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

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CHAPTER 2

T cell metabolism in metabolic disease-associated autoimmunity

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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 survival¹. Tumor cells (and proliferating cells) switch their metabolism from a respiratory towards a glycolytic profile, despite the presence of oxygen² to facilitate proliferation¹. 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 demand⁵. 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 glycolysis⁴, glutaminolysis⁶⁻⁸, 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 switch¹²⁻¹⁴. Accordingly, inadequate nutrient availability is a limiting factor for T cell proliferation, differentiation and function¹⁵⁻¹⁷. 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 inflammation¹⁸, including, diabetes¹⁹, atherosclerosis²⁰, obesity²¹ 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 disease²⁶⁻²⁸. Nevertheless, these diseases are generally associated with a type 1 autoimmune response^{21,25,29,30} and a loss of tolerance by regulatory T cells³¹⁻³⁴. 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 cells³⁵⁻³⁷. 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 ATP³⁸. 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 consuming¹. Activated T cells utilize glucose and glycolysis for cell growth and proliferation⁴⁰. The CD4⁺ T helper (Th) cells Th1, Th2 and Th17, characterized by high levels of interferon- γ (IFN γ), 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 function³⁹ and exhibit relatively high levels of fatty acid oxidation (FAO) and OXPHOS during differentiation and proliferation⁴¹⁻⁴³. During T cell proliferation, glycolysis additionally generates metabolic intermediates for anabolic pathways rather than just energy^{40,44} while OXPHOS is required for energy generation⁹. In activated T cells, glucose influx via GLUT1 is induced by CD28 co-stimulation in synergy with TCR/CD3 crosslinking⁴⁵.

Herein, CD28 acts as an adaptor protein to increase PI3K-Akt signaling which subsequently enhances expression of GLUT1, glucose uptake and glycolysis⁴⁵. 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) control³⁹. 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 proliferation³⁹. 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 FAO⁴². 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 proliferation⁴³. 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 mice⁴⁶.

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 ammonium⁴⁸. Glutamate can enter the mitochondria where it is converted into alpha ketoglutarate (α -KG) by glutamate dehydrogenase (Glu1). 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/CD28⁸ or polyclonal mitogens such as concanavalin A^{7,38} and glutaminase activity increases correspondingly⁴⁸. 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 controls⁴⁸. 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 TGF β dependent manner, even under Th1 polarizing conditions⁵⁴. Upon administration of the (cell-permeable) α -KG analog DMK, Th1 differentiation is rescued under glutamine deprivation conditions⁵⁴, 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 transporters⁵⁶, 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 cells⁴¹. Similarly, Th1, Th2 and Th17 cytokine production is decreased by exogenous FA supplementation while FoxP3 expression and suppressive function in Tregs are increased⁴¹. 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 accordingly⁵⁹, 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 FAO¹²⁻¹⁴. 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 capacity¹³. Etomoxir diminishes proliferation of Tm cells while simultaneously decreasing glycolysis and OXPHOS upon restimulation, as indicated by oxygen consumption- and extracellular acidification rate measurements¹³. 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 stimulation⁶¹⁻⁶³. 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 enzymes⁶⁸. Statins, a class of drugs which inhibit HMGCR, inhibits TCR-driven T cell expansion accordingly⁶⁹. 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 proliferation⁶³. 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 capacity⁷⁰. 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 not⁷¹. 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 reuse⁷³. 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 degraded⁷⁵⁻⁷⁷. 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 secretion⁷⁹. Furthermore, blocking autophagy during stimulation of CD4⁺ T cells decreases ATP production, lactate generation and FAO⁷⁹ 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 cells⁷⁹ as these need functional mitochondria for proper activation⁹. 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 transporters⁸³. 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 T_{eff} subtypes and T_m 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:

