Dyslipidemia, metabolism and autophagy : antigen-independent modulation of T cells in atherosclerosis

Amersfoort, J.

CHAPTER 2

T cell metabolism in metabolic disease-associated autoimmunity

Immunobiology 2017; 222:925-936

J. Amersfoort[1]
J. Kuiper[1]

[1] Division of Biopharmaceutics, LACDR, Leiden University, Leiden, The Netherlands

ABSTRACT

This review discusses the relevant metabolic pathways and their regulators which show potential for T cell metabolism-based immunotherapy in diseases hallmarked by both metabolic disease and autoimmunity. Multiple therapeutic approaches using existing pharmaceuticals are possible from a rationale in which T cell metabolism forms the hub in dampening the T cell component of autoimmunity in metabolic diseases. Future research into the effects of a metabolically aberrant micro-environment on T cell metabolism and its potential as a therapeutic target for immunomodulation could lead to novel treatment strategies for metabolic disease-associated autoimmunity.

KEYWORDS

T cells, cellular metabolism, autophagy, diabetes, dyslipidemia, therapeutics

INTRODUCTION

Metabolism is defined as the complex network of (bio)chemical processes occurring in organs and cells required to sustain life. Metabolism is divided into catabolism and anabolism. Catabolism is the degradation/breakdown of macromolecules, generating energy and/or precursors for anabolic processes. Anabolism is the assembly of macromolecules, an energy consuming process. The activity of and (im)balance between these two processes is crucial for various cellular processes, providing energy and building blocks for cellular proliferation, differentiation, function and survival [1]. Tumor cells (and proliferating cells) switch their metabolism from a respiratory towards a glycolytic profile, despite the presence of oxygen [2] to facilitate proliferation [1]. The bioenergetic and biosynthetic requirements of tumor cells may resemble those of proliferating T cells during clonal expansion [3,4]. Naïve T cells (Tn) are in a metabolically dormant state primarily relying on glucose derived pyruvate, fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) to meet a low bioenergetic and biosynthetic demand [5]. After activation of a Tn cell, through mitogens or by T cell receptor (TCR) stimulation, T cells switch to a metabolic state characterized by high rates of glycolysis [4], glutaminolysis [6–8], and OXPHOS [9,10] to provide energy and metabolic intermediates to generate macromolecules and eventually, new effector T cells (Teff) [5,11]. Differentiation into memory T cells (Tm) after clearance of a pathogen is on its turn characterized by a distinct metabolic switch [12–14]. Accordingly, inadequate nutrient availability is a limiting factor for T cell proliferation, differentiation and function [15–17]. In general, metabolism in T cells facilitates an appropriate inflammatory response to specific antigens in a multitude of inflammatory environments with varying nutrient and oxygen availability.

Many diseases (and their complications) challenging the modern health care system are characterized by metabolic disorders and chronic low-grade inflammation [18], including, diabetes [19], atherosclerosis [20], obesity [21] and resulting cardiovascular disease. These metabolic diseases are characterized by systemic metabolic dysregulation, which creates an abnormal metabolic environment for T cells to cope with in the circulation, lymphoid tissue and at the site of inflammation. An abnormal metabolic environment can result from autoimmunity directed to metabolic tissues, as is the case in type 1 diabetes mellitus (T1DM) [22–25]. Vice versa, an abnormal metabolic environment can be a risk factor for disease development. For example, dyslipidemia in familial hypercholesterolemia (FH) patients increases the risk of developing atherosclerosis and cardiovascular disease [26–28]. Nevertheless, these diseases are generally associated with a type 1 autoimmune response [21,25,29,30] and a loss of tolerance by regulatory T cells [31–34]. Currently, it is relatively unknown if and to what extent a metabolically aberrant micro-environment caused by systemic metabolic defects affects T cell metabolism and whether this contributes to

development or progression of diseases hallmarked by both metabolic disease and an autoimmune-like response.

Reviews on T cell metabolism have already identified its potential as a target for therapeutic intervention as well as how altered systemic metabolism in metabolic diseases might affect cellular metabolism in immune cells [35–37]. We now aim to conceive different ways in which a metabolically aberrant micro-environment might affect T cells during metabolic diseases such as diabetes, familial hypercholesterolemia and obesity and how this could contribute to disease progression. Furthermore, we propose different strategies to target T cell metabolism and how existing pharmaceuticals may be implemented to (specifically) modulate T cell metabolism and inhibit disease progression.

SUBSTRATES OF CELLULAR METABOLISM

Glucose

To understand how a metabolically aberrant environment could influence T cell metabolism directly, the most important substrates for T cell metabolism will be discussed. Glucose is one of the vital substrates for T cells to generate ATP [38]. Glucose is mainly taken up by T cells via glucose transporter 1 (GLUT1) [39] and catabolized in the cytoplasm through glycolysis to produce pyruvate and ATP. Pyruvate can subsequently be converted into lactic acid to provide nicotinamide adenine dinucleotide (NAD^+) for redox reactions in the cytosol (e.g. glycolysis). Alternatively, acetyl-CoA is generated in the mitochondria from pyruvate by pyruvate dehydrogenase. Acetyl-CoA can enter the tricarboxylic acid (TCA) cycle to generate the reducing agents NADH and flavin adenine dinucleotide (FADH2) (through a series of biochemical reactions). The latter agents fuel oxidative phosphorylation (OXPHOS) by providing electrons for the electron transport chain (ETC) and creating a proton gradient across the inner mitochondrial membrane for ATP-synthase to convert ADP to ATP. Glycolysis is considered a rapid but relatively inefficient process for energy production whereas OXPHOS is efficient but time- and oxygen consuming [1]. Activated T cells utilize glucose and glycolysis for cell growth and proliferation [40]. The $CD4^+$ T helper (Th) cells Th1, Th2 and Th17, characterized by high levels of interferon-γ (IFNγ), interleukin-4 (IL-4) and IL-17 respectively, are highly glycolytic and depend on high GLUT1 expression for their function [39,41]. Regulatory T cells (Treg), which dampen inflammatory responses, rely less on GLUT1 expression for their function [39] and exhibit relatively high levels of fatty acid oxidation (FAO) and OXPHOS during differentiation and proliferation [41–43]. During T cell proliferation, glycolysis additionally generates metabolic intermediates for anabolic pathways rather than just energy [40,44] while OXPHOS is required for energy generation [9]. In activated T cells, glucose influx via GLUT1 is induced by CD28 co-stimulation in synergy with TCR/CD3 crosslinking [45].

Herein, CD28 acts as an adaptor protein to increase PI3K-Akt signaling which subsequently enhances expression of GLUT1, glucose uptake and glycolysis [45]. The necessity of adequate membrane GLUT1 levels for $CD4^+$ and $CD8^+$ T cell proliferation is illustrated by a decrease in homeostatic and activation-induced proliferation of GLUT1-deficient T cells as compared to their wildtype (WT) control [39]. GLUT1-deficient T cells also show diminished growth and survival upon *in vitro* stimulation indicating glucose influx is required for adequate blast, prior to T cell proliferation [39]. Interestingly, human inducible Tregs (iTreg) have a metabolic program seemingly distinct from murine iTregs. iTregs are highly glycolytic and rely less on FAO for their differentiation and also for their suppressive capacity, human iTregs are more dependent on glycolysis than on FAO [42]. Accordingly, inhibiting glycolysis with the glucose analog 2-deoxyglucose (2-DG) during generation of iTregs decreases their frequency as well as their capacity to suppress $CD4^+$ T helper cells *in vitro* [42]. *Ex vivo* analysis of isolated human Tregs has shown that they primarily rely on glycolysis in rest, whereas both FAO and glycolysis are crucial for proliferation [43]. These findings suggest that Tregs are not ubiquitously skewed towards FAO to meet their metabolic needs and emphasize the requirement of glycolysis for human Tregs. In the context of aforementioned findings from murine Tregs, these data suggest that cellular metabolism can be context-dependent which is an important consideration in assessing and modulating T cell metabolism. Altogether, glycolysis is generally associated with an immunostimulatory T cell response and inhibiting glycolysis using 2-DG seems feasible to dampen a T cell response as characterized by the use of 2-DG to inhibit the $CD8^+$ T cell response in prediabetic NOD mice [46].

Glutamine

Glutamine which is transported into T cells through various solute carrier transporters (SLC), but primarily by a heterodimer of Slc3a2/Slc7a5 (CD98) [47,48], is a crucial amino acid for rapidly proliferating cells [44,49,50]. During glutaminolysis in the cytoplasm, glutamine is primarily hydrolyzed by the rate-limiting enzyme glutaminase-2 to form glutamate and ammonium [48]. Glutamate can enter the mitochondria where it is converted into alpha ketoglutarate (α-KG) by glutamate dehydrogenase (Glud1). α-KG is anaplerotic (i.e. a substrate for the TCA cycle) and can thus facilitate ATP generation by OXPHOS or by pyruvate synthesis as a precursor for acetyl-CoA formation [50,51]. Alternatively, glutamate can be converted to ornithine and eventually polyamines, which are required for biosynthesis during (T cell) proliferation [52,53]. Like glucose, glutamine uptake increases upon activation of T cells with anti-CD3/CD28 [8] or polyclonal mitogens such as concanavalin A [7,38] and glutaminase activity increases correspondingly [48]. Glutaminase inhibition using 6-Diazo-5-oxo-L-norleucine (DON) illustrates the necessity of glutaminolysis for antigen specific T cell expansion. Treating wildtype (WT) mice with DON results in decreased proliferation of adoptively transferred, OVA-challenged OT-II cells as compared

to vehicle controls [48]. Likewise, glutamine starvation diminishes T cell growth and proliferation *in vitro* [48]. Glutamine availability also affects T cell differentiation as glutamine deprivation or DON treatment in Tn cells specifically increases FoxP3 expression in a TGFβ dependent manner, even under Th1 polarizing conditions [54]. Upon administration of the (cell-permeable) α-KG analog DMK, Th1 differentiation is rescued under glutamine deprivation conditions [54], indicating glutaminolysis is involved in both T cell growth and differentiation. Although an elevated level of circulating glutamine is mostly observed in rare diseases (such as chronic kidney disease) [55], inhibiting glutaminolysis in T cells (e.g. using DON) seems feasible to constrain autoimmunity, possibly in combination with other metabolic pathway inhibiting compounds.

