune responses, they also pose a promising target for therapeutic interventions. Expansion of DC development in combination with TLR ligands or CD40 agonists has been shown to be applicable in cancer therapy in mouse models [75, 164]. However, expanding the DC population also poses the risk of increasing the reservoir for intracellular infections, as seen by the impaired response to *Listeria monocytogenes* and *Mycobacterium tuberculosis* [165]. DC vaccines present the most promising approach in cancer therapy, where moDCs are activated and loaded with peptides before being transferred back to the patient. With the expanding knowledge on how to manipulate DC activation, DC vaccines can both be used to activate antitumor
immune responses and suppress autoimmune diseases by transferring tolerogenic moDCs [166]. Several clinical trials involving tolerogenic DCs from either bone marrow or blood are currently ongoing and are nicely summarized by others [166]. A full comprehension of DC metabolism could present new ways of optimizing DC vaccines by enhancing their metabolic potential to fight cancer.

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ACKNOWLEDGEMENTS
P-CH was supported in part by SNSF project grants (31003A_182470), the European Research Council Starting Grant (802773-MitoGuide), the Swiss Cancer League (RFS-3949-08-2016), the Cancer Research Institute (Lloyd J. Old Star award and CLIP Investigator award), the Melanoma Research Alliance Established Investigator Award, and EMBO Young Investigator award.

FUNDING
Open Access funding provided by Université de Lausanne.

COMPETING INTERESTS
P-CH received research grants from Elixon Immunotherapeutics and serves as scientific advisors for Acepodia, Elixon Immunotherapeutics, and Novartis.

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