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Metabolic switching and fuel choice during T-cell differentiation and memory development

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Summary: Clearance or control of pathogens or tumors usually requires T-cell-mediated immunity. As such, understanding the mechanisms that govern the function, maintenance, and persistence of T cells will likely lead to new treatments for controlling disease. During an immune response, T-cell development is marked by striking changes in metabolism. There is a growing appreciation that these metabolic changes underlie the capacity of T cells to perform particular functions, and this has led to a recent focus on the idea that the manipulation of cellular metabolism can be used to shape adaptive immune responses. Although interest in this area has grown in the last few years, a full understanding of the metabolic control of T-cell functions, particularly during an immune response *in vivo*, is still lacking. In this review, we first provide a basic overview of metabolism in T cells, and then we focus on recent studies providing new or updated insights into the regulation of metabolic pathways and how they underpin T-cell differentiation and memory T-cell development.

Keywords: *T cell, metabolism, immune response*

Conventional view of T-cell metabolism

T cells develop in the thymus and then enter the periphery, where their role is to protect the body from invading pathogens and cancers. Circulating naive T cells are quiescent, but upon T-cell receptor (TCR)-mediated recognition of antigen and the receipt of costimulatory signals, these cells become activated, undergo extensive proliferation, and acquire effector functions, such as the ability to produce cytokines and cytolytic molecules. The differences in functional and phenotypic characteristics of quiescent T cells and activated T cells are supported by distinct metabolic requirements. Specifically, quiescent T cells (i.e. naive or memory T cells) have a catabolic metabolism where nutrients are broken down to fuel cell survival. In contrast, activated T cells adopt an anabolic metabolism, where nutrients will also be used to construct the molecular building blocks that are incorporated into cellular biomass to support

proliferation (1–3). The balance of catabolic and anabolic pathways in a cell determines how much adenosine triphosphate (ATP) is generated versus consumed, the availability of biosynthetic precursors, and the redox status of the cell.

Upon activation, T cells increase in size and undergo a metabolic transition from oxidative phosphorylation (OXPHOS) to glycolysis (4–6) (*Fig. 1*). Signals from growth factor cytokines like interleukin-2 (IL-2) and the ligation of costimulatory CD28 promote this switch to glycolysis by inducing the phosphoinositol 3-kinase (PI3K)-dependent activation of AKT (7, 8). Mamalian target of rapamycin (mTOR) is a key regulator of translation (9) and cell size (10) that stimulates glycolysis and cellular metabolism (11) by increasing glycolytic enzyme activity and enhancing the expression of nutrient transporters. Active AKT promotes the mTOR pathway, thereby enabling increased utilization of glucose and amino acids (2, 12–16). Activated T cells aerobically ferment glucose into lactate, a process that occurs despite the presence of sufficient oxygen to support mitochondrial OXPHOS (5, 6) (*Fig. 1*). This phenomenon is referred to as the Warburg effect, after Otto Warburg who in the 1920s first described this process in cancer cells (17). The current view of metabolism in activated T cells is one where the induction and maintenance of Warburg metabolism, in addition to glutamine uptake and utilization, is essential for activation, proliferation, and the expression of effector functions (5, 7, 18–21) (*Fig. 2*).

Glucose and proliferating T cells

Glucose is an essential nutrient for T cells, and its uptake is regulated by glucose transporter expression (22). TCR stimulation leads to trafficking of the glucose transporter Glut1 from intracellular vesicles to the cell surface (8), while signals via CD28 and growth factor cytokines further increase its expression and cell surface localization (1, 5, 7). Naive T cells isolated from Glut1 overexpressing mice are larger, and these mice have increased the numbers of $CD44^{hi}$ T cells, suggesting that the earliest steps in T-cell activation, the increase in cell size and activation marker expression, are dependent on glucose utilization (18). It has also been shown that in the absence of glucose, T-cell survival and proliferation is impaired, even in the presence of an alternative carbon source such as glutamine (18, 23). In addition, T-cell cytokine production is also thought to be dependent on glucose. For example, glucose deprivation has been shown to strongly inhibit IFN-γ gene expression (18, 24),