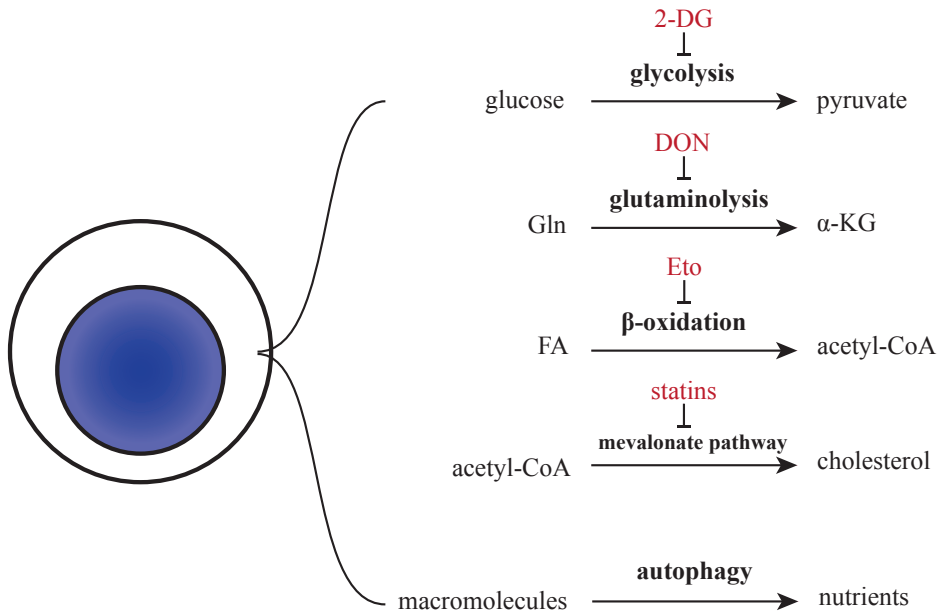


Figure 1 The most relevant metabolic pathways for T cell proliferation and differentiation are depicted with their corresponding inhibitors. The catabolic and anabolic processes which are important for adequate T cell growth, proliferation and differentiation are summarized with the most frequently used 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 survival⁸⁴. 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 (HIF1 α) by increasing its transcriptional activity and its translation, partly by increasing the translation of the 5'UTR sequence of a HIF1 α ^{91,92}. HIF1 α 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}. HIF1 α is upregulated under hypoxic conditions to ensure sufficient energy is generated through anaerobic glycolysis⁹⁵. *In vitro*, HIF1 α -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 HIF1 α is dispensable for acute metabolic programming prior to T cell proliferation. Rather, it is presumed HIF1 α plays a role in maintaining glycolysis when T cells enter mitosis⁴⁸. Apart from regulation of glycolysis through HIF1 α , mTORC1 can indirectly regulate metabolism through regulation of the YY1-PGC1 α 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 metabolism¹⁰⁰. mTORC2 is inhibited by long-term rapamycin treatment in various cell types by inhibiting the assembly of the mTORC2 complex¹⁰¹. 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 proliferation⁴⁸. 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 synthesis⁴⁸. 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 proliferation⁴⁸. 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 conditions⁴⁸. 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 activation⁴⁷. 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 pathways⁴⁸. Myc-inhibition using synthetic inhibitors such as 10058-F4 can prevent Th1 cells from promoting inflammation¹⁰⁷, 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 glycolysis¹⁰⁸⁻¹¹⁰, inhibition of glycogen synthesis and inhibition of gluconeogenesis¹¹¹⁻¹¹³. 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 expression¹¹⁶. 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 conditions¹⁰⁸. Counterintuitively, IFN γ production in CD8⁺ T cells after re-stimulation was higher in *Prkaa1*^{-/-} cells at both physiological (5 mM) and high glucose levels (25 mM) compared to control T cells¹⁰⁸. This is explained by the fact that AMPK inhibits the translation of IFN γ mRNA¹⁰⁸, 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 oxysterols¹¹⁹. Its main action is to induce cholesterol efflux via ABC transporters¹²⁰ thereby, effectively counteracting SREBP¹²¹, 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 LXR β KO mice suggested this particular isoform of LXR is important for proliferation as both CD4⁺ as CD8⁺ T cell proliferation was increased compared to WT⁶². 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 promoter sites in their target genes indicating SREBP1 and SREBP2 co-regulate lipid synthesis⁶³. 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 HIF1 α , 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 cells⁴¹. 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 cells¹²⁵. Rheb-deficient T cells, which lack mTORC1 activity, fail to differentiate into Th1 and Th17 cells¹²⁶. 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 cells¹²⁶. 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-complexes¹²⁶. 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 HIF1 α and Th17 generation is highly dependent on HIF1 α ^{132,133}. HIF1 α -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 differentiation¹³². Lipid biogenesis is especially crucial for Th17 differentiation during which ACC1 and ACC2 play a central role¹³⁴ 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 Tregs¹³⁴ 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 TGF β 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 differentiation¹³⁵. During cholesterol synthesis, desmosterol, a

