#### Review

# Metabolite Transporters—The Gatekeepers for T Cell Metabolism

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## ABSTRACT

Metabolism is the fundamental biological process that drives the survival and death at both organismal and cellular level. It is also intimately involved in all cellular functions, proliferation, differentiation, and response to environmental cues and stress. During infection, successful immune response depends on the proper activation of various cell types. T cells are a key component of adaptive immunity. They remain metabolically quiescent before meeting their cognate antigens, however upon antigen encounter, these activated T cells have increased demands energy and biological building blocks for differentiation, for proliferation, and effector molecules production. These biosynthetic needs are met through metabolic reprogramming. Multilayered metabolite sensing machinery is in place to interact with the environment and coordinates the cellular metabolism with cell signaling and gene expression to meet the cellular demand in a timely manner. As most of the metabolites are cell membrane impermeable and require specialized membrane proteins to facilitate their translocation, metabolite transporters serve as gatekeepers and an important layer of regulation of metabolism in general. In this review, we discuss how key metabolite transporters are involved in T cell metabolism and shape T cell responses.

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Copyright © 2019 by the author(s). Licensee Hapres, London, United Kingdom. This is an open access article distributed under the terms and conditions of <u>Creative Commons Attribution</u> <u>4.0 International License</u>. **KEYWORDS:** T cell metabolism; metabolic reprogramming; metabolites; metabolite transporters; SLC transporters

#### **INTRODUCTION**

For the past two decades, major research effort has been focused on genomics. While genes serve as the blueprint for an organism's biological functions, their expression intimately interacts with environmental factors, diet, life style, and metabolic responses. As a result, it is becoming clear that metabolites influence and are influenced by genetics and are heavily involved in cell survival, differentiation, and functions. At cellular level, each cell receives cues from the dynamic environment by intricate nutrient-sensing modules composed of sensors, transporters and signaling proteins. Once activated, the nutrient-sensing modules re-adjust the metabolic program to match the cellular demands in a context-specific manner.

T cells are a crucial arm of adaptive immunity. They respond to stimulation bv undergoing rapid proliferation antigen and differentiation to produce effector populations. The nature of the T cell response requires these cells to rewire their metabolic program to support the huge and rapid surge in their biosynthetic needs necessary for clonal expansion and effector molecule production. As the immune response subsides, the activated T cell population is reduced by apoptosis, enabling the T cell compartment to return to homeostasis—a process involving metabolic readjustment. The metabolic reprogramming of T cells has drawn intense attention in recent years and has been extensively reviewed elsewhere [1–9]. In brief, naïve T cells are metabolically quiescent while patrolling secondary lymphoid tissues. During this stage, they generate energy mainly through oxidative phosphorylation (OXPHOS) from various nutrients, such as glucose and amino acids. Upon receiving the signal from their cognate antigen, naïve T cells enter activation state followed by differentiation and proliferation to become T effector cells. T effector cells have tremendously increased bioenergetic and biosynthetic needs to support their rapid division and effector molecule production. To meet these cellular demands, T effector cells increase the uptake of glucose and amino acids, and rewire their metabolic programs to enhance the utilization of glucose and glutamine through glycolysis and glutaminolysis. The pentose phosphate pathway (PPP) is also upregulated, working together with glutaminolysis to support the biosynthetic needs. At the same time, these activated T cells also steadily increase their fatty acid uptake to promote lipid synthesis. The metabolic reprogramming in T effector cells is not only important to supply the anabolic metabolism with substrates and ATP, but also modifies gene transcription, post-transcriptional regulation, and cell signaling events ultimately shaping the overall phenotype of the cells.

The metabolic reprogramming for effector T cell differentiation is coordinated by the signaling events mediated by T cell receptor (TCR) and CD28, as well as cytokine receptors. The engagement of TCR and CD28 promotes the activation of the phosphoinositide 3-kinase (PI3K)-AKTmechanistic target of rapamycin (mTOR) signaling axis [10], which leads to the expression of transcription factors such as HIF-1 $\alpha$  and c-Myc that regulate T cell metabolic programs [11–14]. Following the successful control of infection, the activated T cells are deprived and antigen stimulation and pro-survival cytokines and start the contraction phase. The effector T cell population has to decrease its numbers and returns to homeostatic levels. At the same time, it is crucial to generate T memory cells ensuring that the host is protected from reinfection. The formation of T memory cells is