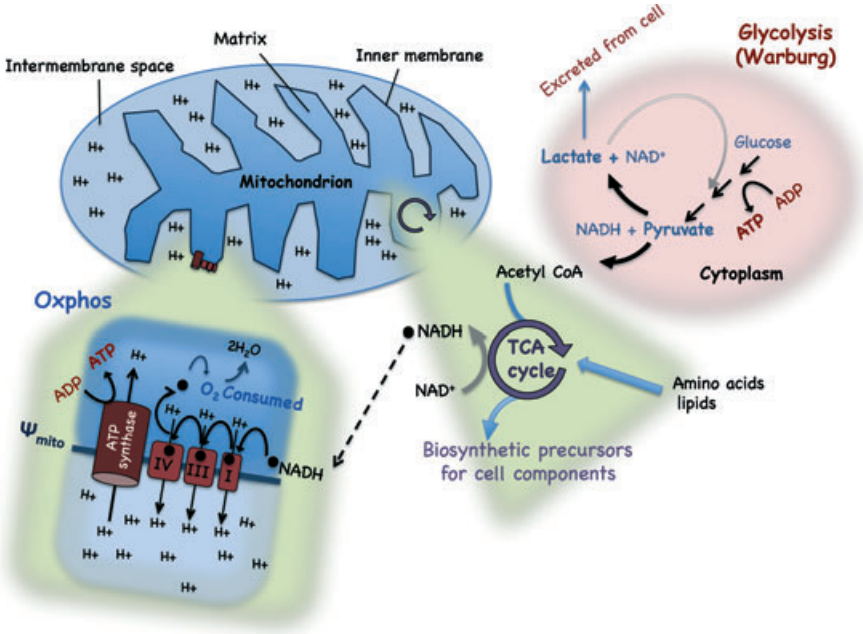

***Fig. 1.* ATP-generating metabolic pathways**. Quiescent T cells mainly use oxidative phosphorylation (OXPHOS) to generate ATP, a process that takes place in mitochondria. A variety of substrates, such as glucose, amino acids, and lipids, are oxidized in the TCA cycle; these reactions generate biosynthetic precursors as well as the reducing agents NADH. The NADH coenzyme, which is used in many biochemical reactions, donates electrons to the electron transport chain for OXPHOS. Upon activation, T cells upregulate glycolysis, the process in which glucose is converted into pyruvate, thereby generating ATP and reducing NAD^+ to NADH. To fuel OXPHOS, pyruvate can be shuttled into the TCA cycle; however, during Warburg metabolism, the majority of pyruvate is converted into lactate, which is excreted from the cell. In the process of converting pyruvate to lactate, NADH is oxidized to NAD^+, which in turn can support continued glycolysis.

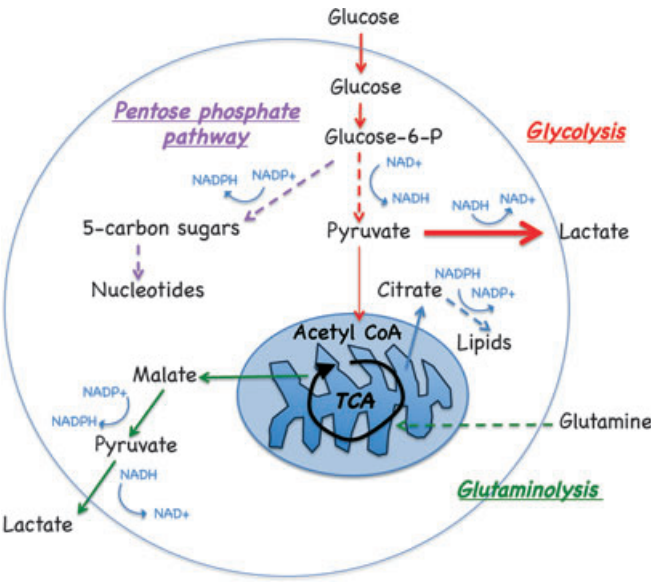

***Fig. 2.* Metabolic pathways in activated T cells.** Activated T cells use glucose and glutamine as their main nutrients to support rapid proliferation and effector functions. Glucose uptake is dependent on glucose transporter expression, and once inside the cell, glucose is converted into glucose-6-phosphate. This intermediate is the starting point for glycolysis, which ultimately leads to the production of pyruvate. In Warburg metabolism, the majority of pyruvate is excreted as lactate. Glutamine uptake and metabolism is also critical in activated T cells. As proliferation requires rapid synthesis of macromolecules for the generation of daughter cells, biosynthetic intermediates provided by the TCA cycle are necessary to support this process. Citrate from the TCA cycle can be excreted into the cytosol and used for lipid synthesis. Lipid synthesis also requires reductive power in the form of NADPH. NADPH is generated by the conversion of malate, excreted from the mitochondria, into pyruvate. Alternatively, using glucose-6-phosphate in the pentose phosphate pathway also generates NADPH. This latter pathway is important for nucleotide synthesis. Dashed arrows indicate multiple steps. Red, green, and purple arrows indicate glycolysis, glutaminolysis, and pentose phosphate pathways, respectively.

whereas transgenic expression of Glut1 leads to enhanced IL-2 and IFN-γ production (18). Also, in the absence of glucose, $CD8^+$ T cells have a defect in cytolytic activity marked by reduced granzyme and perforin production (19). Collectively, many studies illustrate that glucose is an essential nutrient for T cells.

Once inside the cell, glucose is degraded and used to (i) generate energy, either through Warburg metabolism or the TCA cycle and OXPHOS, and (ii) to provide the building blocks for cellular maintenance and proliferation (*Fig.* 1). To produce ATP from OXPHOS, glucose is converted to pyruvate via the glycolytic pathway and then to acetyl CoA, where it can enter the TCA cycle by condensing with oxaloacetate (*Fig.* 2). TCA cycle reactions generate the reduced coenzyme NADH, which acts as an electron donor for the electron transport chain (ETC.), fueling ATP production through OXPHOS. Glycolysis can also generate ATP in the cytoplasm via substrate level phosphorylation. Here, glucose is not fully oxidized in the mitochondria, even though there is sufficient oxygen present to do so (Warburg effect), but rather glucose-derived pyruvate is converted to lactate in the cytoplasm and excreted from the cell. Lactate excretion supports continued Warburg metabolism by regenerating NADH to NAD^+, a coenzyme that facilitates earlier steps of glucose breakdown (2) (*Fig.* 1). As an alternative to glycolysis, glucose-derived glucose-6-phosphate can be directed into the pentose phosphate pathway (*Fig.* 2). The pentose phosphate pathway is important for proliferating cells as it provides precursors for the synthesis of nucleotides and aromatic amino acids, as well as reducing equivalents in the form of NADPH. NADPH is required to maintain the pool of reduced glutathione, as well as to support lipid and cholesterol synthesis (*Fig.* 2).

