Cellular metabolism dictates T cell effector function in health and disease

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Abstract
In a healthy person, metabolically quiescent T lymphocytes (T cells) circulate between lymph nodes and peripheral tissues in search of antigens. Upon infection, some T cells will encounter cognate antigens followed by proliferation and clonal expansion in a context-dependent manner, to become effector T cells. These events are accompanied by changes in cellular metabolism, known as metabolic reprogramming. The magnitude and variation of metabolic reprogramming are, in addition to antigens, dependent on factors such as nutrients and oxygen to ensure host survival during various diseases. Herein, we describe how metabolic programmes define T cell subset identity and effector functions. In addition, we will discuss how metabolic programs can be modulated and affect T cell activity in health and disease using cancer and autoimmunity as examples.

1. T LYMPHOCYTES AND THEIR SUBSETS ARE PIVOTAL CELLS OF THE IMMUNE SYSTEM

The immune system is a host defence system comprising many biological structures and processes within an organism that protects against infections caused by external and internal factors. The ability of the immune system to act optimally depends on its capacity to distinguish foreign and self and to react to non-self. In higher organisms, the immune system is classified into the innate and adaptive immune system. While the innate immune system deals with foreign pathogens in an acute and ‘unspecific’ manner, the adaptive immune system is activated over time and is associated with controlled activation of T and B lymphocytes (T and B cells). B cells are activated to produce immunoglobulins (antibodies; Abs) after interaction with soluble pathogens through the B cell receptor (BCR) and are considered as parts of humoral immunity. T cells, on the other hand, are activated through cell-to-cell interactions when T cell antigen receptor (TCR) complex encounters peptide antigens presented to them by antigen-presenting cells (APC). APCs present antigens through major histocompatibility complex I or II (MHCI and MHCII), which interact with two major subsets of T cells, designated as CD8-positive (CD8+) and CD4-positive (CD4+) T cells, respectively. CD8 + T cells are also called cytotoxic T lymphocytes (CTLs) while CD4 + T cells are designated as T helper cells. A number of CD4 + T cells have been identified. The best-studied are T helper (Th) 1 (Th1), Th2, Th9, Th17, Th22 as well as follicular helper T

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(Th17) cells and regulatory T cells (Tregs). The CD4 + T cell subsets express unique combinations of cell surface receptors, transcription factors and secrete specific combinations of cytokines. Th17 cells are involved in autoimmune and allergy, whereas Th1 cells are involved in immune responses. Th22 cells appear involved in both autoimmune and allergy. In addition, Th17 cells play vital roles in promoting autoimmunity and Th9 cells have been implicated in tumour immunity. The activities of T cell subsets are balanced, in part, by unique Treg CD4 + T cells subpopulation. Tregs are vital to immune homeostasis and tolerance, dampening inflammation and preventing the development of autoimmune disease, but may also be involved in promoting cancer progression. The balance between pro-inflammatory and anti-inflammatory signals is critically important. Exaggerated or inappropriate T cell responses may be associated with diseases such as allergic responses, inflammatory disorders, autoimmunity, cancer and other diseases.

It is widely accepted that the fundamental processes in T cell biology, such as T cell activation, differentiation and effector functions, are closely linked to changes in the cellular metabolism programmes. Key metabolic pathways such as glycolysis, fatty acid synthesis and mitochondrial metabolism play a crucial role in T cell immunometabolism.

2 | T CELL ACTIVATION AND METABOLISM

Naïve T cells enter the circulation from the thymus and are actively maintained in a quiescent G0 state by self-peptide–MHC engagement of the TCR/CD3 and by interleukin (IL)-7 stimulation. When activated to growth, proliferation and clonal expansion, the cells go through a so-called quiescence exit. This process is initiated and regulated by three key factors, perturbation of cell surface receptors, nutrient availability and oxygen levels.