Fatty acids

Fatty acids (FA) form a class of substrates with a high energy density for T cells. FAs can enter the cell through various SLC transporters [56], the low-density lipoprotein receptor (LDLr) [57] or the scavenger receptor fatty acid translocase (FAT/CD36) [58]. Before being oxidatively catabolized during FAO, short-, medium-, and long-chain FAs are activated in the cytosol through acylation by acyl-CoA synthetase to facilitate transport through the outer mitochondrial membrane. Carnitine palmitoyl transferase 1 (CPT1) replaces the acyl group by a carnitine group to facilitate transport across the inner mitochondrial membrane so β-oxidation can occur. Inhibiting CPT1 using etomoxir inhibits β-oxidation accordingly. Through β-oxidation, two carbon-units per cycle are cleaved off through a series of biochemical reactions yielding FADH2, NADH and acetyl-CoA, indicating that β-oxidation is a slow, but highly energetic oxidative process. Interestingly, exogenous FA during *in vitro* Th1 differentiation inhibits the production of Th1 cells [41]. Similarly, Th1, Th2 and Th17 cytokine production is decreased by exogenous FA supplementation while FoxP3 expression and suppressive function in Tregs are increased [41]. Thus, extracellular FA abundance can affect Teff function and Treg abundance and function. During murine graft-versus-host disease, Teff cells require upregulation of the FAO machinery and proliferation of allogenic T cells is inhibited by etomoxir accordingly [59], which indicates that inhibition of FAO is feasible to dampen inflammation in some diseases. Tm cells generally have a lower metabolic demand and rely to a large extent on FAO [12–14]. Interestingly, FAO and OXPHOS are of particular importance for the increased inflammatory capacity of Tm cells compared to Teff cells during primary activation. Upon activation of *in vitro* induced Tm cells, high levels of glycolysis, a large mitochondrial mass, and resulting high levels of FAO and OXPHOS facilitate their rapid recall capacity [13]. Etomoxir diminishes proliferation of Tm cells while simultaneously decreasing glycolysis and OXPHOS upon restimulation, as indicated by oxygen consumption- and extracellular acidification rate measurements [13]. In contrast, induction of FAO using metformin or rapamycin increases

Tm generation, providing a useful metabolic compound to improve vaccination efficiency [12,60].

Cholesterol

Besides glucose and glutamine influx, T cells also rapidly upregulate a program for the biosynthesis of fatty acids and cholesterol and increased uptake of lipids after TCR stimulation and mitogen stimulation [61–63]. Cholesterol is a crucial factor as it is required for cellular growth and proliferation as a component of cell membranes [61,64,65]. Moreover, it is important for lipid raft formation by regulating membrane fluidity, which might play a role in T cell activation by regulating immunological synapse stability [66,67]. *De novo* cholesterol is synthesized in the mevalonate pathway in which 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and squalene epoxidase (SQLE) are the rate-limiting enzymes [68]. Statins, a class of drugs which inhibit HMGCR, inhibits TCR-driven T cell expansion accordingly [69]. After TCR stimulation with anti-CD3, T cells acquire a transcriptional program to decrease cholesterol efflux via ATP- binding cassette (ABC) transporter and increase synthesis through *HMGCR* and *SQLE* expression to ensure adequate cholesterol availability for membrane biogenesis [62,63]. Intracellular cholesterol availability is crucial for ER-membrane biogenesis, an important cell-cycle progression checkpoint, again indicating that (sufficient) cholesterol is required for adequate T cell growth and proliferation [63]. For Tregs, cholesterol synthesis through the mevalonate pathway is particularly important for their function. Treating Tregs with simvastatin decreases their suppressive capacity, whereas additional treatment with mevalonate, the product of HMGCR, restores their suppressive capacity [70]. Remarkably, statin treatment in both healthy individuals and hypercholesterolemia patients appears to increase circulatory Treg numbers and FoxP3 expression [71,72]. Atorvastatin increases Treg generation from peripheral blood mononuclear cells *in vitro* from healthy donors, whereas mevastatin and pravastatin do not [71]. Since the Treg promoting effects appear to be statin-type specific further research is required to evaluate the exact effects of specific statins on Tregs. Altogether, not only T cell blast and proliferation, but also the function of specific T cell subsets depends on adequate cholesterol metabolism.

Autophagy

An intrinsic manner through which T cells can acquire nutrients for bioenergetic and biosynthetic purposes is autophagy. Macroautophagy is the predominant and most-studied form of autophagy and is hereafter simply referred to as autophagy. Autophagy is a catabolic recycling process, through which cytosolic macromolecules (e.g. protein aggregates) and damaged or obsolete organelles can be targeted for lysosomal degradation for self-renewal or nutrient reuse [73]. Autophagy starts when double-membrane structures called phagophores are formed *de novo* through a series of complex processes

involving autophagy-related proteins (ATG), among others [73,74], which subsequently enclose cytoplasmic components to form autophagosomes. Thereafter, autophagosomes fuse with lysosomes to form autolysosomes in which macromolecules are degraded [75–77]. The resulting degradation products can be effluxed into the cytosol when the metabolic demand is high, although the mechanisms behind this process are poorly understood [75,78]. Under metabolic stress (e.g. hypoxia, starvation) autophagy is upregulated to intrinsically cope with a changed environment [79,80]. For example, increased autophagy is required to meet the bioenergetic demand in peripheral $CD4^+$ T cells upon TCR stimulation. Atg7-deficient T cells show decreased activation as measured by proliferation and IL-2 secretion [79]. Furthermore, blocking autophagy during stimulation of $CD4^+$ T cells decreases ATP production, lactate generation and FAO [79] which shows that autophagy plays a critical role in metabolic adaptation upon activation. Autophagy is required for quality control of mitochondria in Tn cells to prevent toxicity from reactive oxygen species [81,82]. Interestingly, mitochondria are largely excluded from autophagosomal degradation upon activation in T cells [79] as these need functional mitochondria for proper activation [9]. Interestingly, macrophages and foam cells require autophagy of lipid droplets (lipophagy) when loaded with lipoproteins *in vitro* to degrade cholesteryl-esters and subsequently efflux cholesterol via ABC transporters [83]. Autophagy might play a similar role in T cells for them to cope with a non-physiological micro-environment in metabolic disease and inhibition of autophagy might therefore not be desirable for therapy as will be elaborated further on.

T cells depend on varying substrates to meet the metabolic demand required to transcend from Tn cells into different Teff subtypes and Tm cells in nutrient- and oxygen rich lymphoid tissue and metabolically restricted tissues. Accordingly, different metabolic pathways can be targeted, also simultaneously, to regulate cellular metabolism in T cells to dampen proliferation and skew differentiation (fig. 1). This is an interesting approach and could be especially interesting for treating diseases in which a single substrate is particularly abundant.

REGULATORS OF METABOLISM

To understand the coupling between different metabolic states and inflammatory phenotypes in T cells, the most noteworthy therapeutic targets for T cell metabolism are summarized. A widely studied and important regulator of cellular metabolism during biogenesis and biosynthesis is the serine/threonine protein kinase mammalian target of rapamycin (mTOR). mTOR combines metabolic and environmental signals to regulate a wide range of processes including cell growth, proliferation and autophagy. It is the catalytic subunit of two structurally and functionally distinct protein complexes:

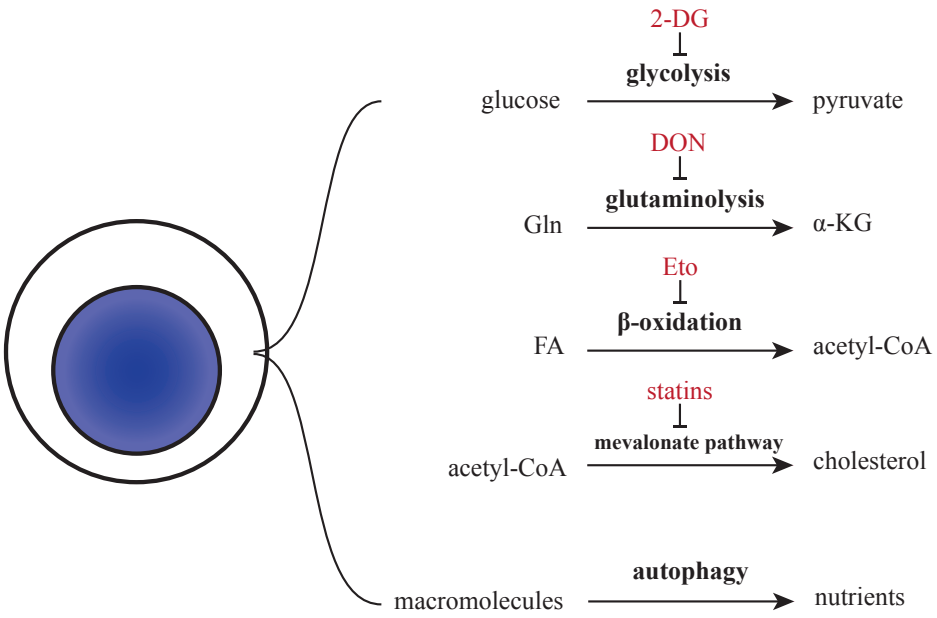

Figure 1 The most relevant metabolic pathways for T cell proliferation and differentiation are depicted with their corresponding inhibitors. The catabolic and anabolic processes which are important for adequate T cell growth, proliferation and differentiation are summarized with the most frequently used corresponding inhibitors. Autophagy is a cellular process T cells upregulate upon activation. Inhibiting T cell autophagy might not be desirable from a therapeutic standpoint. **2-DG**=2-deoxyglucose, **DON**=6-Diazo-5-oxo-L-norleucine, **Eto**=etomoxir.