Glutamine and proliferating T cells

In addition to the switch to glycolysis, several other metabolic pathways are altered upon T-cell activation. Changes include decreased fatty acid oxidation, decreased pyruvate flux into the TCA cycle, increased glucose flux into the pentose phosphate pathway, and increased glutamine metabolism (21, 25, 26) (*Fig.* 2). Similar to glucose uptake, growth factor signaling controls the import of glutamine into the cell. It was recently shown that in an IL-3-dependent cell line glutamine uptake is dependent on glucose flux through the hexosamine pathway (27). The IL-3 receptor, like receptors for other growth factor cytokines, is a glycoprotein whose surface expression is dependent on glucose availability for glycosylation, thus providing an explanation as to why in certain settings glucose is essential for mammalian cells even when there is an abundant supply of amino acids available (28). Glutamine uptake and metabolism has been shown to be critical for T-cell function (7, 20). Metabolism of glutamine, termed glutaminolysis in analogy to glycolysis, is an energy-producing pathway. In contrast with glycolytic energy production, glutaminolytic energy production requires mitochondrial OXPHOS (3). In addition to energy, proliferating T cells, much like tumor cells, require the rapid synthesis of macromolecules that can be incorporated into cellular machinery and membrane components to form daughter cells. Synthesis of macromolecules requires biosynthetic intermediates, which are provided by the TCA cycle through the conversion of metabolites such as pyruvate and glutamine.

As described earlier, pyruvate derived from glycolysis is converted into acetyl CoA, which condenses with oxaloacetate

to generate citrate. Glutamine can enter the TCA via conversion to α-ketoglutarate, which can also be processed to oxaloacetate and subsequently citrate. To generate new lipids, citrate has to be excreted into the cytosol, where it can be converted to acetyl CoA, the backbone for lipid synthesis (29) (*Fig.* 2). Thus, both glucose and glutamine can be used as carbon sources for lipid synthesis. Cells must be capable of rapidly replenishing TCA cycle intermediates if any are drawn off for biosynthesis. Under normoxic conditions, most of the carbon for fatty acid synthesis is derived from glucose; however, glutamine is also important for lipid synthesis, as it replenishes oxaloacetate for continued TCA cycle function (23, 30–32). In addition to carbon, reductive power in the form of NADPH is required for lipid synthesis (29). Along with NADPH production from the pentose phosphate pathway, NADPH is also produced during glutaminolysis when glutamine is metabolized in the TCA cycle to pyruvate and then converted into lactate (31) (*Fig.* 2). The need for NADPH to support lipid and nucleotide biosynthesis as well as for maintenance of reduced glutathione may explain why the majority of glutamine-derived carbon is excreted as lactate in rapidly proliferating cells (31). In addition, high rates of glutamine metabolism in proliferating cells results in excess intracellular nitrogen that must be secreted as alanine or ammonia to avoid toxicity (29). Glutamine also provides a nitrogen source for non-essential amino acids and nucleotides (31, 33).

Quiescent T cells

Much more is known about the metabolic pathways that support T-cell activation and proliferation than the metabolic pathways that support T-cell quiescence. Naive and memory T cells are smaller than activated T cells, have decreased metabolic activity that is primarily catabolic in nature, and are more resistant to apoptosis than activated cells (34). The conventional view suggests that quiescent T cells interchangeably break down glucose, amino acids, and fatty acids to fuel the TCA cycle and generate ATP in their mitochondria via OXPHOS (1, 4). Although TCR stimulation and CD28 costimulation are required for the switch from a quiescent metabolism to the glycolytic metabolism of an activated cell (7, 18), it has been shown that maintaining quiescence in naive T cells is not simply due to a lack of these signals but is an active process that involves metabolic regulation (34–36).

Regulation of metabolic pathways in T cells

The factors that regulate metabolism in cells are highly interconnected. Given the fundamental role of metabolism in virtually every cellular process, there has been accumulating interest in how T-cell metabolism is controlled during different phases of T-cell differentiation. For organizational purposes, we have chosen to discuss some of the latest findings in this field as they pertain to naive T cells, effector T cells, and memory T cells independently.

Naive T cells

KLF and Fox transcription factors

Naive T cells maintain a catabolic metabolism, which is actively enforced (34–36). The Forkhead box (Fox) and Kruppel-like family of transcription factors (KLF) are thought to be responsible for T-cell quiescence by inducing inhibitors of cellular activation (34). Several recent studies have suggested that Fox and KLF2 transcription factors indirectly maintain quiescence by regulating the survival and migration of T cells (37–40) (*Fig.* 3). A study by Takada *et al.* (40) showed that KLF2-deficient $CD8^+$ T cells had only a minimal defect in cell cycle control and displayed normal proliferative capacity. However, expression of the homing receptors $S1P_1$ and CD62L were decreased and thus trafficking of T cells *in vivo* was impaired (40). In another study (41), T cells deficient in