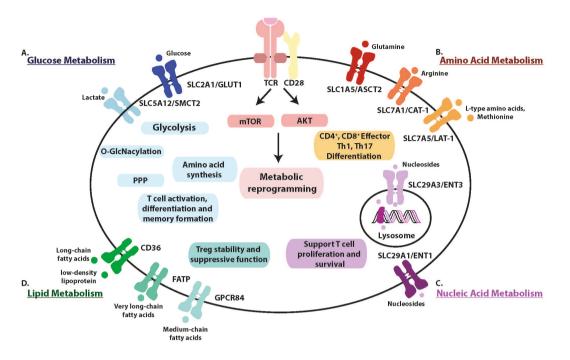


Figure 1. Metabolite transporters that support T cell survival, activation, differentiation and acquisition of effector function. Upon encountering cognate antigen, TCR-mediated signals cause the activation of AKT and mTOR cascade, which promotes the expression of metabolite transporters. These metabolite transporters facilitate the nutrient uptake that is required for the metabolic reprogramming. (A) Glucose transporter SLC2A1 (GLUT1) moves glucose into activated T cells to fuel glycolysis. Glycolysis feeds forward to PPP, amino acid synthesis, and participates in the addition of O-linked N-acetylglucosamin to proteins. Th1, Th2, Th17 and  $T_{FH}$  cells differentiation all rely on the expression of Glut1 and glycolysis. Glycolysis also promotes the formation of effector memory T cells. Lactate is imported into T cells via the lactate transporter SLC5A12 (SMCT2) and the lactate metabolism can interfere with the glycolysis to halt the migratory signal upon activation. (B) Amino acid transporters that are responsible for the influx of glutamine (SLC1A5, ASCT2), arginine (SLC7A1, CAT-1), and L-type amino acids/methionine (SLC7A5, LAT-1), are all upregulated upon TCR stimulation. The elevated levels of intracellular amino acids activate mTORC and are necessary for Th1 and Th17 cell differentiation as well as the effector T cell responses. (C) Although nucleoside transporters (SLC29A1, SLC29A3) are expressed in T cells, the regulatory mechanisms and their roles in T cell function are not well studied. The lysosomally located SLC29A3 (ENT3) has an important role in supplementing the intracellular nucleoside pool upon T cell activation and is required for T cell proliferation and survival. (D) Fatty acids exert multiple effects on T cells. Short-chain fatty acids can inhibit histone deacetylases and facilitate the differentiation of Th1 and Th17 cells. Once imported, the intracellular fatty acids signal through peroxisome proliferator-activated receptors (PPARs) to stabilize the expression of Foxp3, the lineage determinant of T regulatory cells.

The features of metabolism that affect the immune system include (1) metabolic substrate availability, expression of (2) enzymes, (3) transporter proteins, and (4) transcription factors that regulate the catabolism or anabolism of these substrates. Having in mind that the

immune response is a highly dynamic and context-specific process, the metabolic modules in immune cells must remain versatile. While we now know that preferential metabolic programs are used in different T cell populations, there is relatively little discussion on how these programs are being supported by the transporter proteins, which regulate the substrate availability. Without proper expression of these transporters, the cells would lack crucial anabolic substrates or accumulate undesirable metabolites. In this review, we aim to provide an overview of the contribution of different metabolite transporters in supporting T cell immunity. We will discuss these transporters by the metabolic pathways they are involved in (Figure 1).

## **Glucose Metabolism**

Glucose is the key energy source for most of the cells as well as important substrate for multiple biochemical reactions. Once glucose is transported into the cells, it is phosphorylated by the hexokinase and trapped inside the cell for further degradation by glycolysis. During glycolysis, each glucose molecule is broken down to pyruvate with the generation of two ATP molecules. While glycolysis is relatively inefficient in generating ATPs comparing to OXPHOS, it can provide energy rapidly. However, an even more important role of glycolysis is that it also is a source of substrates for various anabolic processes. It feeds glucose to the tri-carbonic acids (TCA) cycle, which fuels amino acid synthesis, PPP for the generation of 5-phosphoribose-1-pyrophosphate (PRPP), the starting material for *de novo* nucleotide synthesis, and one-carbon metabolism, the folate-methionine cycle [18].

## Glucose transporters

As glucose is needed for every cell of the body, glucose transporters are also required for all cells. There are two main types of glucose transporters—sodium-glucose linked transporters (SGLTs) and facilitated diffusion glucose transporters (GLUTs). SGLTs belong to the Solute carrier (Slc) 5 family and move glucose and sodium inside the cell following the existing sodium gradient established by the sodiumpotassium ATPases [19]. They are mostly expressed on intestinal and kidney epithelial cells, and play a role in the absorption of glucose from the food as well as the re-absorption of glucose form urine. GLUTs are members of the Slc2 family of 12 membrane-spanning proteins that can be grouped into three subclasses. These transporters work through facilitated diffusion and differ in their substrate specificity, distribution, and how their expression is regulated [19]. In T cells, only GLUT1, 3, 6 and 8 are expressed, with the latter two having lower levels than GLUT1 and 3 [20]. Most of our knowledge is about the role of GLUT1 in T cells. However, even in the absence of GLUT1, resting T cells and CD8 effector cells can survive, implying GLUT3 and perhaps to lesser degree, GLUT6 and 8, also have important or compensatory function [20].