Together with the TCR molecule, a group of T cell surface co-receptors regulates the magnitude of the acute early T cell response to antigens. The group comprises CD3, CD4, CD8 and the CD28/CTLA4 receptors. As mentioned, CD4 and CD8 molecules directly interact with the MHCI and MHCII molecules, respectively, and influence the early mode of T cell activation. When the MHC-peptide complex is recognized by TCR and the CD4/CD8 co-receptor, the proteins tyrosine kinase (PTK) C-terminal Src kinase (Csk) and lymphocyte-specific protein tyrosine kinase (Lck) are activated. This results in phosphorylation of immunoreceptor tyrosine kinase-based activation motifs (ITAMs) on the ζ-chain of CD3 (Figure 1). This further leads to the recruitment and phosphorylation of the zeta-chain-associated protein kinase (ZAP70). ZAP70 initiates a signalling cascade that activates several signalling pathways including phospholipase Cγ1 (PLCγ1) that promotes calcium mobilization and activation of protein kinase C (PKC) and the RAS pathway. The combination of these signalling cascades promotes activation of several transcription factors, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), nuclear factor of activated T cells (NFAT) and activator protein 1 (AP-1). Together, this induces the production and secretion of T cell-specific paracrine and autocrine growth factor IL-2. The costimulatory molecule CD28 interacts with its ligands CD80 and CD86, which are differentially expressed by APCs. Whereas APC CD86 expression is constitutive, CD80 expression is induced on APCs by Toll-like receptor ligands. Ligation of the CD28 receptor leads to cross phosphorylation of intrinsic CD28 receptor tyrosine residues followed by attachment and activation of phosphatidylinositol kinase 3 (PI3K). PI3K is responsible for phosphorylation of phosphatidylinositol 4, 5-bisphosphate (PIP2) to phosphatidylinositol 3, 4, 5-phosphate (PIP3), followed by activation of the protein kinase B (PKB or Akt) and NF-κB. These are together responsible for the regulation of the B cell lymphoma-extra large (BCL-XL) gene and T cell survival together with Akt-dependent promotion of IL-2 production. In contrast to CD28 interaction with CD80/86, CTLA4 stimulation will, to some extent, suppress TCR/CD3-CD28 induced activation by competing with CD80 for CD86. To this end, CTLA4 stimulation will lead to the recruitment of the protein tyrosine and serine/threonine phosphatases. This will lead to dephosphorylation of several signalling points that are essential for T cell activation to growth, proliferation and clonal expansion.

Naïve T cells have low metabolic activity. During the T cell quiescent exit, receptor perturbation will induce metabolic reprogramming and increased metabolic activity. This is associated with increased nutrient uptake and up-regulation of protein, DNA and lipid synthesis. This can also be induced by TCR/CD3 and CD28 stimulation, which is further associated with activation of calcium calmodulin-dependent protein kinase 2 (CaMK2). CaMK2 is known to activate the energy sensor AMP-dependent protein kinase (AMPK) in T cells. AMPK in most cells is activated by low levels of ATP and LKB1-dependent phosphorylation. However, TCR/CD3-CD28 perturbation increases mitochondrial biogenesis and activation of AMPK even in the presence of ample ATP. This suggests that increased mitochondrial activity and AMPK activation are a prerequisite for energy production needed for T cell growth and proliferation (Figures 1 and 2). Engagement of TCR/CD3-CD28 also promotes recruitment of PI3K.
and activation of Akt at the immune synapse. This controls the activity of the mammalian target of rapamycin, mTOR/raptor complex 1 (mTOR complex 1/mTORC1) through inhibition of the key upstream regulator tuberous sclerosis 2 protein (TSC2), which functions as a GTPase activity protein (GAP) for Ras homolog enriched in brain (Rheb) GTPase.33 mTOR kinase exists in two distinct protein complexes termed; mTORC1 and mTORC2, where the mTORC1 complex is defined as regulatory-associated protein of mTOR (RAPTOR) while mTORC2 is defined as rapamycin insensitive companion of mTOR (RICTOR).33 The GTP-bound form of Rheb directly interacts with mTORC1 and tunes the induction and hence activity of a number of transcription factors regulating anabolic and mitochondrial activity. These include MYC and sterol regulatory element-binding proteins (SREBPs), respectively.34

mTORC2, on the other hand, is more involved in fatty acid oxidation and regulates CD8+ T cell memory differentiation through stabilization of nuclear forkhead box protein O1 (FOXO1), through Akt.35,36 mTORC2 directly phosphorylates Akt on Serine 473, thereby, promoting the expression of glucose transporter 1 (Glut1) and activating hexokinase 2 (HK-2) and phosphofructokinase-1 (PFK-1), consequently promoting glucose uptake and glycolysis.37,38 TCR/CD3-CD28 stimulation is further associated with induction of the A form of lactate-dehydrogenase (LDHA) that converts pyruvate to lactate.39 Because of this metabolic shift, proliferating T cells are associated with aerobic glycolysis and lactate production, even in the presence of sufficient oxygen.40 This process is often referred to as the Warburg effect and supports molecular intermediates for the pentose phosphate pathway (PPP) in order to produce ribose 5-phosphate (R5P) for nucleotide biosynthesis. The Warburg effect is also associated with the production of nicotinamide adenine dinucleotide phosphate (NADPH), which has two main functions; it acts as a redox agent and is essential as an electron donor in anabolic biomass synthesis. It is also important to note that TCR/CD3 and CD4/CD8-induced PTK activity inhibits the
M form of pyruvate kinase (PKM2) leading to the inhibition of the late steps of glycolysis, a key step supporting lactate production. In fact, downregulated PKM2 activity is a key factor together with pyruvate dehydrogenase kinase 1 (PDK1) in preventing pyruvate entering the mitochondrion. This leads to accumulation of pyruvate and hence increased substrate availability for LDHA. Active LDHA will further support enhanced glycolytic flux by removing pyruvate and regenerating NAD⁺ from NADH.