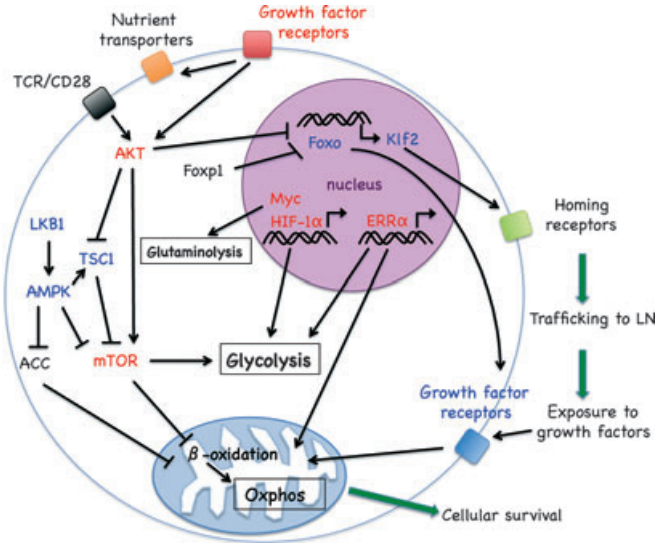

Fig. 3. Factors regulating metabolism in T cells. Metabolism has a fundamental role in virtually every cellular process, and there has been a growing interest in how metabolic pathways regulate T-cell function and fate. This diagram highlights some of the factors recently described in the literature to regulate metabolism in quiescent and activated T cells. Factors promoting anabolic pathways support the metabolic needs of T cells during activation and proliferation (red). Factors that regulate quiescence maintain catabolic mitochondrial metabolism and promote cell survival and persistence (blue). Depending on the context, signaling through growth factor receptors (red) can drive proliferation and effector T-cell differentiation (e.g. IL-2 signals), or promote survival (blue) through enhanced mitochondrial function (e.g. IL-15 and IL-7 signals).

the transcription factor ELF4 showed reduced KLF2 and KLF4 expression, which resulted in diminished CD62L expression and increased homeostatic and antigen-driven proliferation, respectively (41). It was later shown by the same group that mTOR signaling, which is induced during T-cell activation (*Fig.* 3), inhibits ELF4 expression in activated $CD8^+$ T cells, releasing them from their quiescent state (42). Altered homing receptor expression changes trafficking patterns of naive T cells and thereby affects exposure to growth factors and subsequently the metabolic pathways in these cells.

Foxo1 has been found to be essential for IL-7Rα expression in peripheral T cells (38), and IL-7 signaling is critical for naive T-cell survival (43, 44) (*Fig.* 3). In line with these observations, it was recently shown that inactivation of Foxo1 was essential for effector differentiation of $CD8^+$ T cells, a process regulated through the transcription factor T-bet and the mTOR kinase (45). In fasted muscle cells, Foxo1 is activated and will suppress glucose oxidation (46) while enhancing surface levels of the fatty acid translocase FAT/CD36 (47) and thus promoting fatty acid uptake and oxidation. These data suggest that Foxo1 prepares the muscle cell for the increased reliance on fatty acid metabolism that is characteristic of fasting and is consistent with the idea that Foxo1 is contributing to the maintenance of metabolic quiescence in naive T cells. Interestingly, a different Fox factor, Foxp1, has been demonstrated to compete with Foxo1 for the control of IL-7Rα expression, and loss of Foxp1 induced naive T cells to gain an effector phenotype (48). Whereas normal naive T cells do not proliferate in response to IL-7 (49), when Foxp1 was lost, IL-7Rα expression increased and proliferation was induced. In addition to promoting IL-7Rα expression, Foxp1 deficiency induced other changes, including enhanced Erk activation, and inhibition of Erk and MEK severely attenuated the proliferative response of Foxp1 deficient T cells. Thus, Foxp1 appears to affect more than one pathway to maintain naive T-cell quiescence (48).

MST1 is a kinase that directly activates FOXO transcription factors. The MST/FOXO pathway has been shown to mediate oxidative stress responses and extend lifespan in both mammalian neurons and nematodes (50). A recent study reported a primary immunodeficiency phenotype in humans that was associated with the loss of MST1 function and primarily characterized by a progressive decline in naive T cells (51). In T cells, MST-1 was necessary for Foxo1 protein stability and expression, and indeed IL-7Rα and CD62L expression was impaired on T cells from these patients (51). In line with impaired IL-7R signaling, Bcl-2 expression levels were low and cell survival was diminished in MST-deficient T cells (51). Collectively, these observations indicate that effective maintenance of T-cell quiescence and survival is likely a balanced interplay between regulating metabolism and apoptosis, activation, and migration of naive T cells.