The proper activation, differentiation, and memory formation of T cells are all tightly associated with glucose metabolism [21]. To fulfill the metabolic and biosynthetic requirements for proliferation and differentiation, activated T cells are highly glycolytic-~10% of the cellular carbon in these cells can be traced back to glucose, making the increase in the glucose transporter transcriptional induction and protein trafficking to the surface critical steps for the metabolic reprogramming [22]. Glucose is transported into T cells mostly via the high affinity Glut1, which is the major glucose transporter on T cells [23,24]. The sharp induction of Glut1 is a prerequisite step for the activated T cells to meet the drastic need for glucose intake that supports biomass increase and effector functions. Glut1 is regulated not only at the level of transcription, but also by intracellular trafficking. Glut1 molecules that are located in the cytosol can be shuttled to the cell surface upon stimulation [22]. TCR and CD28 co-stimulation are required to increase the expression of Glut1 in T cells, while the PI3K-Akt pathway triggers the translocation of Glut1 from the cytoplasm to cell surface. T cells harboring constitutively active Akt have higher level of surface Glut1 expression, although the total Glut1 protein remains at the same level as control cells [24,25]. Glut1 expression is also responsive to cytokines or growth factors. For example, naïve and activated T cells respond to IL-7-induced STAT5 signaling by directing Glut1 to the cell surface; while insulin stimulation upregulates Glut1 surface expression in memory CD4<sup>+</sup> T cells [26]. Conversely, the engagement of cytotoxic T-lymphocyte antigen-4 (CTLA-4) reduces surface Glut1 levels, and the inhibition of PI3K pathways leads to the internalization and lysosomal degradation of Glut1 [27]. c-Myc, the transcription factor that has been identified as crucial regulator of T cell activation-induced metabolic reprogramming, is also associated with the Glut1 expression [11].

Extensive efforts have been made to understand the functional role of Glut1 in T cells. Even though Th1, Th2, and Th17 cells all express high levels of Glut1, overexpression of Glut1 does not affect T cell development, but enhances T cell proliferation [28]. The Glut1overexpressing T cells have increased cell size, cytokine production, and proliferation upon activation. This augmented T-cell activation eventually leads to the accumulation of activated T cells in aged Glut1 transgenic mice and signs of autoimmunity and autoinflammation [25]. While Glut1 knockout animals are embryonically lethal [29], T-cell specific GLUT1 conditional knockout mice have been instrumental for the understanding of the Glut1 involvement in T cell development and activation. T cells deficient for Glut1 failed to generate Th1, Th2, and Th17 cells both *in vivo* and *in vitro* [20]. The exact contribution of Glut1 in the functional specialization of each effector population is still under investigation, but Glut1 has been suggested to be an integral component for both CD4 and CD8 T cell memory formation and maintenance [26,30].

The increased expression of surface Glut1 upon T cell activation is required to mediate the intense demand for glucose uptake necessary for effector cell proliferation and function. Why do effector cells require glycolysis for their functions? Chang et al. showed that in the absence of glucose, GAPDH shifts away from its role as a glycolytic enzyme and instead binds to and prevents the efficient translation of IFN-y [31]. Glycolytic metabolism also impacts the epigenetics in T cells. Glycolysis generates pyruvate, which is imported into mitochondria via the transport protein pyruvate translocase and further processed to Acetyl-CoA. Acetyl-CoA not only can be oxidized for energy production in mitochondria, but also contributes to the acetylation of proteins including histones. Moreover, the sugar moieties generated downstream from glycolysis can modify metabolite transporters and cytokine receptors via N-linked glycosylation or addition of O-linked Nacetylglucosamine (O-GlcNAcylation) to influence the subcellular localization or activity of these proteins. The cell surface translocation of Glut1 and the glutamine transporters sodium-coupled neutral amino acid transporter (SNAT)1, SNAT2, alanine-, serine-, and cysteine-preferring transporter 2 (ASCT2) on activated T cells all require N-linked glycosylation [32-34]. The glycosylation also affects the effector cell differentiation. For example, polarizing CD4<sup>+</sup> effector cells decreases Nglycan branching under Th17 conditions, while adding UDP-Nacetylglucosamine increases the development of T regulatory cells and inhibits the Th17-associated gene expression [35]. This is because IL-2 receptor a (CD25) needs N-glycan branching for its surface expression, and IL-2 signaling is prerequisite for T regulatory cell development [36]. The multifaceted involvement of glycolysis in T cells attracts high interest in activating or inhibiting this pathway as means to modulate immune responses.