Under conditions where glucose is deprived by many proliferating cells, including TCR/CD3-CD28-stimulated T cells, may adopt profiles associated with oxidative phosphorylation (OXPHOS). This is seen as increased uptake and metabolism of the amino acid glutamine. It is also associated with up-regulation of the glutamine transporter, alanine-serine-cysteine transporter 2 (ASCT2/SLC1A5) concomitant with differential regulation of the glutaminase (GLS) isoforms, kidney glutaminase (GLS_KGA) and glutaminase C (GLS_GAC). During TCR/CD3-CD28 stimulation, GLS_KGA is downregulated and GLS_GAC upregulated. GLS is known to convert glutamine to glutamate that is the key first step in cellular glutaminolysis, a process mostly regulated by MYC. Conversion of glutamine to glutamate is pivotal for proliferating cells in general, as it supports a number of metabolic pathways. These include production of α-ketoglutarate (α-KG) from glutamate during which alanine is produced from glycolytic pyruvate by alanine aminotransferase (ALT). The product α-KG may further be converted to citrate in the tricarboxylic acid (TCA) cycle during which citrate is shunted out of the mitochondrion to support cytosolic acetyl co-enzyme A (AcCoA) levels. AcCoA is a substrate for lipogenesis and mevalonate metabolism as well as cholesterol synthesis. Moreover, glutaminolysis supports PPP-dependent and TCA cycle-derived NADPH synthesis, as well as production of the antioxidant glutathione (GSH). Finally, glutaminolysis supports polyamine and amino acid synthesis in addition to providing nitrogen required for DNA and RNA nucleotide production.

In line with the central role of glutaminolysis, inhibition of glutaminolysis as well as reduced ornithine and putrescine synthesis decreases T cell proliferation.

Overall, this points to glutamine as a critical nutrient for proliferating cells including activated T cells. In fact, exogenous glutamine deprivation and inhibition of glutaminolysis...
are incompatible with T cell activation, proliferation and clonal expansion. As a result, proliferating cells are often referred to as glutamine-addicted. In line with this, we have recently shown that inhibition of glutaminolysis in CD4 + T cells down-regulates extracellular acidification rate (ECAR) and reduced HK activity suggesting that glutaminolysis is rate-limiting for glycolytic activity (Azazul and Skålhegg, unpublished). In fact, others have shown that glutamine-dependent anaplerosis dictates glucose uptake in proliferating cancer cells. The vital importance of glutamine for T cell activation and proliferation is further substantiated by the fact that a number of endogenous amino acids, including proline and arginine, are required and metabolized, when exogenous glutamine is deprived. Extracellular glutamine depletion is further associated with metabolic reprogramming, as glutamine synthetase (GLUL), which converts glutamate to glutamine, is upregulated. Interestingly, it has been demonstrated that transfection and ectopic expression of asparaginase, which is not expressed in mammalian cells, can compensate for extracellular glutamine depletion by restoring TCA activity. However, in this case on the expense of asparagine as substrate for protein synthesis, leading to cell growth and arrest. Other amino acids are also essential to T cell proliferation and clonal expansion. They include cysteine for GSH synthesis and the essential branched-chain amino acid (BCAA) leucine. Leucine regulates the leucine sensor sestrin 2 (SESN2), which forms an inhibitory complex with the GTPase-activating protein towards Rags 2 (GATOR2) when leucine is depleted. Loss of the leucine transporter SLC7A5/LAT1 (CD98) limits T cell activation and effector maturation owing to impairments in mTORC1 activity. Furthermore, serine supports one-carbon metabolism and purine synthesis through the B vitamin 10-formyltetrahydrofolate. Analogous to folate metabolism, serine also supports 5-methyltetrahydrofolate and generation of the methyl donor S-adenosyl methionine. Finally, L. monocytogenes-infected mice fed a serine- and glycine-free diet show reduced pathogen-specific effector T cell responses due to insufficient de novo nucleotide biosynthesis. The role of glycine as an important support of T cell effector function is further in line with our results demonstrating depletion of endogenous glycine upon exogenous glutamine deprivation and inhibition of glutaminolysis (Azazul and Skålhegg, unpublished).