Tsc and mTOR

Several recent studies investigated the role of the tumor suppressor Tsc1, a regulator of the mTOR pathway, in maintaining naive T-cell quiescence (*Fig.* 3). It was found that Tsc1 established a quiescent gene-expression program linking cell cycle and metabolism with the expression of immune response genes in T cells (35). T cells that lacked Tsc1 were larger and proliferated more than control T cells, indicating a loss of quiescence and a more activated state, which resulted from increased mTORC1 activation (35, 52). Loss of Tcs1 also led to more ROS production and thus enhanced apoptosis and lower numbers of peripheral T cells (35, 36, 52, 53). Unexpectedly, although Tsc1 deficiency caused hyperactive responses to TCR stimulation *in vitro*, expansion of Tsc1-deficient $CD8^+$ T cells upon bacterial infection *in vivo* was impaired, demonstrating that maintenance of T-cell quiescence is important for a productive immune response (35). Failure to mount an efficient T-cell response *in vivo* was also observed when the excessive apoptosis evident in Tsc1-deficient T cells was blocked by transgenic expression of Bcl2, suggesting that Tsc1 deficiency impairs T-cell responses through a mechanism that is largely independent of cell survival. It was previously shown that hematopoietic stem cells (HSCs) deficient for Tsc1 have increased expression of many mitochondrial genes and higher rates of OXPHOS that leads to increased levels of ROS (54). Loss of Tsc1 function resulted in reduced hematopoiesis and self-renewal of HSCs. Treatment with a ROS antagonist *in vivo* restored HSC numbers and function, suggesting that the Tsc1-mTOR pathway serves to suppress mitochondrial respiration and ROS production, and thereby maintains quiescence in hematopoietic stem cells (54). In a separate study, it was also shown that scavenging ROS decreased cell death of Tsc1-deficient peripheral $CD4^+$ T cells (36). Together, these studies illustrate how the Tsc1-mTOR pathway maintains quiescence by regulating cellular metabolism.

Lkb1 and AMPK

Evidence is emerging that the liver kinase B1 (LKB1) is also important in controlling T-cell metabolism and quiescence (*Fig.* 3). LKB1 is a serine/threonine kinase and is part of an evolutionarily conserved energy-sensing pathway (55, 56). It was first identified as the tumor repressor responsible for Peutz-Jeghers syndrome (57) and was recently described to

regulate quiescence and metabolism in hematopoietic stem cells (58–60). LKB1 can exert its effects on cellular metabolism via the phosphorylation of the α subunit of AMP-activated protein kinase (AMPK), and AMPK activation by LKB1 is essential under conditions of bioenergetic stress (61, 62). AMPK has a central role in the regulation of energy homeostasis by mediating glucose, protein, and fatty acid metabolism (63). In contrast with the P13K/AKT/mTOR pathway, which promotes anabolism, AMPK supports catabolism and inactivates growth-inducing pathways. As quiescent cells have a catabolic metabolism and mostly use OXPHOS for their energy, the regulatory effects of AMPK in quiescent cells are likely to merge with the oxidation of fatty acids to supply energy needs. Activation of AMPK can promote the oxidation of fatty acids in two ways (64, 65): through suppression of mTOR activity (66) and by phosphorylating and thereby inactivating acetyl-CoA carboxylase (ACC) (67, 68) (*Fig.* 3). Although it was shown that AMPK was not the critical effector of LKB1 in the maintenance of hematopoiesis (58–60), LKB1 regulated mitochondrial function is at least partly AMPK dependent (58, 60). Recently, it was found that LKB1 is essential for the survival of thymocytes and development of T-cell progenitors (69, 70) and is required for $CD4^+$ and $CD8^+$ T-cell development and peripheral homeostasis (71). LKB1-deficient peripheral T cells were shown to have enhanced glucose uptake and a higher glycolytic rate (71), whereas LKB1-deficient thymocytes were shown to have reduced expression of the CD98 subunit of the amino L-acid transporter (70). These results suggest that LKB1 is critical for matching metabolism to energy demand and in this way acts as an important regulator of cellular quiescence. Future studies are required to further elucidate the role of mTOR and AMPK signaling in maintaining quiescence in T cells and its precursors.

Effector T cells

AKT, LKB1, and AMPK

T cells increase their metabolism in response to immune stimulation via the serine-threonine kinase AKT (7, 8) (*Fig.* 3). AKT is rapidly activated in response to TCR triggering or common γ-chain cytokines, such as IL-2 and IL-15 (72–75). However, it was recently reported that AKT-independent pathways also control T-cell metabolism, survival, and proliferation (76). AKT was shown to be dispensable for the regulation of glucose uptake in effector $CD8^+$ T cells (77) but rather maintained a critical role in T-cell effector functions by regulating the expression of cytolytic molecules and the repertoire of cytokine/chemokine-receptors and adhesion molecules (77). In addition to describing the role of LKB1 in controlling cellular quiescence, a recent study by MacIver *et al.* (71) investigated the role of LKB1 in T-cell development and peripheral function. In response to activation, LKB1-deficient T cells displayed defects in proliferation, viability, and altered metabolism. Loss of LKB1 resulted in enhanced glycolytic gene expression and impaired oxidation of lipids in response to metabolic stress induced by IL-2 withdrawal. In line with enhanced glucose metabolism, LKB1-deficient T cells had greater expression of surface activation markers and increased production of IFN-γ. Furthermore, T cells deficient for AMPK displayed similar defects in activation, metabolism, and cytokine production. As expected, both LKB1 and AMPK-1α-deficient T cells showed increased phosphorylation of mTOR targets, and rapamycin blocked the enhanced IFN-γ production in these cells (71). This study demonstrates that the metabolic effects of LKB1 and AMPK are mTOR dependent (71) and highlights the multifaceted role of these proteins in regulating T-cell development and function through metabolism.