# Lactate transporters

Lactate is produced mainly in the cytoplasm from pyruvate, the endproduct of glycolysis, via the action of lactate dehydrogenase (LDH). Its normal concentrations in serum are 0.5–2 mM—second only to glucose in abundance [37,38]. However, it accumulates to high levels under hypoxic conditions or at inflammatory sites. For a long time, lactate has been considered as by-product of cell metabolism, however, recent studies suggest that it is the major source of carbon for the TCA cycle and, thus, for energy in most tissues [38]. Lactate can be sensed through a G-protein coupled receptor, GPR81 [39], however, most of its effects on immune cells are mediated through the lactate transporters on the cell surface. So far six lactate transporters have been described. Four of them belong to the solute carrier 16 (SLC16) family of proton-linked monocarboxylic acid transporters (MCTs): MCT1 (SLC16A1), MCT2 (SLC16A7), MCT3 (SLC16A8), and MCT4 (SLC16A3). All of the MCTs can transport lactate bidirectionally depending on its concentration gradient, but MCT1 is mostly involved in lactate import, while MCT4 functions mostly as a lactate exporter [40]. Two additional lactate transporters are members of the SLC5 family and function as sodium-coupled lactate co-transporters (SLC5A12, SLC5A8) [41]. All lactate transporters share conserved sequence motifs, and have differential affinity to lactic acid and sodium lactate. The efficiency of each transporter depends on multiple factors such as pH, intra-, and extracellular lactate concentrations as well as other substrates such as pyruvate. According to the IMMGEN database, of all lactate transporters, only MCT1 is expressed at appreciable levels on mouse T lymphocytes (www.immgen.org), however, there are reports of MCT4 expression on human peripheral blood mononuclear cells and SLC5A12 on human CD4<sup>+</sup> T cells [37,42]. MCT1 is up-regulated upon T cell receptor stimulation, however the regulation of its expression is poorly understood [43].

The production of lactate from pyruvate is accompanied by conversion of NAD<sup>+</sup> to NADH by LDH. As lactate export through MCTs is coupled with proton export, it prevents acidification of the cytosol and ensures that glycolysis can proceed. High levels of lactate in the extracellular environment reverse the transport direction and lactate and protons enter the cytosol with profound metabolic implications such as consumption of NADH and glycolysis inhibition [44]. As effector T cells are critically dependent on glycolysis, high concentrations of lactate, for example in the tumor microenvironment, have been shown to inhibit the proliferation, cytokine production and cytotoxicity of human cytotoxic T lymphocytes (CTLs)[43,45,46]. Inhibition of MCT1 function had the same effect [43]. Interestingly, regulatory T cells are resistant to this effect of lactate due to their expression of FoxP3 and predominant reliance on oxidative phosphorylation [45]. Lactate concentrations can also influence lymphocyte motility. Lactate is present in the extracellular space as lactic acid at low pH or as sodium lactate at higher pH. These two molecules can exert different effects on target cells. Sodium lactate is imported into human CD4<sup>+</sup> T cells via the SLC5A12 and interferes with the glycolytic pathway, which is required for migration in response to chemokine signals [42]. In addition to retaining the activated CD4<sup>+</sup> T cells in the lactate-rich microenvironment, sodium lactate also prompts CD4<sup>+</sup> T cells to produce IL-17 but not IFN-y. On the other hand, lactic acid exerts its effect on CD8<sup>+</sup> T cells through MCT1 and leads to the impairment of cytolytic function [42]. The dual acts of lactate on T cells have been implicated in the pathogenesis of rheumatoid arthritis (RA)[47]. Upregulation of MCT4 in RA synovial fibroblasts promotes the acidification of synovial fluid. It is proposed that the high concentration of lactate in synovial fluid traps the IL-17 producing CD4<sup>+</sup> T cells in the synovial joint and may in turn result in the formation of ectopic lymphoid-like structures in RA patients. These results together make targeting lactate transporters become a promising therapeutic avenue in oncology as well as in autoimmune diseases.