mTORC1 and mTORC2. In mTORC1, mTOR is in a protein-complex with, among others, regulatory associated protein of mTOR (RAPTOR) while in mTORC2 it is complexed to rapamycin-insensitive companion of mTOR (RICTOR). mTORC1 is involved in nutrient sensing, energy metabolism and protein synthesis, contributing to cell growth, whereas mTORC2 is mainly involved in cytoskeletal rearrangements and cell survival [84]. Based on these properties, it is apparent mTOR signaling is crucial for adequate T cell growth, proliferation and migration [85–87]. Moreover, mTOR is a potent inhibitor of autophagy [88–90], which, together with its role in regulating metabolism, makes it a particularly interesting target for therapy. In regulating glycolysis, mTORC1 can act through hypoxia inducible factor 1 alpha (HIF1α) by increasing its transcriptional activity and its translation, partly by increasing the translation of the 5'UTR sequence of a HIF1α [91,92]. HIF1α regulates the expression of GLUT1 and enzymes required for glycolysis while simultaneously favoring glycolysis over OXPHOS through induction of pyruvate dehydrogenase kinase 1 (PDK1), which prevents pyruvate from entering the TCA cycle [93,94]. HIF1α is upregulated under hypoxic conditions to ensure sufficient energy is generated through anaerobic glycolysis [95]. *In vitro,* HIF1α-deficient T cells show a moderate decrease in expression of hexokinase 2

(HK2) and lactate dehydrogenase A (LDHa) with similar effects on glycolytic rates (within 72h after stimulation) as compared to control T cells, indicating HIF1α is dispensable for acute metabolic programming prior to T cell proliferation. Rather, it is presumed HIF1α plays a role in maintaining glycolysis when T cells enter mitosis [48]. Apart from regulation of glycolysis through HIF1α, mTORC1 can indirectly regulate metabolism through regulation of the YY1-PGC1α complex whose main targets are genes involved in mitochondrial biogenesis and mitochondrial respiration [96,97]. mTORC1 can also promote *de novo* pyrimidine synthesis through its downstream target S6 kinase [98,99]. The role of mTORC2 in metabolism still remains relatively unexplored. Interestingly, PTEN-deficient Tregs show increased glycolysis and decreased mitochondrial fitness suggesting mTORC2 function might also modulate T cell metabolism [100]. mTORC2 is inhibited by long-term rapamycin treatment in various cell types by inhibiting the assembly of the mTORC2 complex [101]. As the function and regulation of mTORC2 are still poorly understood and target-specific compounds are scarce, exploration of the metabolic effector function will be crucial in predicting the therapeutic potential of long-term mTOR modulation.

Myc is another well-recognized protein involved in T cell metabolism and proliferation [48]. Myc is a highly conserved transcription factor, which induces cell cycle progression by regulating p27, cyclins, and CDKs [102,103]. During T cell activation, Myc induces glycolysis and glutaminolysis as Myc is a transcription factor for GLUT1, HK2, pyruvate kinase (PK), and LDHa (which are important for glycolysis) [48,104]. Furthermore, Myc increases the expression of glutamine transporters and mitochondrial glutaminase expression, the latter by repressing microRNAs 23a and 23b [105,106]. The paper of Wang et al. specifically examined the various roles of Myc in the metabolic programming preceding T cell proliferation. Myc-driven glutaminolysis is severely abrogated in *Myc*-deficient T cells with a concomitant decrease in genes and metabolic intermediates involved in polyamine synthesis [48]. Furthermore, Myc-deficient T cells display decreased glycolysis, most likely via decreased expression of HK2 and PK isoform M2 and $CD4^+$ and $CD8^+$ T cell proliferation is severely decreased, indicating Myc-dependent transcriptional programs are required for T cell proliferation [48]. A decrease in the phosphorylation and protein levels of downstream targets of mTOR (4E-BP and S6) is observed in activated Myc-deficient T cells and T cells activated under glutamine starvation conditions [48]. This is particularly interesting as mTOR activity is regulated through CD98 and intracellular L-glutamine levels as L-glutamine efflux induces the influx of essential amino acids and ultimately mTORC1 activation [47]. This suggests that, during T cell activation, Myc-induced glutamine influx is required for mTOR activation and thus crosstalk might exist between the Myc- and mTOR pathways [48]. Myc-inhibition using synthetic inhibitors such as 10058-F4 can prevent Th1 cells from promoting inflammation [107], indicating the feasibility of Myc as a therapeutic target.

Another important sensor and regulator of cellular metabolism is the heterotrimeric

serine/threonine kinase complex AMP-activated kinase complex (AMPK). AMPK can regulate glucose metabolism in various manners, through increased glucose uptake and increased glycolysis [108–110], inhibition of glycogen synthesis and inhibition of gluconeogenesis [111–113]. A major function of AMPK is to inhibit fatty acid synthesis by inactivating acetyl-CoA carboxylase 1 (ACC1) through phosphorylation and inhibiting sterol regulatory element-binding protein 1 (SREBP1) [114,115]. Furthermore, AMPK drives FAO as it inhibits ACC2 by phosphorylation which is in turn an inhibitor of CPT1A expression [116]. Deficiency in *Prkaa1,* encoding the catalytic subunit of AMPK, does not affect T cell proliferation at a high glucose concentration (25 mM) as compared to WT T cells but at low glucose concentrations (3-6 mM) *Prkaa1*-deficient $CD4^+$ T cells are less proliferative, indicating the necessity for adequate AMPK signaling for proliferation in nutrient-restricted conditions [108]. Counterintuitively, IFNγ production in $CD8^+$ T cells after re-stimulation was higher in $Prkaa^{-/-}$ cells at both physiological (5 mM) and high glucose levels (25 mM) compared to control T cells [108]. This is explained by the fact that AMPK inhibits the translation of IFNγ mRNA [108], which supports immunomodulatory effects of stimulation of AMPK in T cells. Another mechanism by which AMPK can induce ATP production is by promoting autophagy through inhibition of mTORC1 and through phosphorylation and subsequent activation of ULK1 [117,118].

Lastly, lipid metabolism is tightly regulated by the counteracting transcriptional regulators liver-X-receptor (LXR) and SREBP. LXR is a transcription factor, which is activated by various oxysterols [119]. Its main action is to induce cholesterol efflux via ABC transporters [120] thereby, effectively counteracting SREBP [121], regulating adequate cholesterol availability under quiescent or activated conditions. Upon T cell activation, simultaneous downregulation of LXR target gene expression and upregulation of target genes of SREBP-1 and -2 ensures cholesterol efflux is decreased and lipid synthesis is increased, respectively [62,63]. Mitogen-induced and TCR-induced proliferation in LXRβ KO mice suggested this particular isoform of LXR is important for proliferation as both $CD4^+$ as $CD8^+$ T cell proliferation was increased compared to WT [62]. LXR activation with natural or synthetic ligand inhibits proliferation accordingly [62,122,123]. SREBP is a zinc finger helicase mainly involved in fatty acid and cholesterol synthesis and uptake during activation through its target genes *Hmgcr*, *Hmgcs*, *Acaca*, *Fasn*, *LDLr* [124]. During T cell activation, SREBP1 and SREBP2 are simultaneously enriched at some of the promotor sites in their target genes indicating SREBP1 and SREBP2 co-regulate lipid synthesis [63]. Interestingly, pretreating T cells with the mTOR inhibitor rapamycin blocked SREBP mediated lipogenesis during TCR mediated stimulation suggesting a crosstalk between mTOR and SREBP pathways [63,91].

In conclusion, multiple modulators of metabolism in T cells represent suitable candidates for therapeutic intervention. As mTOR is involved in the effector function of other metabolic modulators which generally have an important role in facilitating T cell growth,

proliferation and function, including HIF1α, Myc and SREBP, T cell specific inhibition of mTOR appears most feasible. As activation of AMPK has anti-inflammatory effects, partly through inhibition of mTOR, compounds such as metformin are additionally appealing for T cell modulation. Naturally, this depends on which T cell response is to be inhibited or enhanced in which specific micro-environment.

COUPLING METABOLIC TO INFLAMMATORY PHENOTYPE

Differentiation of T cells into specific subtypes of T helper cells is primarily dependent on the inflammatory context and the ability of T cells to adjust their metabolism. $CD4^+$ Th1 are highly glycolytic and display relatively low FAO, a profile similar to the even more glycolytic Th2 cells [41]. As mTOR regulates upregulation of glycolysis upon activation, mTOR deletion in T cells inhibits Th1, Th2 and Th17 generation while simultaneously favoring the induction of $FoxP3^+$ Tregs upon TCR stimulation of $CD4^+$ T cells [125]. Rheb-deficient T cells, which lack mTORC1 activity, fail to differentiate into Th1 and Th17 cells [126]. Interestingly, mTORC2 is important for Th2 development as is characterized by the ability of RICTOR-deficient T cells to differentiate into Th1 and Th17 but not Th2 cells [126]. As Treg differentiation was unaltered in both Rheb- as well as RICTOR-deficient T cells the increased Treg differentiation observed in mTOR-deficient T cells is dependent on inhibition of both mTOR-complexes [126]. mTOR inhibition using rapamycin and DKM1 induces Treg differentiation [127,128], which is indicative of a Treg suppressive function of mTOR, as inhibition of mTOR induces Treg generation both *in vitro* and *in vivo* [129–131].
In Th17 cells, glycolysis is regulated through HIF1α and Th17 generation is highly dependent on HIF1α [132,133]. HIF1α-deficiency impairs the upregulation of genes involved in glycolysis while decreasing the Th17/Treg ratio. Similarly, inhibiting glycolysis in Tn cells using 2-DG or rapamycin shifts T cell differentiation from Th17 cells towards Treg differentiation [132]. Lipid biogenesis is especially crucial for Th17 differentiation during which ACC1 and ACC2 play a central role [134] by regulating *de novo* FA synthesis from acetyl-CoA. Therefore, ACC1 is important for Th17 development as it can couple glycolysis and pyruvate to lipogenesis, thus facilitating membrane biosynthesis. Inhibiting ACC1 and ACC2 using soraphen under Th17 polarizing conditions skews differentiation towards Tregs [134] indicating the intricate link that exists between these $CD4^+$ T cell subtypes. This also emphasizes the potential for metabolic signals to overrule inflammatory signals. The fact that differentiation of Tn cells into Th17 as well as Treg cells largely depends on the pleiotropic cytokine TGFβ certainly explains the potential of metabolic modulation to induce Tregs in the appropriate environment. Sterols represent another class of environmental regulators of Th17 differentiation. Cholesterol uptake and synthesis are increased during Th17 differentiation [135]. During cholesterol synthesis, desmosterol, a

precursor for cholesterol, serves as an endogenous agonist for RORγ, a key transcription factor for Th17 development [135]. This is surprising as desmosterol is a low-affinity LXR agonist [136] and LXR activation with synthetic ligands inhibits Th17 differentiation [137]. During differentiation, Th17 cells increase SULT2B1 expression which catalyzes sulfate conjugation to sterols, thereby inactivating them as LXR agonists [138,139], while desmosterol sulfate retains its RORγ-binding properties. Altogether, the inhibition of glycolysis or induction of sterol efflux seems feasible to skew Tn cells away from Th17 differentiation, thereby dampening inflammation.