HIF-1 and Myc

Recently, the metabolism of various $CD4^+$ T-cell subsets has been explored. It was shown that upon activation, Th1, Th2, Th17, and Treg cells all increased their glycolytic rates but that this occurred to a much greater extent in Th1, Th2, and Th17 cells than in Treg cells (78, 79). Hypoxia inducible factor 1α (HIF-1α) is a transcription factor that regulates the expression of genes that encode for glycolytic enzymes (80) as well as downregulates mitochondrial oxygen consumption by blocking the entrance of pyruvate in the TCA cycle (81) (*Fig.* 3). A recent study showed that HIF-1α was strongly induced in Th17 cells but not in Th1 or Th2 cells and that Th17 cell differentiation *in vitro* and *in vivo* was impaired in the absence of this transcription factor (79). These results demonstrated that HIF-1α controls the glycolytic activity that is essential for Th17 cell development. Interestingly, the addition of fatty acids to Th1, Th2, and Th17 cells suppressed lineage-specific cytokine production (78). Although this effect appeared to be at least in part due to enhanced cell death, precisely how fatty acids impair viability and cytokine production in these cells remains to be determined. In addition to the idea that the presence of a lipid fuel source alters cellular metabolism from glycolysis to lipid oxidation, it is also possible that the

presence of fatty acids results in signaling that is not compatible with the glycolytic program of these cells. Alternatively, lipids could be promoting lipotoxicity, as non-adipose tissues have a limited capacity to store lipids, that when exceeded, cellular dysfunction and death may result (82). In contrast with $CD4^+$ effector T cells, Treg cells demonstrated high rates of fatty acid oxidation, and blocking this type of metabolism impaired Treg development (78). Inhibiting mTOR with rapamycin also resulted in an increased frequency of Treg cells, an effect that was dependent on fatty acid oxidation (78). In line with this finding, Treg cells demonstrated elevated AMPK activation when compared with effector and naive $CD4^+$ T cells, and administration of the AMPK activator metformin reduced Glut1 expression, promoted fatty acid oxidation, and increased the percentage and number Treg cells (78). Together these results demonstrate that HIF-1α is essential for Th17 cell development and associated glycolytic metabolism, while the absence of HIF-1α drives Treg cell differentiation and fatty acid oxidation.

In contrast with the findings by Shi *et al.* (79) showing that HIF-1α was strongly and selectively induced only in the Th17 cells subset, a recent study by Wang *et al.* (21) showed that HIF-1α expression was highly induced upon activation in a mixed culture of $CD4^+$ and $CD8^+$ T cells. Despite its quick induction, HIF-1α appeared dispensable for the initiation of metabolic changes and activation-associated proliferation in these T cells (21). This study went on to show that Myc, a transcription factor that regulates glycolytic metabolism in transformed cells (83, 84), was highly induced upon T-cell activation, and its expression was required for activation-induced metabolic programming (21) (*Fig.* 3). Acute Myc deletion inhibited proliferation and cell growth upon T-cell activation, while leaving AKT and ERK signaling pathways intact. Consistent with the important role for glycolysis and glutaminolysis in T-cell activation, Myc-deficient T cells showed impaired activation-induced glycolysis and glutaminolysis and decreased activation of the downstream targets of mTOR. Furthermore, Myc-driven glutaminolysis was tightly coupled to the biosynthesis of polyamines that are essential for T-cell proliferation (21). In agreement with these findings, Carr *et al.* (20) recently showed that T cells grown without glutamine did not increase in size to the same extent as in cells cultured in media with glutamine, implicating that this amino acid is essential for optimal cell growth. Interestingly, in renal carcinoma cells and fibroblasts, other effects of Myc and HIF-1α signaling have been described. It has been shown that Myc stimulates nuclear-encoded mitochondrial genes and mitochondrial biogenesis (85), and HIF-1α inhibits these changes (86). Although metabolic processes in cancer cells can closely mimic those in activated T cells, future studies in T cells are required to determine the relative expression and dominance of Myc and HIF-1α upon activation and how this may affect mitochondrial changes.

mTOR

The conserved kinase mTOR has a central role in the regulation of metabolism by sensing and integrating signals in response to nutrients, growth factors, energy, and stress (87) (*Fig.* 3). mTOR is present in two distinct complexes: mTOR complex 1 (mTORC1) and mTORC2 (88). It was previously shown that mTOR signaling is required for $CD4^+$ effector T-cell differentiation, as mTOR-deficient T cells fail to develop into Th1, Th2, and Th17 cells both *in vitro* and *in vivo*. However, mTOR-deficient T cells do differentiate into Treg cells (89). More recently it was shown by this same group that mTORC1 signals specifically regulate Th1 and Th17 lineage differentiation, whereas mTORC2 signaling drives Th2 development (90). In line with their previous observation, inhibition of both mTORC1 and mTORC2 was required for spontaneous Treg induction (90). These studies provide a strong link between metabolic control and $CD4^+$ T-cell differentiation. Future studies are needed to further elucidate the pathways by which mTORC1 and mTORC2 differentially affect helper T-cell development and metabolism.