#### Amino Acid Metabolism

Once T cells are activated, their clonal expansion and acquisition of effector functions are accompanied by changes in nutrient uptake and cellular metabolism. Amino acids are the basic building blocks for protein synthesis, so it is not surprising that deficiency in either dietary proteins or amino acids impairs immune function and increases susceptibility to infection. With recent advances, it is becoming clear that amino acids are not merely the building blocks for protein synthesis, but are also actively involved in the regulation of multiple cellular processes such as metabolism, protein translation, and cell growth and proliferation. Thus, their transporters are in a position to regulate many aspects of cell biology. An important feature of amino acid transporters is that they often transport multiple structurally similar metabolites. For the purposes of clarity, we will discuss the amino acid transporters by their substrates.

## Leucine and glutamine transporters

Leucine cannot be synthesized in the body and must be obtained from the diet in humans. It is transported across the cell membrane by a family of L-type amino acid transporters (LATs). LAT family has four Na<sup>+</sup>-independent neutral amino acid transporters—LAT1-4 (also known as SLC7A5, SLC7A8, SLC43A1, SLC43A2). Each LAT family member has unique properties: LAT1 and LAT2 exist as heterodimers, in complex with a constant heavy chain, SLC3A2 (CD98), to which they are linked by disulfide bridges [48]. They are obligatory anti-porters that move one amino acid into the cytosol in exchange for the efflux of another amino acid [49]. In contrast, LAT3 and LAT4 are symmetrical uniporters, whose direction of transport depends on the concentration gradient [50]. Their preferred substrates are leucine, isoleucine, valine, phenylalanine and methionine. LAT1 and LAT2 transport a broader range of neutral amino acids than LAT3 and LAT4.

LAT1 (SLC7A5) is preferentially expressed on activated lymphocytes and its function in facilitating the utilization of leucine has been studied the most. The transport of leucine requires intracellular glutamine as an efflux substrate. It has been shown that the expression of Slc7a5 is regulated by TCR activation via ERK/MAPK and nuclear factor of activated T cells (NFAT) signaling in T cells [51]. Just like for Glut1, c-Myc, can also regulate Slc7a5 expression putting this transcription factor at the crossroads of multiple metabolic pathways [11]. Although Slc7a5 can transport several amino acids, its key role is in the influx of leucine, because leucine is essential for the activity of mTORC1. The entire mechanism of mTOR activation is still not completely elucidated, but two sets of GTPases are essential for its function—Rag that mediates its lysosomal localization and Rheb that stimulates its kinase activity. The GTPase activity of Rag is stimulates by two GTPase activating protein (GAP) complexes—GATOR1 and folliculin-folliculin interacting protein 2 (FLCN-FNIP2)[52,53]. GATOR1 is negatively regulated by GATOR2 complex. Leucine is a ligand for the GATOR2 interacting protein Sestrin2. Leucine binding to Sestrin2 disrupts its interaction with GATOR2, which promotes Rag activity by inhibiting GATOR1 [54]. Conversely, under leucine-depleted conditions, Sestrin 2 binds to and inhibits GATOR2, which disables Rag and mTORC1 activity [55]. The importance of this transporter is further highlighted by the Slc7a5-deletion animal model [51]. The Slc7a5-null T cells fail to undergo clonal expansion or differentiation into CD4<sup>+</sup> and CD8<sup>+</sup> effector T cells. Similar observations have emerged for the importance of SLC7A5 in human T cells. Disruption of this gene with siRNA reduced essential amino acids uptake and decreased cytokine production [56]. Similar to Scl7a5, the expression of leucine metabolic enzymes such as branched-chain aminotransferase (BCAT) is also regulated by the TCR, suggesting that leucine uptake and metabolism are critical for T cell activation [57]. These data match well with the drastic increase in leucine uptake in effector CD8 T cells upon Listeria monocytogenes infection [51]. Interestingly, follicular helper T cells (TFH) from a lupus prone mouse strain, expressed lower levels of Slc3a2, the heterodimerizing partner of LAT1 and LAT2 [58]. Whether this phenomenon has anything to do with the transport of leucine or other amino acids remains to be established.

Glutamine is the most abundant amino acid in the circulation and can participate in multiple metabolic pathways. It can be converted to  $\alpha$ -ketoglutarate to fuel either the TCA cycle or acetyl-CoA production, or used as a starting point for polyamine, glutathione and serine biosynthesis. Glutathione, for example is one of the most abundant non-enzymatic antioxidant systems in the cells. Although it is not needed for early T cell activation, but it is required for T cell growth and supports the mTOR and NFAT activity and primes T cell for inflammation [59]. Removing glutaminase, the key enzyme converting glutamine to glutamate, diminishes T cell activation, proliferation, and differentiation of Th17 cells [60].