3 T CELL ACTIVATION AND THE ROLE OF HYPOXIA

Many lymphoid organs, including spleen, thymus and lymphatic fluids experience reduced levels of oxygen often referred to as hypoxia. Under physiological conditions, this is defined as oxygen tensions lower than < 4% O2. Within this context, T cells, which are highly mobile in nature, encounter a wide range of oxygen levels in the body. For example, during thymus development, thymocytes inhabit within relatively low oxygen (< 1%) whereas circulating T cells experience a relatively high oxygen level, up to 20% in the lungs and 15% in arterial blood. Moreover, T cells residing within the lymphatic system experience a wide range of oxygen levels from < 1 to > 10%. Given the metabolic challenges of a low oxygen environment, hypoxia is known to elicit a range of adaptive responses at the cellular, tissue and systemic level. To this end, it is widely accepted that oxygen availability influences T cell differentiation, function and survival, a response orchestrated in large by the transcription factor hypoxia-inducible factor (HIF) or more specifically, HIF-1α. HIF-1α which is a basic helix-loop-helix transcription factor is induced and heterodimerizes with the constitutively expressed HIF-1β (or aryl hydrocarbon receptor nuclear transporter, Arnt) under hypoxic conditions. The HIF complex translocates into the nucleus where it binds to hypoxia response elements (HREs). In the presence of oxygen, HIF-1α is rapidly degraded and the HIF complex has thus limited transcriptional activation capacity. Metabolically, HIF-1α promotes the adaptation of cells to hypoxia primarily by lowering oxygen consumption through 1) regulation of LDHA expression in order to alter the capacity to regenerate NAD + and 2) regulation of PDK1 expression to prevent pyruvate to enter the mitochondrion. In response to hypoxia, HIF-1α will regulate these metabolic checkpoints along with increasing the expression of other glycolytic enzymes to shift cellular metabolism away from OXPHOS and O2 dependence. This implies that cells under these conditions rely solely on ATP from glycolysis. Reduced pyruvate availability will further lead to dampened TCA cycle activity and reduced ability to produce citrate. To compensate for this, cells at hypoxia produce citrate and cytosolic ACCoA required for fatty acid synthesis through glutaminolysis. In this case, α-KG is metabolized to citrate through the TCA cycle in a reverse fashion by reductive carboxylation. These reactions are catalyzed by the two isocitrate dehydrogenase isozymes, IDH1 and IDH2, which are induced by low oxygen. Taken together, this demonstrates that energy and biomass for T cell proliferation and clonal expansion at hypoxia are supported through HIF-1α-induced increased glycolytic flux and reversed TCA cycle.

4 T CELL SUBSETS DISPLAY DIFFERENT METABOLIC PROGRAMMES

Over the last decade, it has become clear that metabolic profiles define T cell subsets. Metabolic reprogramming from naïve T cells to T cell subsets is orchestrated through key signalling hubs and downstream transcription factors (Figure 3). As mentioned above, during the T cell quiescence
exit, receptor-dependent regulation of mTOR complex activation is pivotal. However, a key question is whether and how mTORC1 and mTORC2 activities can modulate T cell effector maturation under different physiological conditions, including antigen stimulation as well as nutrient and oxygen availability.

Loss of mTOR signalling in naïve T cells has been ascribed to impaired metabolic reprogramming and T cell differentiation. To this end, mTORC2 has been shown to regulate Tfh differentiation by facilitating the inducible T cell co-stimulator (ICOS)-dependent increase in anabolic metabolism.74 mTORC1 alters cellular metabolism by directly regulating the activity of transcriptional regulators MYC and HIF-1α, key drivers of metabolic reprogramming in T cells.75 The primary effector of hypoxic condition is the induction of HIF-1α. However, HIF-1α is also upregulated after cMYC/PI3K/AKT/mTOR and FOXO1-induced TCR/CD3-CD28 stimulation in CD4+ T cells. The latter suggests an alternative way of stimulating HIF-1α even at normoxia.60,66 This may suggest that HIF-1α is, as is the case with AMPK, induced as a prerequisite for early T cell reprogramming, preparing the cell for metabolic changes required for proliferation and clonal expansion. In this picture, MYC-dependent control of cellular metabolism and growth involves regulating the expression of a number of enzymes involved in glycolysis and glutaminolysis including the key enzymes HK, FFK-1 and GLSGAC.47 Despite it has been shown that HIF-1α promotes Th17 differentiation through inhibition of glycolytic activity in vitro, the complete mechanisms of how Th17 cells are polarized in vivo and are not fully understood. However, naïve T cells polarize into Th17 cells by HIF-1α induction of the master transcription factor RORγt through the aryl hydrocarbon receptor (AhR) pathway. This occurs only in the presence of cytokines like transforming growth factor-β (TGF-β), IL-23, IL-6 and IL-1β.77-81 Interestingly, HIF-1α promotion of Th17 differentiation also prevents Treg differentiation implying HIF-1α as a key factor regulating the ratio of Th17 and Treg in time and space in vivo.82 Finally, loss of the von-Hippel-Lindau tumour suppressor (VHL), a master negative regulator of HIF-1α, potentiates effector CD8+ T cell responses suggesting that HIF-1α is also involved in CD8+ T cell differentiation.83