The exact potential of autophagy modulation to affect T cell differentiation from Tn cells remains to be elucidated. While increased autophagy is required for adequate $CD4^+$ T cell proliferation and cytokine secretion, induction of autophagy in immune cells is often associated with an anti-inflammatory profile [140] and dysfunctional autophagy disrupts Treg function [141,142]. Atg7-deficient Tregs show impaired cell survival and stability with corresponding increases in apoptosis markers and decreased FoxP3 stability [141]. Moreover, Atg7-deficient Tregs have increased glycolytic metabolism and increased expression of IFNγ and IL-17 as compared to Atg7 WT Tregs [141]. $Foxp3^{Cre}Atg5^{fl/fl}$ mice have higher IFNγ expression in $CD4^+$ and $CD8^+$ T cells and lower Treg percentages compared to $Foxp3^{Cre}Atg5^{+/fl}$ mice, underlining the requirement of functional autophagy for Tregs to maintain immune homeostasis [141]. Likewise, ATG16L-deficient Tregs show decreased survival and increased glycolysis. Presumably, autophagy functions to degrade intracellular lipid droplets (lipophagy) and increase FA abundance for FAO, thereby improving Treg survival [142]. Therefore, (Treg-specific) stimulation of autophagy using metformin or rapamycin might prove a useful approach to stabilize Tregs and increase Treg abundance to diminish autoimmunity. Differentiated Th1 cells require autophagy upon TCR stimulation for cytokine secretion and proliferation as these parameters decrease upon pharmaceutical inhibition of autophagy using 3-MA and NH_4Cl [79]. Likewise, acute deletion of Atg7 after Th1 differentiation results in diminished IFNγ secretion [79]. As ATP production is severely inhibited in Atg7-deficient T cells it is likely that autophagy plays a role in metabolic adaptations for differentiation and function of more T cell subsets. More research on the intricate link between autophagy and metabolism is necessary to predict the outcome of therapeutic intervention in both processes for each type of T cell.

CELLULAR METABOLISM-BASED T CELL MODULATION

Since cellular metabolism is a determining factor in a T cell response, it provides a non-antigen-specific window for T cell therapeutics in the context of diseases characterized by metabolic disease. While a similar phenomenon has been proposed earlier in metabolic and cardiovascular disease [35,36] here we speculate more in detail about the different

mechanisms through which a metabolically altered micro-environment might influence cellular metabolism and immunological phenotype.
We propose five ways through which a metabolically altered micro-environment might fuel T cell metabolism and thereby contribute to disease progression: 1) through increased substrate abundance in the extracellular micro-environment, 2) through increases in intracellular substrate reservoirs, 3) through skewing of substrate dependence, which could alter the activity of bifunctional enzymes or, 4) skew differentiation from Tn cells into Th, Treg or Teff into Tm cells and lastly, 5) through selective metabolic restriction.

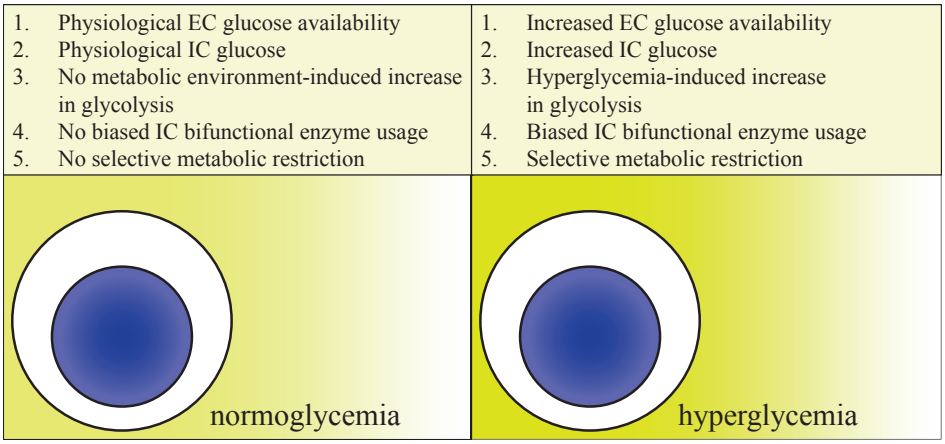

Figure 2 Non-antigen-specific manners through which a metabolically altered micro-environment caused by hyperglycemia might fuel the inflammatory effector functions of T cells and thereby contribute to disease progression. Through these mechanisms, a T cell in a hyperglycemic micro-environment might be more potent in driving inflammation than a T cell with the same cognate antigen in a normoglycemic micro-environment. Similar mechanisms might be applicable to other metabolic diseases, such as dyslipidemia. **EC**=extracellular, **IC**=intracellular.

The proposed mechanisms might be well applicable to diabetes and hyperglycemia (fig. 2). T1DM is a disease in which hyperglycemia caused by $CD4^+$ Th1 and $CD8^+$ T cell mediated autoimmunity against pancreatic islets is one of the hallmarks [22–24]. Insulin insufficiency develops as a result of pancreatic islets degradation, causing T cells to be exposed to prolonged hyperglycemia, which potentially exerts detrimental metabolic effects by one (or several) of the mechanisms described above. As described, *in vitro* hyperglycemia increases IFNy secretion by $CD8^+$ T cells [108]. Although prolonged hyperglycemia in diabetes patients is much milder (fasting plasma glucose ≥ 7 mM) this effect of glucose availability on cytokine secretion does suggest that increased extracellular substrate abundance can indeed fuel T cell mediated autoimmunity. Similarly, culturing T cells under low glucose concentrations decreases the extracellular acidification rate,

a measure for glycolysis, in a dose-dependent manner [108], illustrating the extracellular substrate abundance can dictate the rate at which a certain metabolic pathway is used. Hyperglycemia could increase glycolytic activity in activated T cells which skews GAPDH, an enzyme involved in glycolysis as well as a translational inhibitor of cytokines such as IFNγ [9], availability towards glycolysis. Similarly, increased glycolysis in Tregs might bias enolase-1 (another enzyme from the glycolysis pathway) activity, thereby preventing enolase-1 from binding to the *FoxP3* promotor and FoxP3 CNS2 where it can induce splicing variants of FoxP3 which result in less functional Tregs [42]. Thus, hyperglycemia, and other metabolic diseases, might have anti-inflammatory effects on T cells (or specific T cell subsets) as well, but this review only focuses on the inflammation driving effects for the sake of simplicity. Summarizing, as a result of hyperglycemia, a T cell clone would exhibit increased glycolysis and increased cytokine secretion as compared to the same clone in a normoglycemic environment, thus driving inflammation in a non-antigen-specific manner.

Additionally, selective metabolic restriction might play a pathological role. Metabolic competition exists between progressing tumors and cytotoxic T lymphocytes as highly glycolytic tumors restrict tumor infiltrating lymphocytes from glucose, resulting in a relatively anergic T cell population, unable to properly fight the tumor [143]. A similar discrepancy between supply and demand of certain nutrients for T cells might be present in hypoxic tissues such as atherosclerotic plaques [144–146]. T cell subtypes which are highly glucose consuming with high expression of GLUT1 (e.g. Th1 cells) would benefit more from hyperglycemia than subtypes with an FAO and OXHPOS-dependent profile (e.g. Tregs). Thereby, Th1 cells could deprive Tregs of glucose, an effect which would be particularly pronounced after hyperglycemia has 'primed' Th1 cells through indicated mechanisms and in a micro-environment with nutrient and oxygen scarcity.

Elucidating the mechanisms behind the interplay between altered systemic metabolism and cellular metabolism in T cells would contribute to further understanding T1DM pathology and its comorbidities, for example, an increased risk of atherosclerosis and CVD in T1DM patients [147]. Therapeutic intervention might be possible by inhibiting metabolic pathways, which drive inflammation or by inducing metabolic pathways with an immunomodulatory effect.

Although various immune cells are at the basis of the inflammatory response against pancreatic islets, one central phenomenon contributing to the pathology of T1DM is loss of tolerance by Tregs [31]. Metformin, an AMPK agonist, which is already routinely used as an anti-diabetic is well recognized to modulate T cell metabolism, induce autophagy and induce Treg expansion [41,148,149]. Accordingly, it exerts anti-inflammatory effects on $CD4^+$ T cells in various models of autoimmune disease, including systemic lupus erythematosus, experimental autoimmune encephalomyelitis and arthritis [150–152]. Another AMPK agonist, 5-Aminoimidazole-4-carboxamide ribonucleoside, shows

promising inflammation dampening properties by decreasing T cell proliferation and secretion of pro-inflammatory cytokines, including IFNγ and tumor necrosis factor α [153]. Besides metabolism modulation and its anti-inflammatory effects, metformin might induce autophagy in T cells through inhibition of mTOR, which might show additional beneficial effects as compared to metabolism modulators primarily targeting one specific metabolic pathway (e.g. glycolysis).

Dyslipidemia is another metabolic disease, which might affect T cell metabolism. Dyslipidemia in the form of (familial) hypercholesterolemia and/or hypertriglyceridemia is a major risk factor for atherosclerosis [154], an inflammatory disease characterized by lipid accumulation and subsequent leukocyte infiltration in the wall of medium and large sized arteries. It is the main underlying pathology of CVD and, as a chronic autoimmune-like disease, it has a large component which is $CD4^+$ T cell (mainly Th1) mediated [29,155]. Dyslipidemia in FH patients is characterized by hypercholesterolemia, mainly caused by elevated low-density lipoproteins [156]. Hypercholesterolemia might drive T cell-mediated autoimmunity by providing substrate for membrane synthesis, as cholesterol supplementation *in vitro* is known to drive T cell proliferation [157]. Moreover, diet-induced dyslipidemia could alter the lipid content of T cells intracellularly as these lipids are stored in lipid droplets, or shuttled to the cell membrane, thereby potentially driving membrane raft formation which affects stability of the immunological synapse [158]. Interestingly, prolonged diet-induced dyslipidemia in low density lipoprotein receptor knockout mice altered the membrane lipid composition in T cells which increased T cell activation status [159]. Modulation of T cell lipid metabolism in dyslipidemia patients might therefore help dampen T cell mediated autoimmunity.