Glutamine can be transported by across the plasma membrane by multiple transporters that belong to four families of SLC: SLC1, SLC6, SLC7, and SLC38 [61]. Activated T cells control the expression of glutamine transporters Slc1a5, Slc3a2, Slc7a5, Slc38a1 and glutaminolysis via c-Myc-mediated pathway [11]. Slc1a5 (Alanine-Serine-Cysteine Transporter, ASCT2) has been identified as an important mediator of glutamine uptake following T cell activation in CD4, but not CD8 T cells [62]. Using ASCT2 deficient animals, it was found that this transporter is dispensable for CD8<sup>+</sup> T cell development, but important for Th1 and Th17 cell differentiation [62]. However, if other glutamine transporters have distinct contribution to T cell function remains to be explored. Slc7a5, described in detail above, usually facilitates the influx of leucine at the expense of efflux of glutamine from the cell.

## Arginine transporters

L-Arginine is a conditionally essential amino acid. It is used for the biosynthesis of proteins, nitric oxide (NO), polyamines, creatine and agmatine. L-Arginine is transported into the cell via the cationic amino acid transporters 1-4 (CAT1-4) that belong to the Slc7 family [63]. In addition to L-Arginine, CATs also transport L-Lysine and L-Ornithine. Similar to other Slc7 family members, CATs are thought to function mostly as exchangers [64]. However, in contrast to LAT1 and LAT2 (Slc7A5 and Slc7A8), they function as monomers at the plasma membrane [65]. Once L-Arginine is imported inside the cell, it is further metabolized into several directions. Arginase converts it into urea and L-Ornithine, while nitric oxide synthase (NOS) turns it into NO and L-Citrulline. Arginine decarboxylase metabolizes arginine to agmatine. Both ornithine and agmatine are precursors to the polyamines such as spermidine, spermine and putrescine. In addition, L-Arginine is also a precursor of creatine and creatinine. Deficiency of L-Arginine increases the expression levels of several multi-amino acid transporters including CAT-1 through enhanced transcription of the genes and stability of the mRNAs [66,67], resulting in greater import of cationic amino acids into the cell. T cell activation enhances the metabolism of L-arginine through the Arginase 2 pathway. This metabolic change is important for antitumor immunity as well as supporting CD4<sup>+</sup> and CD8<sup>+</sup> T cell survival [68]. Starvation of L-Arginine leads to activated T cell cycle arrest in the G<sub>0</sub>–G<sub>1</sub> phase, associated with the inability to upregulate cyclin D3 and cyclindependent kinase 4 (CDK4)[69]. The key role of arginine transporters in cell physiology is underscored by the phenotype of the CAT1 (the main L-Arginine transporter in most cells) knock-out mice. These animals experience severe anemia and prenatal death due to its essential role for both differentiation and proliferation of erythrocytes [70]. The mechanism of how T cells sense arginine and regulate the expression of CAT-1 remains to be described.

# Methionine transporter

Methionine is an essential amino acid in humans and must be obtained through diet or recycling from existing proteins. In addition to its role in protein synthesis, methionine is critically involved in methylation of both proteins and nucleic acids. For example, histone and DNA methylations are key epigenetic modifications that regulate gene transcription [71]; mRNA cap methylation controls the translation initiation by affecting mRNA binding to the eukaryotic translation initiation factor 4E (eIF4E) [72,73]; the methylation of adenosine in RNA is important for translation, splicing and stability of mRNA [74]. Moreover, the methylation of arginine has recently been suggested to play important part in T cell activation [75].

Seven mammalian methionine transporters have been identified to date—Slc1a5, Slc7a6, Slc7a7, Slc7a8, Slc38a1 and Slc38a2 [76,77]. However, Slc7a6 and Slc7a7 do not seem to be expressed in T cells [78]. All other methionine transporters are notably upregulated upon T cell stimulation. Slc7a5 and Slc38a2 (SNAT2) are the most abundantly expressed methionine transporters in activated T cells, but inhibition of Slc7a5 with excess Alanine or of Slc38a2 with 2-methylaminoisobutiric acid (MeAIB) had little impact on the methionine transport in these cells [78]. In contrast, blocking Slc7a5 with 2-aminobicyclo-(2,2,1)-heptane-2carboxylic acid (BCH) demonstrated that the methionine delivery through this transporter is the rate limiting step for the methionine cycle, protein synthesis and RNA methylation in T cells [78]. The Slc7a5 null CD4<sup>+</sup> T cells failed to increase the intracellular methionine level upon TCR-activation and were unable to sustain the methionine metabolism required for T cell activation and differentiation [78]. These findings underline that methionine transport licenses the methionine usage in multiple fundamental biological processes that support T cell proliferation and differentiation.