precursor for cholesterol, serves as an endogenous agonist for ROR γ , a key transcription factor for Th17 development¹³⁵. This is surprising as desmosterol is a low-affinity LXR agonist¹³⁶ and LXR activation with synthetic ligands inhibits Th17 differentiation¹³⁷. 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 ROR γ -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 profile¹⁴⁰ 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 stability¹⁴¹. Moreover, Atg7-deficient Tregs have increased glycolytic metabolism and increased expression of IFN γ and IL-17 as compared to Atg7 WT Tregs¹⁴¹. Foxp3^{Cre}Atg5^{fl/fl} mice have higher IFN γ expression in CD4⁺ and CD8⁺ T cells and lower Treg percentages compared to Foxp3^{Cre}Atg5^{+/+} mice, underlining the requirement of functional autophagy for Tregs to maintain immune homeostasis¹⁴¹. 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 survival¹⁴². 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 NH₄Cl⁷⁹. Likewise, acute deletion of Atg7 after Th1 differentiation results in diminished IFN γ secretion⁷⁹. 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.

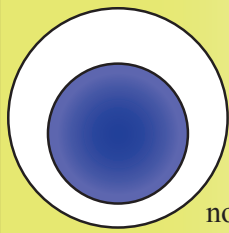
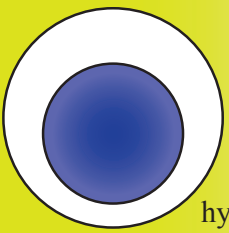
1. Physiological EC glucose availability	1. Increased EC glucose availability
2. Physiological IC glucose	2. Increased IC glucose
3. No metabolic environment-induced increase in glycolysis	3. Hyperglycemia-induced increase in glycolysis
4. No biased IC bifunctional enzyme usage	4. Biased IC bifunctional enzyme usage
5. No selective metabolic restriction	5. Selective metabolic restriction
	
normoglycemia	hyperglycemia

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 hallmarks²²⁻²⁴. 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 IFN γ secretion by CD8⁺ T cells¹⁰⁸. 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 manner¹⁰⁸, 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 IFN γ 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* promoter and *FoxP3* CNS2 where it can induce splicing variants of *FoxP3* which result in less functional Tregs⁴². 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 tumor¹⁴³. A similar discrepancy between supply and demand of certain nutrients for T cells might be present in hypoxic tissues such as atherosclerotic plaques¹⁴⁴⁻¹⁴⁶. 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 patients¹⁴⁷. 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 Tregs³¹. 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 arthritis¹⁵⁰⁻¹⁵². 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 IFN γ 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 atherosclerosis¹⁵⁴, 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 lipoproteins¹⁵⁶. Hypercholesterolemia might drive T cell-mediated autoimmunity by providing substrate for membrane synthesis, as cholesterol supplementation *in vitro* is known to drive T cell proliferation¹⁵⁷. 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 synapse¹⁵⁸. 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 status¹⁵⁹. 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 disease¹⁶⁰. 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 simvastatin¹⁶⁵. 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 disease¹⁶⁶⁻¹⁶⁸.