The role of metabolism in polarization of more conventional subsets of T cells Th1 and Th2 has also been described. Despite the fact that Th2 cell differentiation is dependent on mTOR-mediated metabolic transition from OXPHOS to aerobic glycolysis, the complete role of metabolism in Th2 polarization is not fully understood. Reprogramming of glycolysis and OXPHOS in Th2 cells relies on the activity of the small GTPase RhoA.84 Upon TCR/CD3 perturbation and activation, RhoA localizes to the immune synapse where it engages in asymmetric sorting of MYC and mTOR signalling promoting differentiation of Th1 and Th2 cells.85,86 To this end, it is expected that RhoA mainly regulates Th2 skewing, since RhoA-deficient T cells exhibit impairment in Th2 differentiation but not Th1 cell differentiation.84 This observed reduction in Th2 cell differentiation is due to greater dependence of Th2 cells on RhoA for maintenance of T cell polarity. mTORC2 is known to regulate RhoA activity and hence essential for Th2 cell generation.87,88 As described, mTORC2, but not mTORC1 activity, is reduced in RhoA-deficient T cells, confirming that glycolytic engagement during T cell differentiation is not identical between Th1 and Th2.84 Moreover, mTORC2 deficiency impairs Th2, but not Th1 and Th17 cell differentiation in vivo and in vitro.89 So, what are the requirements for Th1 differentiation? Whereas the role of mTRC2 is unclear, it is likely

![Figure 3](https://example.com/figure3.png)

**Figure 3** Graded metabolic states of T cells in cancer and autoimmunity. T cells residing in the tumour microenvironment (TME) and in inflamed tissues appears to display anti-inflammatory (tolerant) T regulatory (Treg) and pro-inflammatory Th1, Th2, Th9 and Th17 like metabolic features, respectively. This includes low metabolic rate associated with low glycolytic and glutaminolytic rate and increased fatty acid (FA) combustion in T cells residing in TME. In autoimmunity, metabolic rate is elevated with increased glucose and glutamine consumption concomitant with lactate production.
that mTORC1 is indispensable for Th1 T cell phenotype, as CD4+ T cells lacking Rheb, a critical regulator of mTORC1 signalling, fail to differentiate towards Th1 phenotype under Th1 skewing conditions.\(^9\)\(^7\) Moreover, in addition to MYC and HIF-1α, the transcription factor interferon regulatory factor 4 (IRF4) is vital. IRF4 is pivotal in the glycolytic reprogramming and differentiation of Th1 cells and has the ability to block the expression of glycolytic enzymes through regulation of the transcriptional repressor B cell lymphoma 6 (BCL-6).\(^9\)\(^0\),\(^9\)\(^1\) This regulation not only affects Th1 cell differentiation, recently it has been shown to affect CTLs differentiation and function as well.\(^9\)\(^2\),\(^9\)\(^3\)

Together, this reveals that extensive knowledge of metabolic programs is emerging that may contribute to explain how metabolism dictates Th1, Th2, Treg and Th17 differentiation and function. However, metabolic programmes regulating other T cell subsets such as Th9 and Th22 are still elusive and need further investigation.

### 5 \ | \ METABOLIC REPROGRAMMING OF T CELLS SUPPORTS DISEASE DEVELOPMENT IN CANCER AND AUTOIMMUNITY

As discussed above, an increasingly refined picture has emerged of how cell metabolism affects and critically determines activation of T cells into differentiation and effector maturation. We have very briefly described how T cell metabolism under normal conditions regulates immune responses. Below we will describe T cell metabolism in aberrant immune responses in health and disease. We would like to address to what extent dysfunctional T cell metabolism is a driving force in disease development using cancer and autoimmunity as examples.