Statins might be particularly successful doing this as these are quite successful in primary prevention of coronary heart disease [160]. While the main therapeutic effect of statins is aimed at inhibition of hepatic cholesterol synthesis, T cells are also directly modulated, although the underlying mechanism is sometimes unclear. Atorvastatin inhibits T cell proliferation in mice, an effect, which is overruled by the addition of mevalonate or its precursor farnesyl-PP [161,162]. Moreover, cerivastatin, simvastatin, lovastatin, and atorvastatin induce a Th2 biased differentiation and a decreased Th1 differentiation, as measured by cytokine secretion, although the effects of atorvastatin are not observed in human primary T cells [163,164]. Simvastatin inhibits *ex vivo* T cell proliferation in CVD patients on simvastatin [165]. Modulating LXR through synthetic agonists in T cells, thereby depriving them of cholesterol, might prove successful as well. Systemic administration of LXR agonists GW3965 or T0901317 dampens atherosclerosis development in experimental models of disease [166–168].

mTOR inhibitors such as sirolimus (rapamycin), everolimus and other rapalogs (functional analogs of rapamycin) are well known for their immunosuppressive capacity in graft-versus-host disease and have shown to reduce plaque size in various animal models, as

was extensively reviewed by others [169]. Moreover, mTOR inhibitors, for example in the form of drug-eluting stents with everolimus, are used in the clinic after percutaneous coronary intervention to inhibit restenosis [170].

mTOR signaling also plays a role in obesity-associated low grade inflammation [21]. Inflamed visceral adipose tissue (VAT) contains a disproportionally low amount of Tregs displaying a dysfunctional phenotype, as opposed to Tregs from lean VAT [34]. A significant part of the dysfunction of VAT Tregs is through an insulin induced decrease in IL-10 expression and secretion via the Akt/mTOR pathway [171]. Increasing the Treg population in inflamed VAT has been shown to reduce HFD-induced obesity and insulin resistance [172]. Rapamycin abolishes the negative effects of insulin on mTOR signaling in Tregs, suggesting also in obesity related insulin resistance, T-cell specific modulation of mTOR may have beneficial effects [171]. Direct exposure during priming of $CD4^+$ T cells to the saturated fatty acid palmitate modulates the PI3K-p110 -Akt axis thereby causing a decrease in C-C chemokine receptor type 7 and L-selectin [173]. This contributes to biased differentiation of inflammatory $CD4^+$ T cells which is observed during chronic low grade inflammation in obesity [173].

Although long-term rapamycin treatment improves the metabolic state in animal models [174], a potential side-effect of rapamycin is dyslipidemia which occurs in 40-75% of the patients receiving rapamycin treatment [175–177]. Detrimental effects of rapamycin in mice are found early in treatment [174] indicating treatment duration might be an important factor. Ongoing research and developing other rapalogs should further improve the applicability of these compounds for preventative therapy.

A final important point to address is the implementation of modulators to target T cell metabolism in the pathologies, which were discussed. Systemic administration is most readily available as some of the compounds discussed (e.g. metformin, rapamycin) are already used in the clinic for other therapeutic means. The United Kingdom Prospective Diabetes Study showed the use of metformin as an antidiabetic drug in T2DM patients decreased the risk of myocardial infarction with 33% as compared to a conventional-intervention group, 10 years after cessation of randomized intervention [178]. Although the immune-mediated part of this beneficial effect of metformin remains speculative, it indicates the feasibility of this compound to diminish cardiovascular disease in an aged population. Combined therapy of lifestyle intervention and metformin in newly diagnosed T2DM patients has been shown to decrease serum IL-17 levels, indicating metformin dampens Th17 cells [179]. In patients with multiple sclerosis and metabolic syndrome, disease activity of multiple sclerosis as measured by brain magnetic resonance imaging was decreased in metformin treated patients as compared to non-treated control patients [180]. Additionally, circulating Treg percentage was increased in metformin treated patients, as well as Treg suppressive capacity and IL-10 secretion [180]. However, the possible off-target effects during long-term treatment that could be detrimental

to disease progression call for a more T cell-specific approach. Although metformin is generally considered a safe and low-cost compound for treatment of diabetes, detrimental gastrointestinal side-effects are observed relatively frequently [181]. Lactic acidosis is an infrequently observed complication of metformin usage but is potentially lethal [182]. To overcome potential off-target effects as the ones discussed above for metformin

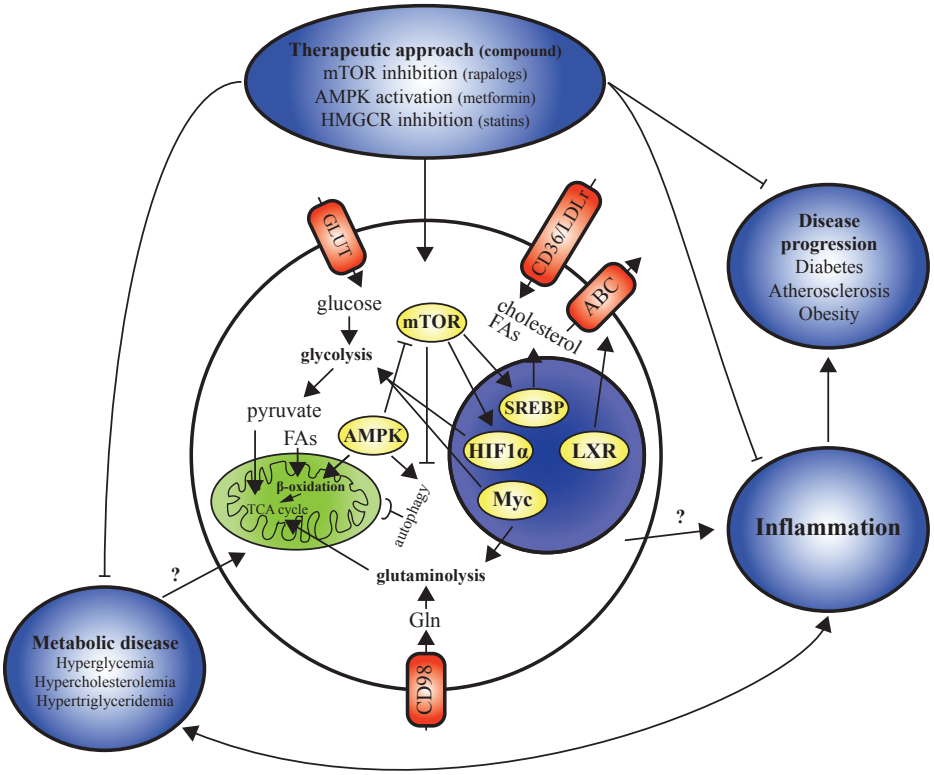

Figure 3 T cell metabolism as a potential driving force of inflammation and target for immunomodulation in metabolic disease-associated autoimmunity. A simplified scheme of T cell metabolism is presented as a hub whose (specific) modulation could prove a valuable therapeutic target. Whether T cell metabolism is modulated by a metabolically aberrant micro-environment (e.g. during hyperglycemia) and how this affects the inflammatory response remains elusive but probably provides an interesting novel therapeutic target. Systemic administration of clinically available compounds (such as rapalogs) could be used to therapeutically modulate T cell metabolism and subsequently dampen T cell-mediated autoimmunity and inflammation. This approach might also contribute to disease through off-target effects. T cell specific ex vivo therapy requires further investigation but would overcome possible off-target effects. Additionally, inhibition of one or multiple metabolic pathways might also be feasible, depending on the specific metabolic micro-environment T cells are exposed to (e.g. 2-DG in hyperglycemia). **GLUT**=glucose transporter, **CD36**=cluster of differentiation 36/fatty acid translocase, **LDLr**=low-density lipoprotein receptor, **ABC**= ATP-binding cassette transporter, **CD98**=cluster of differentiation 98/dimer Slc3a2/Slc7a5, **mTOR**=mammalian target of rapamycin, **HIF1α**=hypoxia inducible factor 1 alpha, **AMPK**= 5' AMP-activated protein kinase, **LXR**=liver-X-receptor, **SREBP**=sterol regulatory element-binding protein.

and rapamycin, *ex vivo* treatment of T cells isolated from peripheral blood with the suggested compounds, followed by an adoptive transfer, seems feasible as T cells are specifically targeted and any patient heterogeneity in off-target effects are abolished. $CD4^{+}CD127^{lo/-}$ T cells from peripheral blood of recent-onset T1DM patients can be successfully expanded *in vitro* into Tregs in the presence of rapamycin [183]. A disadvantage of this approach is that multiple time-consuming treatments would likely be necessary. *In vivo*, specifically targeting T cells using micro- or nanoparticles seems challenging due to the limited phagocytic capacity T cells have. A possibility lies in the endocytotic processes which ensue in T cells upon cytokine stimulation, for example after binding of IL-7 to CD127 [184]. Although this remains speculative and this approach is not entirely T cell specific, drug-cytokine tandems have been described in literature [185] and their development and therapeutic application might be achievable in the future. Regardless, modulation of T cell metabolism could form the hub in the treatment of diseases characterized by metabolic disease and autoimmunity (fig. 3).

CONCLUSION

The importance of cellular metabolism for T cell proliferation, differentiation and function is indisputable. For T cell metabolism-based therapy, there are multiple approaches to modulate metabolic pathways directly or to activate/inhibit modulators of metabolism to ultimately treat metabolic disease-associated autoimmunity. Examination of the metabolic pathways, which are likely to be modulated in T cells, during metabolic disease might reveal novel therapeutic targets for treatment of prevalent diseases such as diabetes, atherosclerosis, obesity and resulting cardiovascular disease. T cell-specific *in vivo* or *ex vivo* treatment might improve the general applicability of clinically available and novel compounds. Herein, a challenge lies in translating the findings from experimental (disease) models to human disease.