ERRα

Another important regulator of transcription of metabolic genes is the nuclear hormone receptor, estrogen-related receptor-α (ERR-α) (91, 92) (*Fig.* 3). Recently, it was shown that this protein acts as a metabolic regulator of effector $CD4^+$ T-cell homeostasis and function by broadly affecting metabolic gene expression and glucose metabolism (93). ERR-α-deficient $CD4^+$ T cells exhibited reduced activation and proliferation, which was accompanied by impaired glucose uptake and mitochondrial metabolism. Consistent with these observations in T cells, it was recently shown that the expression of the *Drosophila* ortholog of ERR mid-embryogenesis induced a switch in gene expression that promoted a metabolic program associated with proliferating cells, supporting the dramatic growth that occurs during larval development (94). Interestingly, the failure of T cells to become activated in the presence

of an ERR-α inhibitor was a result of impaired glucose metabolism, as this defect was rescued by the addition of exogenous fatty acids, but not pyruvate, indicating that ERR-α positively regulates pyruvate flux into the TCA cycle. This illustrates that mitochondrial metabolism, and not only glycolysis, is important for the early phase of T-cell activation. This is consistent with another study showing that mitochondrial biogenesis is induced in the early phase after TCR activation (95). The inability to direct pyruvate into the TCA cycle may lead to decreased lipid synthesis and cell growth and thus impaired activation and proliferation. Although fatty acid oxidation could rescue $CD4^+$ T-cell proliferation during initial activation, lipids were not sufficient to optimally restore cell division and effector function of Th1, Th2, and Th17 cells upon ERR-α inhibition. However, as would be expected from cells that mainly use lipid metabolism, Treg cells were efficiently generated in the presence of an ERR-α inhibitor. As ERRs have been shown to associate with HIF-1α and regulate its function in tumors (96), future studies will need to determine how nuclear hormone receptors and transcription factors like HIF-1α and Myc interact in $CD4^+$ T cells.

Targeting effector T-cell metabolism for immunotherapy

Implicit in the fact that metabolism underlies the functional capacity of T cells is the idea that manipulating T-cell metabolism *in vivo* will alter the outcome of adaptive immune responses. In a recent study, the bioenergetics of alloreactive T cells was examined (97). It was found that bone marrow cells that were transplanted into irradiated syngeneic hosts proliferated and adopted a glycolytic metabolism, as evidenced by increased Glut1 expression and lactate production when compared with naive bone marrow cells. Oxygen consumption by these cells, however, was unaffected, suggesting that glycolysis was the predominant form of metabolism rather than OXPHOS in bone marrow cells transplanted into irradiated syngeneic hosts. In contrast, lymphocytes that proliferated in response to alloantigens in a model of graft versus host disease (GVHD) also showed increased Glut1 expression and lactate production but had elevated oxygen consumption levels as well. Although large increases of intracellular acylcarnitines suggested changes in fatty acid metabolism, it was not directly determined if fatty acid oxidation was responsible for the increase in oxygen consumption. It was also observed that when compared with the transplanted bone marrow cells, alloreactive T cells proliferated faster, had decreased amounts of glutathione, hyperpolarized mitochondrial membrane potential, and increased superoxide levels. Use of Bz-423, a compound that promotes superoxide production by inhibiting the mitochondrial F_1F_0-ATPase, selectively induced apoptosis in alloreactive T cells but not resting T cells or proliferating bone marrow cells. The authors speculate that this was most likely a result of the lack of sufficient anti-oxidant reserves in the alloreactive cells to cope with increased superoxide. Importantly, Bz-423 has therapeutic potential, as its use arrested ongoing GVHD by selectively inducing apoptosis in the alloreactive T cells. This study highlights the value of targeting T-cell metabolism for therapeutic benefit. To what extent the bioenergetics of alloreactive T cells differs from T cells activated by an infection or cancer needs further exploration.

Memory T cells

Fatty acid oxidation and memory T cells

The metabolic demands of proliferating T cells in culture have been studied extensively, and the importance of glucose is well documented. Much less is known about metabolic requirements of memory T cells. Several years ago we published a study showing that $CD8^+$ T cells that lacked TRAF6 were impaired in their ability to form a stable population of antigen-specific memory T cells after bacterial infection, and microarray analysis suggested that this was due to defects in several catabolic pathways of metabolism, including fatty acid oxidation (98). We demonstrated that augmenting fatty acid oxidation in $CD8^+$ T cells by treating mice with either the AMPK activator metformin or the mTOR inhibitor rapamycin, two drugs that are known to induce fatty acid oxidation, promoted the development of memory $CD8^+$ T cells after immunization (64–68). This study was the first to suggest that targeting catabolic pathways of metabolism, such as fatty acid oxidation, after vaccination could enhance the development of stable $CD8^+$ T-cell memory. Our findings showing that rapamycin promoted $CD8^+$ T-cell memory development were supported by another study published at the same time demonstrating that this drug also promoted immunity to viral infection (99). Importantly, this study by Araki *et al.* (99) established mTOR as a cell-intrinsic regulator of $CD8^+$ T-cell memory development. As rapamycin is traditionally used clinically for its immuosuppressive properties, these findings generated considerable interest.

Focusing on this ability of rapamycin to enhance immunity, it was more recently shown by Rao *et al.* (100) that $CD8^+$ T cells activated and cultured in the presence of rapamycin for 3 days and subsequently transferred into recipient mice formed better memory T cells in terms of quantity and quality in comparison with control T cells. Furthermore, it was found that mTOR regulated the expression of the transcription factors T-bet and Eomesodermin, which are known to direct effector and memory $CD8^+$ T-cell fates, respectively (100–103), further illustrating how this central metabolic regulator specifies T-cell fate. Interestingly, a recent paper from this same laboratory (45) showed that the ability of rapamycin to augment $CD8^+$ memory T-cell development was ablated when Foxo1 was silenced. Inactivation of Foxo1 appeared to be important for T-bet mediated effector functions, whereas active Foxo1 resulted in a switch to more Eomesodermin transcription factor activation, and more $CD8^+$ memory precursor T cells. Given these findings, it will be interesting to determine whether Eomesodermin regulates the expression of metabolism genes that dictate a quiescent phenotype in $CD8^+$ T cells.