# Kynurenine transporters

Kynurenine is a tryptophan metabolite that has immunomodulatory properties. High concentrations of Kynurenine can activate the aryl hydrocarbon receptor (AHR) and inhibit tetrahydrobiopterin (BH4) recycling [79]. Kynurenine can be transported by several members of the Slc7a family, but the critical transporter for its uptake is Slc7a5 [80]. In fact, the ability of Kynurenine to activate AHR targets, correlates precisely with the expression of Slc7a5 on the surface of T cells. Interestingly, AHR seems to be able to up-regulate the expression of additional Kynurenine transporters such as Slc7a8 and Slc36a4, initiating a positive feedback loop [81].

## Nucleotide Metabolism

Nucleotides are needed for various biological processes and are constantly synthesized and recycled in the cells. When cells undergo differentiation or proliferation, it is an essential requirement to increase the nucleotide availability fulfilling the demand for DNA replication and RNA production to support protein synthesis. Nucleotides can be generated by *de novo* synthesis or through the salvage pathway. Nucleotide *de novo* synthesis involves multiple critical transcription factors that regulate the expression of genes encoding enzymes in the nucleotide biosynthetic pathways and in the feeder pathways for the production of the precursors of all nucleotides [82]. Salvage pathway, on the other hand, relies largely on the nucleoside transporters to recycle nucleotide building blocks such as nucleosides and nucleobases [83].

## Nucleoside transporters

Nucleotides are hydrophilic molecules and require specialized transporters for their translocation across lipid membranes. Several transporter systems exist that can be classified into two groups: concentrative nucleoside transporters (CNTs), encoded by Slc28 family and equilibrative nucleoside transporters (ENTs) encoded by Slc29 family. CNTs transport nucleosides in one direction only and are Na<sup>+</sup>dependent [84]. They are found in greatest abundance on intestinal epithelia where they facilitate the acquisition of dietary nucleosides. Virtually nothing is known about CNTs in T cells, although according to a public database (www.immgen.org), at least one member, CNT2, is expressed in these cells. ENTs work in bidirectional fashion as facilitators and are expressed in most tissues. Recently, a lot of attention has been focused on ENTs, because of their participation in the metabolism of anticancer nucleoside analogs [85,86]. There are four members in the ENT family. While ENT1, 2, and 4 are situated on the plasma membrane, ENT3 has been reported to have endosomal/lysosomal and mitochondrial membrane localization [87,88]. Functionally, owing to its ability to transport adenosine, ENT1 has been associated with modulation of adenosine levels [89] and considered as a potential therapeutic target for treating Huntington disease [90]. It is shown that patients with ENT1 mutations are refractory to nucleoside analogs such as Cytarabine (Ara-C) or purine nucleoside analogs (PNAs) treatment [91,92]. In addition, secondary malignant leukemia cells often significantly downregulate surface-expressed ENT1 [93], leading to resistance to treatment. Nuclear ENT2 has been considered as a key element controlling the nucleoside and nucleotide pool for effective DNA synthesis and cell cycle progress [94], and high levels of ENT2 expression have been correlated with advanced stages in different tumors [95,96]. People harboring mutations in ENT3 are linked to a group of heterogeneous hereditary diseases, including H syndrome [97], Faisalabad histiocytosis(FD)[98], pigmentary hypertrichosis and non-autoimmune insulin dependent diabetes mellitus (PHID) syndromes [99], and Rosai-Dorfman disease [100], and deficiency of ENT3 in mice leads to myeloid proliferative phenotype due to defective lysosomal function and disturbed M-CSFR signaling [101]. How ENTs participate in the activation of the cells of the immune system is not clear at the moment, although a number of studies have shown that nucleosides and their derivatives play important roles in T cells [102– 104]. Thus, evidence is mounting that the metabolite transporters responsible for the nucleoside availability should have vital roles in the activation and survival of T cells.

Our group found that ENT3 is highly expressed in peripheral T cells and identified ENT3 as a vital metabolite transporter that supports T cell homeostasis and activation. Mechanistically, we showed that absence of ENT3 results in formation of abnormal and enlarged lysosomes that leads to mitochondrial build up, increase in the reactive oxygen species (ROS), and, eventually, DNA damage in T cells exposed to high  $O_2$  tension. Together, these data point to ENT3 being an important contributor to the survival of activated T cells through its role of regulating nucleoside availability and lysosomal integrity [105]. Further understanding on the function of different ENTs in T cells may hopefully point to new therapeutic targets.