REFERENCES

1. Vander Heiden, M. G., Cantley, L. C. & Thompson, C. B. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324, 1029–33 (2009).
2. Warburg, O. On the origin of cancer cells. Science 123, 309–14 (1956).
3. Fox, C. J., Hammerman, P. S. & Thompson, C. B. Fuel feeds function: energy metabolism and the T-cell response. Nat Rev Immunol 5, 844–52 (2005).
4. Warburg, O., Gawehn, K. & Geissler, A. W. [Metabolism of leukocytes]. Z Naturforsch B 13B, 515–6 (1958).
5. MacIver, N. J., Michalek, R. D. & Rathmell, J. C. Metabolic regulation of T lymphocytes. Annual review of immunology 31, 259–83 (2013).
6. Ardawi, M. S. & Newsholme, E. A. Glutamine metabolism in lymphocytes of the rat. Biochem J 212, 835–42 (1983).
7. Brand, K. Glutamine and glucose metabolism during thymocyte proliferation. Pathways of glutamine and glutamate metabolism. Biochem J 228, 353–61 (1985).
8. Carr, E. L. et al. Glutamine Uptake and Metabolism Are Coordinately Regulated by ERK/MAPK during T Lymphocyte Activation. The Journal of Immunology 185, 1037–1044 (2010).
9. Chang, C. H. et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell 153, 1239–51 (2013).
10. Sena, L. A. et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. Immunity 38, 225–36 (2013).
11. Pearce, E. L., Poffenberger, M. C., Chang, C.-H. & Jones, R. G. Fueling immunity: insights into metabolism and lymphocyte function. Science (New York, N.Y.) 342, 1242454 (2013).
12. Pearce, E. L. et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature 460, 103–107 (2009).
13. van der Windt, G. J. et al. CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. Proc Natl Acad Sci U S A 110, 14336–41 (2013).
14. van der Windt, G. J. et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity 36, 68–78 (2012).
15. Jacobs, S. R. et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. J Immunol 180, 4476–86 (2008).
16. Cham, C. M., Driessens, G., O'Keefe, J. P. & Gajewski, T. F. Glucose deprivation inhibits multiple key gene expression events and effector functions in CD8+ T cells. Eur J Immunol 38, 2438–50 (2008).
17. Cham, C. M. & Gajewski, T. F. Glucose availability regulates IFN-gamma production and p70S6 kinase activation in CD8+ effector T cells. J Immunol 174, 4670–7 (2005).
18. WHO. Global status report on noncommunicable diseases 2014. (2014).
19. Duncan, B. B. et al. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes 52, 1799–805 (2003).
20. Libby, P., Ridker, P. M. & Maseri, A. Inflammation and atherosclerosis. Circulation 105, 1135–43 (2002).
21. Gregor, M. F. & Hotamisligil, G. S. Inflammatory mechanisms in obesity. Annual review of immunology 29, 415–45 (2011).
22. Gepts, W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 14, 619–33 (1965).

23. Itoh, N. et al. Mononuclear cell infiltration and its relation to the expression of major histocompatibility complex antigens and adhesion molecules in pancreas biopsy specimens from newly diagnosed insulin-dependent diabetes mellitus patients. J Clin Invest 92, 2313–22 (1993).
24. Sibley, R. K., Sutherland, D. E., Goetz, F. & Michael, A. F. Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. Lab Invest 53, 132–44 (1985).
25. Coppieters, K. T. et al. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med 209, 51–60 (2012).
26. Cybulsky, M. & Gimbrone, M. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 251, 788–791 (1991).
27. Brown, M. S. & Goldstein, J. L. A receptor-mediated pathway for cholesterol homeostasis. Science 232, 34–47 (1986).
28. Ross, R. & Harker, L. Hyperlipidemia and atherosclerosis. Science 193, 1094–100 (1976).
29. Zhou, X., Nicoletti, A., Elhage, R. & Hansson, G. K. Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. Circulation 102, 2919–2922 (2000).
30. Zhou, X., Paulsson, G., Stemme, S. & Hansson, G. K. Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. Journal of Clinical Investigation 101, 1717–1725 (1998).
31. Buckner, J. H. Mechanisms of impaired regulation by CD4+CD25+FOXP3+ regulatory T cells in human autoimmune diseases. Nature Reviews Immunology 10, 849–859 (2010).
32. de Boer, O. J., van der Meer, J. J., Teeling, P., van der Loos, C. M. & van der Wal, A. C. Low Numbers of FOXP3 Positive Regulatory T Cells Are Present in all Developmental Stages of Human Atherosclerotic Lesions. PLoS ONE 2, e779 (2007).
33. Gotsman, I. et al. Impaired regulatory T-cell response and enhanced atherosclerosis in the absence of inducible costimulatory molecule. Circulation 114, 2047–55 (2006).
34. Feuerer, M. et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nature Medicine 15, 930–939 (2009).
35. Norata, G. D. et al. The Cellular and Molecular Basis of Translational Immunometabolism. Immunity 43, 421–434 (2015).
36. Mauro, C., Fu, H. & Marelli-Berg, F. T cell trafficking and metabolism: novel mechanisms and targets for immunomodulation. Current Opinion in Pharmacology 12, 452–457 (2012).
37. O'Sullivan, D. & Pearce, E. L. Targeting T cell metabolism for therapy. Trends in Immunology 36, 71–80 (2015).
38. Greiner, E. F., Guppy, M. & Brand, K. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. J Biol Chem 269, 31484–90 (1994).
39. Macintyre, A. N. et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. Cell metabolism 20, 61–72 (2014).
40. Hume, D. A., Radik, J. L., Ferber, E. & Weidemann, M. J. Aerobic glycolysis and lymphocyte transformation. Biochem J 174, 703–9 (1978).
41. Michalek, R. D. et al. Cutting Edge: Distinct Glycolytic and Lipid Oxidative Metabolic Programs Are Essential for Effector and Regulatory CD4+ T Cell Subsets. The Journal of Immunology 186, 3299–3303 (2011).
42. De Rosa, V. et al. Glycolysis controls the induction of human regulatory T cells by modulating the expression of FOXP3 exon 2 splicing variants. Nature Immunology (2015). doi:10.1038/ni.3269

43. Procaccini, C. et al. The Proteomic Landscape of Human Ex Vivo Regulatory and Conventional T Cells Reveals Specific Metabolic Requirements. Immunity 44, 406–421 (2016).
44. Newsholme, E. A., Crabtree, B. & Ardawi, M. S. The role of high rates of glycolysis and glutamine utilization in rapidly dividing cells. Biosci Rep 5, 393–400 (1985).
45. Frauwirth, K. A. et al. The CD28 signaling pathway regulates glucose metabolism. Immunity 16, 769–777 (2002).
46. Garyu, J. W. et al. Characterization of diabetogenic CD8+ T cells: immune therapy with metabolic blockade. J Biol Chem (2016). doi:10.1074/jbc.M115.713362
47. Nicklin, P. et al. Bidirectional transport of amino acids regulates mTOR and autophagy. Cell 136, 521–34 (2009).
48. Wang, R. et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity 35, 871–82 (2011).
49. Newsholme, E. A., Crabtree, B. & Ardawi, M. S. M. Glutamine metabolism in lymphocytes: its biochemical, physiological and clinical importance. Quarterly journal of experimental physiology 70, 473–489 (1985).
50. Ardawi, M. S. & Newsholme, E. A. Maximum activities of some enzymes of glycolysis, the tricarboxylic acid cycle and ketone-body and glutamine utilization pathways in lymphocytes of the rat. Biochem J 208, 743–8 (1982).
51. Lund, P. Glutamine metabolism in the rat. FEBS Lett 117 Suppl, K86-92 (1980).
52. Langkamp-Henken, B., Johnson, L. R., Viar, M. J., Geller, A. M. & Kotb, M. Differential effect on polyamine metabolism in mitogen- and superantigen-activated human T-cells. Biochim Biophys Acta 1425, 337–47 (1998).
53. Windmueller, H. G. & Spaeth, A. E. Uptake and metabolism of plasma glutamine by the small intestine. J Biol Chem 249, 5070–9 (1974).
54. Klysz, D. et al. Glutamine-dependent alpha-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. Sci Signal 8, ra97 (2015).
55. Fadel, F. I. et al. Some amino acids levels: glutamine,glutamate, and homocysteine, in plasma of children with chronic kidney disease. Int J Biomed Sci 10, 36–42 (2014).
56. Stahl, A. A current review of fatty acid transport proteins (SLC27). Pflugers Arch 447, 722–7 (2004).
57. Sudhof, T. C., Goldstein, J. L., Brown, M. S. & Russell, D. W. The LDL receptor gene: a mosaic of exons shared with different proteins. Science 228, 815–22 (1985).
58. Greenwalt, D. E. et al. Membrane glycoprotein CD36: a review of its roles in adherence, signal transduction, and transfusion medicine. Blood 80, 1105–15 (1992).
59. Byersdorfer, C. A. et al. Effector T cells require fatty acid metabolism during murine graft-versus-host disease. Blood 122, 3230–3237 (2013).
60. Turner, A. P. et al. Sirolimus enhances the magnitude and quality of viral-specific CD8+ T-cell responses to vaccinia virus vaccination in rhesus macaques. Am J Transplant 11, 613–8 (2011).
61. Chen, H. W., Heiniger, H. J. & Kandutsch, A. A. Relationship between sterol synthesis and DNA synthesis in phytohemagglutinin-stimulated mouse lymphocytes. Proc Natl Acad Sci U S A 72, 1950–4 (1975).
62. Bensinger, S. J. et al. LXR Signaling Couples Sterol Metabolism to Proliferation in the Acquired Immune Response. Cell 134, 97–111 (2008).
63. Kidani, Y. et al. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. Nature immunology 14, 489–99 (2013).