### 6 \ | \ TUMOUR CELL-DEPENDENT REPROGRAMMING OF T CELL METABOLISM CONTRIBUTES TO TUMOUR TOLERANCE

Despite that we know of more than 200 different types of cancer, most of them share common features, such as abnormal and uncontrolled cell growth. A number of cancers are defined as solid tumours constituting a microenvironment defined by nearly confined bidirectional relationships between malignant and non-malignant components and cell types. This confinement is often referred to as the tumour microenvironment (TME).\(^9\)\(^4\) The TME is often nutrient restricted and hypoxic as the centre of the tumour gets less blood supply due to the rapid increase in biomass. Furthermore, TME is characterized by low pH, as rapidly growing tumour cells are mostly glycolytic and produce lactate. Based on these features, current studies support the notion that the TME enforces dysfunctional cellular metabolism in tumour-infiltrating lymphocytes.\(^9\)\(^5\),\(^9\)\(^6\) In fact, lactic acid, tumour acidosis and hypoxia are key factors dictating tumour immune tolerance, and are induced by numerous mechanisms. HIF-1α induced increased lactate production, which in tumours is associated with activation of HIF2α and induction of anti-inflammatory genes. Lactate acts in different compartments in macrophages (MΦ). In the nucleus, lactate acts via histone lacylation, which promotes the transcription of homeostatic genes. At mitochondria, lactate binds to mitochondrial anti-viral–signalling protein (MAVS) and prevents its interaction with retinoic acid-inducible gene I (RIG-I).\(^9\)\(^7\) Under normoxia, lactate does not prevent MAVS from binding RIG-I, which is then free to induce histone demethylase activity by activating lysine (K)-specific demethylase (KDM) 6A (KDM6A) and KDM5A. This occurs upon interferon beta (IFN-β) stimulation at normoxia.\(^9\)\(^8\) Recently, it was demonstrated that hypoxia is a key factor in tumour development as chronic hyperoxia (60% O\(_2\)) prevents development of pulmonary tumours and prevents tumour metastasis. This effect was closely associated with abrogated CD4, CD8 and natural killer (NK) cell levels suggesting that oxygen levels are crucial for immune cell differentiation and activity. This is supported by the fact that depletion of T cell subsets or NK cells prior to tumour inoculation impaired or completely abrogated the anti-tumour effects of increased oxygenation of tumour microenvironment.\(^9\)\(^9\) The effects of hypoxia on T cell subset differentiation are in line with our own studies where we have shown that differentiation of Th17 cells from naïve T cells in vitro is abrogated by normoxia in vitro.\(^1\)\(^0\)\(^0\),\(^1\)\(^0\)\(^1\)

Oxygen-dependent metabolic reprogramming is only one of several ways tumour cells reprogramme immune cells in the TME.\(^9\)\(^6\) It has been shown that TME hypoxia induces immune suppression by upregulation of CD39 and CD73, which are ectonucleotidases that convert ATP via AMP to adenosine, a major adenosine receptor (A2A) agonist (Figure 4). A2A receptor signalling on T cells is coupled to formation of endogenous cyclic adenosine 3’ 5’ monophosphate (cAMP) and activation of protein kinase A (PKA).\(^1\)\(^0\)\(^2\) Others and we have shown that PKA is expressed as a tetrameric holoenzyme consisting of two catalytic (C) subunits and a regulatory (R) subunit dimer in T, B and NK cells.\(^1\)\(^0\)\(^3\),\(^1\)\(^0\)\(^4\) Moreover, we have shown that cAMP and activation of PKA are strong negative regulators of TCR/CD3-CD28-induced T cell activation to proliferation and IL-2 production as well as differentiation into effector function.

Recently, it was shown that lactate-induced acidosis (H\(^+\)) signals in the TME via G-protein coupled receptors (GPCR) conveying their effects through the cAMP/PKA in immune cells (Figure 4). Tumour acidosis induces the transcription
factor inducible cAMP early repressor (ICER) in tumour-associated MΦ that leads to their functional polarization towards a non-inflammatory phenotype and promotes tumour growth. Together, this demonstrates that TME hypoxia, lactate and activation of cAMP/PKA pathway in MΦ will induce a tumour-tolerant phenotype.\textsuperscript{107,108} To what extent this is similar for T cells is unclear and needs further investigation. However, hypoxia and nutrient deficiency in the TME impair CD8 + T cell activity and differentiation through altering metabolism of the major energy source for CD8 + T cells, fatty acids. This occurs through enhanced peroxisome proliferator-activated receptors (PPAR\(\alpha\)) activity.\textsuperscript{109} Despite that, these reports, in part, explain how immune tolerance may be enforced by tumour-derived metabolites and hypoxia in the TME, and there is one more aspect which needs to be addressed.

The TME is often characterized by immune checkpoint blockades. Immunological checkpoints are known to suppress immune responses following antigen clearance to avoid pathological autoimmunity. Today, it is known that there is an interplay between immune checkpoint proteins and cellular metabolism.\textsuperscript{110} Immune checkpoint proteins can affect cellular metabolism and immune cell activation through direct cancer and immune cell receptor/ligand ligation as well as through nutrient competition, and cancer cell-produced metabolites. These signalling processes can suppress the immune cells leading to immune escape and tumour surveillance. Cancer cells aberrantly express ligands for receptors on lymphocytes and their interaction impairs T cell effector functions by inhibiting signalling downstream of the TCR. As mentioned, CTLA4 negatively regulates effector functions to maintain immunological homeostasis \textsuperscript{111} (Figure 5A). In TME, metabolically highly proliferating cancer cells consume large amounts of glucose and amino acids to fuel glycolysis. Higher glycolysis rate results in increased production of lactate, which acidifies the TME and suppress immune cell effector function. It has been reported that ligation of CTLA4 inhibits glycolysis through targeting effectors of PI3K, and prevents activation and differentiation of CTLs, resulting in the reduction of TME acidification.\textsuperscript{112} Programmed cell death-1 (PD-1) is a T cell co-inhibitory receptor that interacts with its ligands PD-ligand 1 (PD-L1) and PD-L2. PD-L1 is normally expressed by self-tissues to regulate peripheral tolerance and is often expressed by cancer cells to impair T cells.\textsuperscript{113} The expression of both PD-1 and PD-L1 is highly dependent on TME conditions and involves production of adenosine, tissue hypoxia and H\(^+\). Moreover, agonistic targeting of PD-1 prevents metabolic reprogramming of T cells into a glycolytic phenotype, TME acidification and restricts the T cells to fatty acid oxidation, a hallmark of naïve T cells and CTLs.