## Lipid Metabolism

Fatty acids (FAs) are an important source of energy. Once insaide the cell, FAs are further converted to acyl-CoA, which after conjugation to carnitine by carnitine palmitoyl transferase 1 (CPT1) can pass the outer and inner mitochondrial membranes with the help of carnitine-acylcarnitine translocase, and participate in the  $\beta$ -oxidation in the mitochondrial matrix. FAs are used as precursors to produce complex lipids such as cholesterol and membrane phospholipids as well. FAs can be incorporated into hormones and function as signaling moieties themselves. Through nuclear receptors, FAs also are known to mediate signals that lead to the survival or death of a cell [106]. While some FAs can be directly incorporated into the plasma membrane through passive diffusion, most of their uptake cells required dedicated transporters. Next, we will discuss the roles of fatty acid transporter in CD4 and CD8 T cell differentiation and function.

# Fatty acid transporter

Free fatty acids (FFAs) have simple structure of a varying length aliphatic chain linked to a carboxyl group. Based on their length, FFAs can be classified into short-chain fatty acids (SCFAs—2–6 carbons), medium chain fatty acids (MCFAs—7–12 carbons), long chain fatty acids (LCFAs—13–18 carbons) and very long chain fatty acids (VLCFAs—>20 carbons). Essential FFAs are predominately obtained either directly from diet or through dietary fiber fermentation by gut microbiota (mostly SCFAs). In the serum, they are usually bound to proteins such as albumin. They are commonly esterified and form larger molecules such as triglycerides (TGs) or phospholipids that associate with chylomicrons or very low-density lipoproteins (VLDL).

Extracellular FAs can be recognized and taken up by G proteincoupled receptors (GPCRs), CD36 (also known as collagen type I receptor or thrombospondin receptor), fatty acid-binding protein TM (FABP<sub>TM</sub>) and fatty acid transport proteins (FATPs). There are five known FA GPCRs: GPCR40, 41, 43, 84, and 120, which have different affinities to various length of FAs [106]. The medium-chain FA receptor, GPCR84, is expressed by both CD4 and CD8 T cells [107]; while the short-chain FA receptor, GPCR43, is reported to be expressed on T regulatory cells in the colon [108].

Both SCFA and LCFA have been suggested to participate in regulating T cell responses. The SCFAs generated mostly from dietary fiber

breakdown by the intestinal microbiota bind to GPCR43 on T regulatory cells and support the differentiation and function of these cells by promoting stable Foxp3 expression [108,109]. In this way, the SCFAs act as a mediator maintaining the intestinal homeostasis. On the other hand, the LCFAs increase Th1 and Th17 differentiation through the p38 and JNK1 pathway [110]. CD36 is an integral plasma membrane glycoprotein that has the function of fatty acid translocase—it transports LCFA into the cell. As a member of the class B scavenger receptor family, CD36 binds not only to FA but also oxidized phospholipids, and oxidized low-density lipoprotein (LDL)[111]. In T cells, it has been suggested that CD36-FABP<sub>TM</sub>-cytoplasmic FABP forms a complex that facilitates the diffusion and stabilization of FAs into the cells [112]. FATPs (Slc27 family) have six family members and transport VLCFAs into the cell where they are metabolically trapped by esterification [113].

Although the importance of fatty acid metabolism in the regulation of T cell function is well-recognized [114], but the role of fatty acid transporters in the process has not been extensively investigated. This is likely due to the impediment in dissecting the contribution of *de novo* FA synthesis versus utilizing extracellular FA in the environment. Considering the pleiotropic functions of FA in T cell response, further understanding and evidence are needed for exploiting FA sensing pathways as therapeutic targets.

## **CONCLUDING REMARKS**

Microenvironment nutrient availability and cellular metabolism regulate and modulate the differentiation and function of T cells in both physiological and disease settings. The recent advances immunometabolism have proven that the cellular metabolism in T cells is not only a constant energy generating process, but also a highly dynamic driving force for cell fate decision, and eventually the outcome of the immune response. Since most of the metabolites cannot freely pass through biological membranes, metabolite transporters are positioned at strategically important points to regulate these processes. Having in mind that metabolic pathways are interconnected and working together responding to clues from the microenvironment, focusing on the surface metabolite transporters may be a chance to find the pebble that causes the ripples, instead of chasing the extensive and amplified ripple patterns. Future studies employing metabolite transporter deficiency models or functional blockade, and the resulting compensatory effects such as upregulation of alternative metabolic activation will undoubtedly provide insights in the development of potential therapeutics interventions targeting T cell metabolism and functions.

## AUTHOR CONTRIBUTIONS

CLH formulated the idea and co-wrote the paper with ILD. Both authors read and approved the final manuscript.

## **CONFLICTS OF INTEREST**

The authors declare no competing financial interests.

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