64. Brown, M. S. & Goldstein, J. L. Suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and inhibition of growth of human fibroblasts by 7-ketocholesterol. J Biol Chem 249, 7306–14 (1974).
65. Chen, H. W., Kandutsch, A. A. & Waymouth, C. Inhibition of cell growth by oxygenated derivatives of cholesterol. Nature 251, 419–21 (1974).
66. He, H. T., Lellouch, A. & Marguet, D. Lipid rafts and the initiation of T cell receptor signaling. Semin Immunol 17, 23–33 (2005).
67. Janes, P. W., Ley, S. C., Magee, A. I. & Kabouridis, P. S. The role of lipid rafts in T cell antigen receptor (TCR) signalling. Semin Immunol 12, 23–34 (2000).
68. Thurnher, M. & Gruenbacher, G. T lymphocyte regulation by mevalonate metabolism. Science Signaling 8, re4–re4 (2015).
69. Chakrabarti, R. & Engleman, E. G. Interrelationships between mevalonate metabolism and the mitogenic signaling pathway in T lymphocyte proliferation. J Biol Chem 266, 12216–22 (1991).
70. Zeng, H. et al. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. Nature 499, 485–490 (2013).
71. Mausner-Fainberg, K. et al. The effect of HMG-CoA reductase inhibitors on naturally occurring CD4+CD25+ T cells. Atherosclerosis 197, 829–39 (2008).
72. Rodriguez-Perea, A. L., Montoya, C. J., Olek, S., Chougnet, C. A. & Velilla, P. A. Statins increase the frequency of circulating CD4+ FOXP3+ regulatory T cells in healthy individuals. J Immunol Res 2015, 762506 (2015).
73. Galluzzi, L., Pietrocola, F., Levine, B. & Kroemer, G. Metabolic Control of Autophagy. Cell 159, 1263–1276 (2014).
74. Russell, R. C. et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. Nat Cell Biol 15, 741–50 (2013).
75. Mizushima, N. Autophagy: process and function. Genes Dev 21, 2861–73 (2007).
76. Epple, U. D., Suriapranata, I., Eskelinen, E. L. & Thumm, M. Aut5/Cvt17p, a putative lipase essential for disintegration of autophagic bodies inside the vacuole. J Bacteriol 183, 5942–55 (2001).
77. Teter, S. A. et al. Degradation of lipid vesicles in the yeast vacuole requires function of Cvt17, a putative lipase. J Biol Chem 276, 2083–7 (2001).
78. Yang, Z., Huang, J., Geng, J., Nair, U. & Klionsky, D. J. Atg22 recycles amino acids to link the degradative and recycling functions of autophagy. Mol Biol Cell 17, 5094–104 (2006).
79. Hubbard, V. M. et al. Macroautophagy regulates energy metabolism during effector T cell activation. Journal of immunology (Baltimore, Md. : 1950) 185, 7349–57 (2010).
80. Li, C. et al. Autophagy Is Induced in CD4+ T Cells and Important for the Growth Factor-Withdrawal Cell Death. The Journal of Immunology 177, 5163–5168 (2006).
81. Willinger, T. & Flavell, R. A. Canonical autophagy dependent on the class III phosphoinositide-3 kinase Vps34 is required for naive T-cell homeostasis. Proc Natl Acad Sci U S A 109, 8670–5 (2012).
82. Pua, H. H., Guo, J., Komatsu, M. & He, Y.-W. Autophagy Is Essential for Mitochondrial Clearance in Mature T Lymphocytes. The Journal of Immunology 182, 4046–4055 (2009).
83. Ouimet, M. et al. Autophagy Regulates Cholesterol Efflux from Macrophage Foam Cells via Lysosomal Acid Lipase. Cell Metabolism 13, 655–667 (2011).
84. Laplante, M. & Sabatini, D. M. mTOR signaling in growth control and disease. Cell 149, 274–93 (2012).
85. Finlay, D. K. et al. PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8 $^{+}$ T cells. The Journal of Experimental Medicine 209, 2441–2453 (2012).

86. Powell, J. D., Lerner, C. G. & Schwartz, R. H. Inhibition of cell cycle progression by rapamycin induces T cell clonal anergy even in the presence of costimulation. J Immunol 162, 2775–84 (1999).
87. Colombetti, S., Basso, V., Mueller, D. L. & Mondino, A. Prolonged TCR/CD28 engagement drives IL-2-independent T cell clonal expansion through signaling mediated by the mammalian target of rapamycin. J Immunol 176, 2730–8 (2006).
88. Ganley, I. G. et al. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem 284, 12297–305 (2009).
89. Hosokawa, N. et al. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. Mol Biol Cell 20, 1981–91 (2009).
90. Jung, C. H. et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol Biol Cell 20, 1992–2003 (2009).
91. Duvel, K. et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. Mol Cell 39, 171–83 (2010).
92. Sun, Q. et al. Mammalian target of rapamycin up-regulation of pyruvate kinase isoenzyme type M2 is critical for aerobic glycolysis and tumor growth. Proceedings of the National Academy of Sciences 108, 4129–4134 (2011).
93. Kim, J. W., Tchernyshyov, I., Semenza, G. L. & Dang, C. V. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3, 177–85 (2006).
94. Papandreou, I., Cairns, R. A., Fontana, L., Lim, A. L. & Denko, N. C. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 3, 187–97 (2006).
95. Wang, G. L., Jiang, B. H., Rue, E. A. & Semenza, G. L. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 92, 5510–4 (1995).
96. Cunningham, J. T. et al. mTOR controls mitochondrial oxidative function through a YY1–PGC-1α transcriptional complex. Nature 450, 736–740 (2007).
97. Handschin, C. & Spiegelman, B. M. Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr Rev 27, 728–35 (2006).
98. Ben-Sahra, I., Howell, J. J., Asara, J. M. & Manning, B. D. Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. Science 339, 1323–8 (2013).
99. Robitaille, A. M. et al. Quantitative phosphoproteomics reveal mTORC1 activates de novo pyrimidine synthesis. Science 339, 1320–3 (2013).
100. Shrestha, S. et al. Treg cells require the phosphatase PTEN to restrain TH1 and TFH cell responses. Nature Immunology 16, 178–187 (2015).
101. Sarbassov, D. D. et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 22, 159–68 (2006).
102. Eilers, M. & Eisenman, R. N. Myc's broad reach. Genes Dev 22, 2755–66 (2008).
103. Dang, C. V. et al. The c-Myc target gene network. Semin Cancer Biol 16, 253–64 (2006).
104. Osthus, R. C. et al. Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. J Biol Chem 275, 21797–800 (2000).
105. Gao, P. et al. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature 458, 762–5 (2009).
106. Wise, D. R. et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci U S A 105, 18782–7 (2008).

107. Bandukwala, H. S. et al. Selective inhibition of CD4+ T-cell cytokine production and autoimmunity by BET protein and c-Myc inhibitors. Proc Natl Acad Sci U S A 109, 14532–7 (2012).
108. Blagih, J. et al. The Energy Sensor AMPK Regulates T Cell Metabolic Adaptation and Effector Responses In Vivo. Immunity 42, 41–54 (2015).
109. Marsin, A. S., Bouzin, C., Bertrand, L. & Hue, L. The stimulation of glycolysis by hypoxia in activated monocytes is mediated by AMP-activated protein kinase and inducible 6-phosphofructo-2-kinase. J Biol Chem 277, 30778–83 (2002).
110. MacIver, N. J. et al. The liver kinase B1 is a central regulator of T cell development, activation, and metabolism. J Immunol 187, 4187–98 (2011).
111. Hardie, D. G. AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. Genes Dev 25, 1895–908 (2011).
112. Carling, D. & Hardie, D. G. The substrate and sequence specificity of the AMP-activated protein kinase. Phosphorylation of glycogen synthase and phosphorylase kinase. Biochim Biophys Acta 1012, 81–6 (1989).
113. Jorgensen, S. B. et al. The alpha2-5'AMP-activated protein kinase is a site 2 glycogen synthase kinase in skeletal muscle and is responsive to glucose loading. Diabetes 53, 3074–81 (2004).
114. Li, Y. et al. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. Cell Metab 13, 376–88 (2011).
115. Munday, M. R. Regulation of mammalian acetyl-CoA carboxylase. Biochem Soc Trans 30, 1059–64 (2002).
116. Abu-Elheiga, L. et al. The subcellular localization of acetyl-CoA carboxylase 2. Proc Natl Acad Sci U S A 97, 1444–9 (2000).
117. Kim, J., Kundu, M., Viollet, B. & Guan, K.-L. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nature Cell Biology 13, 132–141 (2011).
118. Gwinn, D. M. et al. AMPK Phosphorylation of Raptor Mediates a Metabolic Checkpoint. Molecular Cell 30, 214–226 (2008).
119. Janowski, B. A., Willy, P. J., Devi, T. R., Falck, J. R. & Mangelsdorf, D. J. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature 383, 728–31 (1996).
120. Repa, J. J. et al. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science 289, 1524–9 (2000).
121. Brown, M. S. & Goldstein, J. L. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89, 331–40 (1997).
122. Solt, L. A., Kamenecka, T. M. & Burris, T. P. LXR-mediated inhibition of CD4+ T helper cells. PLoS One 7, e46615 (2012).
123. Geyeregger, R. et al. Liver X receptors interfere with cytokine-induced proliferation and cell survival in normal and leukemic lymphocytes. J Leukoc Biol 86, 1039–48 (2009).
124. Horton, J. D., Goldstein, J. L. & Brown, M. S. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. The Journal of clinical investigation 109, 1125–1131 (2002).
125. Delgoffe, G. M. et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity 30, 832–44 (2009).
126. Delgoffe, G. M. et al. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. Nat Immunol 12, 295–303 (2011).
127. Ballou, L. M., Selinger, E. S., Choi, J. Y., Drueckhammer, D. G. & Lin, R. Z. Inhibition of mammalian target of rapamycin signaling by 2-(morpholin-1-yl)pyrimido[2,1-alpha]isoquinolin-4-one. J Biol Chem 282, 24463–70 (2007).