A third immunological checkpoint marker is CD52, a glycosphatidylinositol (GPI)-anchored glycoprotein, also known as CAMPATH-1.\textsuperscript{114} CD52 is released in soluble form following CD4 + T cell activation. It may bind to the inhibitory sialic acid-binding immunoglobulin-like lectin-10 (Siglec-10) receptor on nearby T cells \textsuperscript{115} (Figure 5A). In this way, CD52-expressing cells may attenuate effector T cell activation by impairing downstream activation of Lck and Zap70 in Siglec-10-expressing T cells. However, if the CD52 marker is targeted, for example with an antibody, the CD52 peptide will not be released and immune tolerance aborted. Interestingly, in vitro polarized Th17 cells under hypoxia downregulated CD52 expression level. Re-oxygenation to normoxia restored CD52 expression, suggesting that hypoxia reprogrammes Th17 cells to form immunosuppressive-like Th17 cells. We have shown that such Th17 cells are Treg-like and produce IL-10.\textsuperscript{100} Taken together with other reports, these results provide examples of how cellular metabolism can be differentially modulated to impair cancer cell growth and proliferation and support T cell proliferation.\textsuperscript{116}

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\textbf{FIGURE 4} Hypoxia and adenosine support immune suppression in the TME. The mechanism by which hypoxia is thought to be associated with immune tolerance is through HIF-1\(\alpha\)-dependent expression of the cell surface markers CD39 and CD73 on the tumour cells. CD39 and CD73 are ectonucleotidases that convert ATP via AMP to adenosine. Adenosine in turn exerts its tumour-promoting effects by activating the adenosine receptor (A2A).\textsuperscript{102} A2A is expressed by cells of the TME including CTLs and naïve CD4 + T cells. Stimulation of A2A activates the cyclic adenosine 3’ 5’ monophosphate (cAMP) and protein kinase A (PKA) system, which are strong regulators of both early activation and long-term effector function of CD4 + and CD8 + T cell as well as NK cell cytotoxicity.\textsuperscript{108}

\textbf{7} \quad \textbf{T CELLS, METABOLISM AND AUTOIMMUNITY}

The genetic origins, environmental drivers and clinical manifestations of autoimmunity are enormous. Despite this,
many autoimmune diseases share common features that contribute to pathogenesis. One such commonality is the activation and generation of pathogenic effector CD4+ and CD8+ T cells, which are present in major forms of autoimmunity including systemic lupus erythematosus (SLE), psoriasis, multiple sclerosis (MS), inflammatory bowel disease and rheumatoid arthritis (RA). In the case of RA, the nature of the inflammatory insult, that is the presence of cytokines in the microenvironment, directly induces T cell differentiation into pathogenic effector cells. In the blood of patients with RA, cytokine levels such as IL1-β, IL-6, transforming growth factor-β (TGF-β) and IL-23 are often found to be elevated which promotes pathogenic Th17 cell differentiation and worsens disease outcome. The lactate transporter SLC5A12 is induced on naïve T cells promoting differentiation into Th17 cells through PKM2 activity. This reduces glycolysis and upregulated fatty acid (FA) synthesis. Blockage of SLC5A12 and prevention of lactate uptake lead to reduced symptoms of arthritis in mice.