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 others¹⁶⁹. 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 restenosis¹⁷⁰.

mTOR signaling also plays a role in obesity-associated low grade inflammation²¹. Inflamed visceral adipose tissue (VAT) contains a disproportionately low amount of Tregs displaying a dysfunctional phenotype, as opposed to Tregs from lean VAT³⁴. 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 pathway¹⁷¹. Increasing the Treg population in inflamed VAT has been shown to reduce HFD-induced obesity and insulin resistance¹⁷². 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 effects¹⁷¹. 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-selectin¹⁷³. This contributes to biased differentiation of inflammatory CD4⁺ T cells which is observed during chronic low grade inflammation in obesity¹⁷³.

Although long-term rapamycin treatment improves the metabolic state in animal models¹⁷⁴, a potential side-effect of rapamycin is dyslipidemia which occurs in 40-75% of the patients receiving rapamycin treatment¹⁷⁵⁻¹⁷⁷. Detrimental effects of rapamycin in mice are found early in treatment¹⁷⁴ 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 intervention¹⁷⁸. 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 cells¹⁷⁹. 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 patients¹⁸⁰. Additionally, circulating Treg percentage was increased in metformin treated patients, as well as Treg suppressive capacity and IL-10 secretion¹⁸⁰. 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 frequently¹⁸¹. Lactic acidosis is an infrequently observed complication of metformin usage but is potentially lethal¹⁸². To overcome potential off-target effects as the ones discussed above for metformin

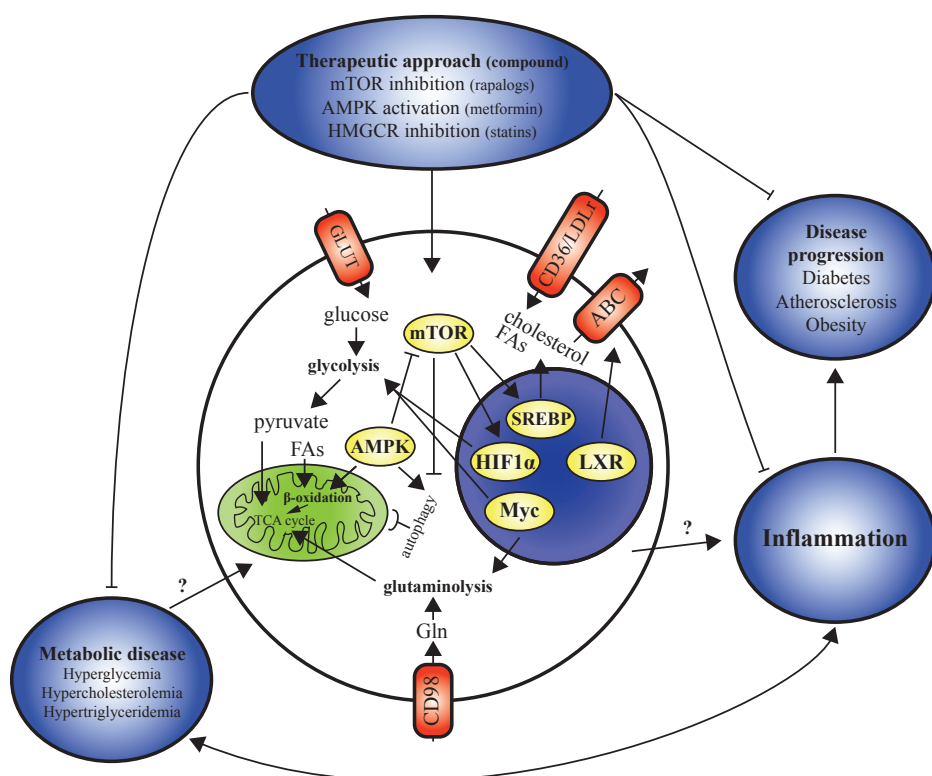


Figure 3 T cell metabolism as a potential driving force of inflammation and target for immunomodulation in metabolic disease-associated autoimmunity. A simplified scheme of T cell metabolism is presented as a hub whose (specific) modulation could prove a valuable therapeutic target. Whether T cell metabolism 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, **HIF1 α** =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 rapamycin¹⁸³. 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 CD127¹⁸⁴. Although this remains speculative and this approach is not entirely T cell specific, drug-cytokine tandems have been described in literature¹⁸⁵ 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.

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