128. Battaglia, M. Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. Blood 105, 4743–4748 (2005).
129. Haxhinasto, S., Mathis, D. & Benoist, C. The AKT-mTOR axis regulates de novo differentiation of CD4+Foxp3+ cells. J Exp Med 205, 565–74 (2008).
130. Kang, J., Huddleston, S. J., Fraser, J. M. & Khoruts, A. De novo induction of antigen-specific CD4+CD25+Foxp3+ regulatory T cells in vivo following systemic antigen administration accompanied by blockade of mTOR. J Leukoc Biol 83, 1230–9 (2008).
131. Kopf, H., de la Rosa, G. M., Howard, O. M. & Chen, X. Rapamycin inhibits differentiation of Th17 cells and promotes generation of FoxP3+ T regulatory cells. Int Immunopharmacol 7, 1819–24 (2007).
132. Shi, L. Z. et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. The Journal of experimental medicine 208, 1367–76 (2011).
133. Dang, E. V. et al. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. Cell 146, 772–84 (2011).
134. Berod, L. et al. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. Nature medicine 20, (2014).
135. Hu, X. et al. Sterol metabolism controls TH17 differentiation by generating endogenous RORγ agonists. Nature Chemical Biology 11, 141–147 (2015).
136. Yang, C. et al. Sterol intermediates from cholesterol biosynthetic pathway as liver X receptor ligands. J Biol Chem 281, 27816–26 (2006).
137. Cui, G. et al. Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation. J Clin Invest 121, 658–70 (2011).
138. Bai, Q. et al. Sulfation of 25-hydroxycholesterol by SULT2B1b decreases cellular lipids via the LXR/SREBP-1c signaling pathway in human aortic endothelial cells. Atherosclerosis 214, 350–6 (2011).
139. Chen, W., Chen, G., Head, D. L., Mangelsdorf, D. J. & Russell, D. W. Enzymatic reduction of oxysterols impairs LXR signaling in cultured cells and the livers of mice. Cell Metab 5, 73–9 (2007).
140. Deretic, V., Saitoh, T. & Akira, S. Autophagy in infection, inflammation and immunity. Nat Rev Immunol 13, 722–37 (2013).
141. Wei, J. et al. Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. Nature Immunology (2016). doi:10.1038/ni.3365
142. Kabat, A. M. et al. The autophagy gene Atg16l1 differentially regulates Treg and TH2 cells to control intestinal inflammation. eLife 5, e12444 (2016).
143. Chang, C.-H. et al. Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression. Cell (2015). doi:10.1016/j.cell.2015.08.016
144. McNamee, E. N., Korns Johnson, D., Homann, D. & Clambey, E. T. Hypoxia and hypoxia-inducible factors as regulators of T cell development, differentiation, and function. Immunol Res 55, 58–70 (2013).
145. Levin, M. et al. Mapping of ATP, glucose, glycogen, and lactate concentrations within the arterial wall. Arterioscler Thromb Vasc Biol 23, 1801–7 (2003).
146. Bjornheden, T., Levin, M., Evaldsson, M. & Wiklund, O. Evidence of hypoxic areas within the arterial wall in vivo. Arterioscler Thromb Vasc Biol 19, 870–6 (1999).
147. Kannel, W. B. & McGee, D. L. Diabetes and cardiovascular disease. The Framingham study. JAMA 241, 2035–8 (1979).
148. Xie, Z., He, C. & Zou, M. H. AMP-activated protein kinase modulates cardiac autophagy in diabetic cardiomyopathy. Autophagy 7, 1254–5 (2011).

149. Song, Y. M. et al. Metformin alleviates hepatosteatosis by restoring SIRT1-mediated autophagy induction via an AMP-activated protein kinase-independent pathway. Autophagy 11, 46–59 (2015).
150. Yin, Y. et al. Glucose Oxidation Is Critical for CD4+ T Cell Activation in a Mouse Model of Systemic Lupus Erythematosus. J Immunol 196, 80–90 (2016).
151. Nath, N. et al. Metformin attenuated the autoimmune disease of the central nervous system in animal models of multiple sclerosis. J Immunol 182, 8005–14 (2009).
152. Kang, K. Y. et al. Metformin downregulates Th17 cells differentiation and attenuates murine autoimmune arthritis. Int Immunopharmacol 16, 85–92 (2013).
153. Nath, N. et al. 5-aminoimidazole-4-carboxamide ribonucleoside: a novel immunomodulator with therapeutic efficacy in experimental autoimmune encephalomyelitis. J Immunol 175, 566–74 (2005).
154. Jellinger, P. S. et al. American Association of Clinical Endocrinologists' Guidelines for Management of Dyslipidemia and Prevention of Atherosclerosis. Endocr Pract 18 Suppl 1, 1–78 (2012).
155. Hansson, G. K., Jonasson, L., Holm, J. & Claesson-Welsh, L. Class II MHC antigen expression in the atherosclerotic plaque: smooth muscle cells express HLA-DR, HLA-DQ and the invariant gamma chain. Clin Exp Immunol 64, 261–8 (1986).
156. Hovingh, G. K., Davidson, M. H., Kastelein, J. J. & O'Connor, A. M. Diagnosis and treatment of familial hypercholesterolaemia. Eur Heart J 34, 962–71 (2013).
157. Armstrong, A. J., Gebre, A. K., Parks, J. S. & Hedrick, C. C. ATP-Binding Cassette Transporter G1 Negatively Regulates Thymocyte and Peripheral Lymphocyte Proliferation. The Journal of Immunology 184, 173–183 (2010).
158. Fessler, M. B. Regulation of Adaptive Immunity in Health and Disease by Cholesterol Metabolism. Current Allergy and Asthma Reports 15, (2015).
159. Pollock, A. H. et al. Prolonged Intake of Dietary Lipids Alters Membrane Structure and T Cell Responses in LDLr $^{-/-}$ Mice. The Journal of Immunology 196, 3993–4002 (2016).
160. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 344, 1383–9 (1994).
161. Dunn, S. E. Isoprenoids determine Th1/Th2 fate in pathogenic T cells, providing a mechanism of modulation of autoimmunity by atorvastatin. Journal of Experimental Medicine 203, 401–412 (2006).
162. Blank, N. et al. Atorvastatin Inhibits T Cell Activation through 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase without Decreasing Cholesterol Synthesis. The Journal of Immunology 179, 3613–3621 (2007).
163. Hakamada-Taguchi, R. et al. Inhibition of hydroxymethylglutaryl-coenzyme a reductase reduces Th1 development and promotes Th2 development. Circ Res 93, 948–56 (2003).
164. Coward, W. & Chow, S. C. Effect of atorvastatin on TH1 and TH2 cytokine secreting cells during T cell activation and differentiation. Atherosclerosis 186, 302–9 (2006).
165. Hillyard, D. Z. et al. Simvastatin inhibits lymphocyte function in normal subjects and patients with cardiovascular disease. Atherosclerosis 175, 305–13 (2004).
166. Joseph, S. B. et al. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. Proc Natl Acad Sci U S A 99, 7604–9 (2002).
167. Tangirala, R. K. et al. Identification of macrophage liver X receptors as inhibitors of atherosclerosis. Proc Natl Acad Sci U S A 99, 11896–901 (2002).

168. Kappus, M. S. et al. Activation of liver X receptor decreases atherosclerosis in Ldlr(-)/(-) mice in the absence of ATP-binding cassette transporters A1 and G1 in myeloid cells. Arterioscler Thromb Vasc Biol 34, 279–84 (2014).
169. Martinet, W., De Loof, H. & De Meyer, G. R. Y. mTOR inhibition: A promising strategy for stabilization of atherosclerotic plaques. Atherosclerosis 233, 601–607 (2014).
170. Lavigne, M. C., Grimsby, J. L. & Eppihimer, M. J. Antirestenotic mechanisms of everolimus on human coronary artery smooth muscle cells: inhibition of human coronary artery smooth muscle cell proliferation, but not migration. J Cardiovasc Pharmacol 59, 165–74 (2012).
171. Han, J. M., Patterson, S. J., Speck, M., Ehses, J. A. & Levings, M. K. Insulin Inhibits IL-10-Mediated Regulatory T Cell Function: Implications for Obesity. The Journal of Immunology 192, 623–629 (2014).
172. Winer, S. et al. Normalization of obesity-associated insulin resistance through immunotherapy. Nature Medicine 15, 921–929 (2009).
173. Mauro, C. et al. Obesity-Induced Metabolic Stress Leads to Biased Effector Memory CD4 + T Cell Differentiation via PI3K p110δ-Akt-Mediated Signals. Cell Metabolism 25, 593–609 (2017).
174. Fang, Y. et al. Duration of rapamycin treatment has differential effects on metabolism in mice. Cell metabolism 17, 456–62 (2013).
175. Pallet, N. & Legendre, C. Adverse events associated with mTOR inhibitors. Expert Opinion on Drug Safety 12, 177–186 (2013).
176. Brattstrom, C. et al. Hypertriglyceridemia in renal transplant recipients treated with sirolimus. Transplant Proc 30, 3950–1 (1998).
177. Morrisett, J. ., Abdel-Fattah, G. & Kahan, B. . Sirolimus changes lipid concentrations and lipoprotein metabolism in kidney transplant recipients. Transplantation Proceedings 35, S143–S150 (2003).
178. Holman, R. R., Paul, S. K., Bethel, M. A., Matthews, D. R. & Neil, H. A. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 359, 1577–89 (2008).
179. Sumarac-Dumanovic, M. et al. Therapeutic improvement of glucoregulation in newly diagnosed type 2 diabetes patients is associated with a reduction of IL-17 levels. Immunobiology 218, 1113–8 (2013).
180. Negrotto, L., Farez, M. F. & Correale, J. Immunologic Effects of Metformin and Pioglitazone Treatment on Metabolic Syndrome and Multiple Sclerosis. JAMA Neurology 73, 520 (2016).
181. DeFronzo, R. A. & Goodman, A. M. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. N Engl J Med 333, 541–9 (1995).
182. Bailey, C. J. & Turner, R. C. Metformin. N Engl J Med 334, 574–9 (1996).
183. Putnam, A. L. et al. Expansion of human regulatory T-cells from patients with type 1 diabetes. Diabetes 58, 652–62 (2009).
184. Faller, E. M., Ghazawi, F. M., Cavar, M. & MacPherson, P. A. IL-7 induces clathrin-mediated endocytosis of CD127 and subsequent degradation by the proteasome in primary human CD8 T cells. Immunol Cell Biol 94, 196–207 (2016).
185. Brooks, B. M., Flanagan, B. F., Thomas, A. L. & Coleman, J. W. Penicillin conjugates to interferon-gamma and reduces its activity: a novel drug-cytokine interaction. Biochem Biophys Res Commun 288, 1175–81 (2001).