As mentioned above, effector T cells are profoundly dependent on microenvironment derived carbohydrates, proteins and lipids. Hence, limited nutrient availability can severely constrain effector T cell responses and promote a tolerogenic environment as observed when the immune system meets the cancer microenvironment. In this context, it should be noted that inflammation induced in autoimmune diseases such as in the semi-contained synovium in RA or in adipose tissues of obese individuals triggers metabolic alterations both at a systemic and localized level. To this end, substantial evidence suggests a direct link...
between dysregulated glucose, amino acid and lipid metabolism in lymphocytes and autoimmunity. This is supported by the fact that increased glucose uptake by T cells is sufficient to drive IFNγ and IL-2 production as shown in mice that overexpress GLUT1 in T cells. Moreover, deletion of the glucose transporter Glut1 reduced CD4 + T cells glycolytic activity, diminished effector CD4 + T cell generation and protected against piroxicam-induced colitis by reducing severity of colitis. In addition, naive CD4 + T cells from lupus-prone mice exhibit higher glycolytic rates as compared to age-matched control cells. Finally, blocking glycolysis at the level of HK using the Glut1 inhibitor 2-deoxy glucose (2-DG) improved clinical outcome in mice with experimental autoimmune encephalomyelitis (EAE). In contrast to EAE, activated T cells from RA patients exhibit lower levels of glycolytic activator 6-phosphofructokinase-2-kinase/fructose-2,6-bisphosphatase (PFKFB3) as compared to healthy controls. PFKFB3 is known to catalyse the production of fructose-2, 6-biphosphate, an activator of PFK. Pharmacological and genetic inhibition of PFKFB3 in T cells induces RA-like phenotype in healthy human T cells, implying that that metabolic drives may be different in different autoimmune diseases.

Above, we discussed the effects of lactate accumulation and acidosis in the TME. The physiological lactate concentration in blood and healthy tissues is approximately 1.5-3 mM. However, in inflamed and more confined tissues such as arthritic joints, atherosclerotic plaques and adipose tissue in obese individuals, lactate can rise to 10-40 mM. Interestingly, in non-confined tissues elevated levels of lactate are reported in the serum of MS patients. In these patients, increased levels of lactate correlates with fatigue and exercise intolerance.

In synovitis of RA patients, the inflammatory infiltrate contains mostly monocyte and/or MΦ grouped in follicular structures. Synovitis also harbours confined secondary lymphoid organs (SLO) like features. These compartments contain infiltrating T cells, and defined structures referred to as ectopic lymphoid-like structures (ELS). These structures are thought to play a key pathogenic role in RA as they are high in lactate. Lactate at the site of chronic inflammation reshares and exacerbates CD4 + T cell effector functions, and worsens disease outcome. This occurs by induction of the lactate transporter SLC5A12 on naïve T cells promoting differentiation into Th17 cells through PKM2 activity, reducing glycolysis and upregulating fatty acid synthesis. Interestingly, in a murine model blockage of SLC5A12 leads to reduced symptoms of arthritis (Figure 5B).

During an inflammatory response, immune cells as described above require increased intracellular pools of amino acids to support a broad range of cellular processes. This includes protein synthesis as well as one-carbon metabolism and nucleic acid, (GSH), NADPH and amino acid synthesis. High amino acid availability upregulates signal transduction by mTORC1 and induces stress-response pathways in T cells, whereas restriction differentially affects T cell activation, proliferation and clonal expansion. As discussed above, T cell activation under glucose-restricted conditions effectively inhibits T cell proliferation as well as IL-2 and IFNγ production. Furthermore, serine, which is used in the one-carbon metabolism, has effects resembling those of methotrexate (MTX). MTX, the most versatile anti-rheumatic drug used today, inhibits folate-dependent one-carbon metabolism, which is pivotal to nucleotide biosynthesis. It has been suggested that serine restriction can be used in conjunction with MTX to treat RA leading to inhibition of Th17 differentiation and effector function.

Finally, targeting de novo lipogenesis with an acetyl CoA carboxylase (ACC1) inhibitor reduces Th17 cell differentiation and proliferation. This is in line with the fact that de novo fatty acid and cholesterol synthesis are a requisite for rapidly dividing T cells. It should be noted that dyslipidaemia is observed in SLE, MS and RA patients, and statins (HMG-CoA reductase inhibitors) ameliorate EAE progression through inhibiting Th17 cell differentiation and lowering RA patient mortality.

**8 | CONCLUSIONS AND THOUGHTS**

We have briefly discussed how metabolic programs may set the stage for T cell effector functions. We have touched on some components of the environment including extracellular signalling compounds, nutrients and oxygen as determinant for regulating normal and aberrant T cell effector function in health and disease. We expect that expanding this knowledge, including a deeper understanding of metabolic T cell flexibility in vivo, will certainly provide new insight that can be used to develop therapeutics and therapeutic strategies for many diseases associated with aberrant immune reactions.

**CONFLICT OF INTEREST**

The authors declare no competing interests.

**AUTHORS’ CONTRIBUTIONS**

Bjørn Steen Skålhegg, Shrikant S Kolan and Gaoyang Li contributed to conceive the review, designed, collected relevant data, drafted, revised and finalized the manuscript. Shrikant S Kolan and Gaoyang Li both contributed equally to the work. Jonas Aakre Wik, Giulia Malachin, Shuai Guo and Pratibha Kolan revised and finalized the draft.
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