Mitochondrial spare respiratory capacity

Research in our laboratory continues to focus on the role of metabolism in the development of memory $CD8^+$ T cells after infection. In a recent study (104), we showed that memory $CD8^+$ T cells maintain significant mitochondrial spare respiratory capacity (SRC) in comparison with naive or effector T cells. SRC is the extra mitochondrial capacity available in a cell to produce energy under conditions of increased work or stress and is thought to be important for long-term cellular survival and function (105–109). We found that greater SRC was underscored by enhanced mitochondrial biogenesis in memory $CD8^+$ T cells, and that this process was modulated by IL-15. In addition, we demonstrated that SRC was abrogated when carnitine palmitoyltransferase 1a (CPT1a), the mitochondrial protein that is the rate-limiting step for β-oxidation of fatty acids in the mitochondria (110–112) was inhibited. Our findings led us to a model where bioenergetics regulates memory T-cell development after infection (Fig. 4). As effector $CD8^+$ T cells rapidly proliferate during an immune response, they primarily use glycolysis to support bioenergetic needs. We speculate that during this process, the majority of $CD8^+$ effector T cells fail to maintain and/or induce mitochondrial biogenesis resulting in a reduction in the ratio between mitochondrial mass and overall cell mass, and thereby lose reserve energy-generating capacity, i.e. SRC. Reduced mitochondrial mass increases dependence on glycolysis and renders effector T cells bioenergetically unstable due to a decline in their ability to create energy from diverse substrates (e.g. fatty acids) via OXPHOS, and thus are unable to maintain viability when infection-associated signals decline and elements such as IL-2 that support glycolysis dissipate. Some antigen-specific T cells from the primary infection survive as long-lived memory T cells, because these cells have maintained mitochondrial function via exposure to IL-15 or other growth factor cytokines. More mitochondrial mass allows greater use of fatty acids for energy, thereby facilitating cell survival in the absence of pro-glycolysis signals. Ongoing experiments in our laboratory will determine whether, in addition to simply more mitochondrial mass, there are qualitative differences between mitochondria in effector T cells compared with memory T cells. For example, increased expression of ETC complexes or CPT1a on a per mitochondrion basis could facilitate the

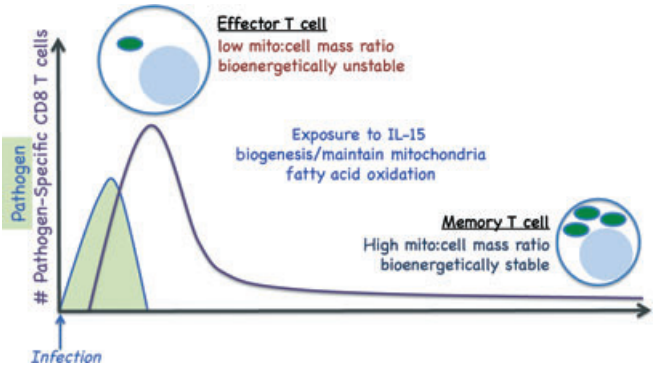

Fig. 4. Bioenergetic model of memory $CD8^+$ T-cell survival after infection. As effector $CD8^+$ T cells rapidly proliferate during an immune response, they primarily use glycolysis to support bioenergetic needs. We speculate that during this process, the majority of $CD8^+$ effector T cells fail to maintain and/or induce mitochondrial biogenesis resulting in a reduction in the ratio between mitochondrial mass and overall cell mass and thereby lose reserve energy-generating capacity, i.e. spare respiratory capacity. Reduced mitochondrial mass increases dependence on glycolysis and renders effector T cells bioenergetically unstable due to a decline in their ability to create energy from diverse substrates via OXPHOS, and thus are unable to maintain viability when infection-associated signals decline and elements such as IL-2 that support glycolysis dissipate. Some antigen-specific T cells from the primary infection survive as long-lived memory T cells, because these cells have maintained mitochondrial mass via exposure to IL-15 or other growth factor cytokines. More mitochondrial mass allows greater use of fatty acids for energy via OXPHOS, thereby facilitating cell survival in the absence of pro-glycolysis signals. Moreover, the enhanced spare respiratory capacity provided by increased mitochondrial mass might allow memory T cells to respond quickly if pathogen is reencountered.

use of fatty acids for energy via OXPHOS. Given that mitochondria undergo significant changes in T cells during various phases of the immune response we think that it is very likely that there will also be qualitative differences in these organelles between these cell types.

Our studies revealed that maintaining/increasing mitochondrial mass is critical for $CD8^+$ T-cell memory development. Unlike the case for effector T cells which rely predominantly on glycolytic metabolism, memory T cells maintain substantial mitochondrial mass, which provides the flexibility to use diverse substrates for energy generation, such as the oxidation of fatty acids. Our earlier microarray analyses comparing 'memory-impaired' TRAF6-deficient $CD8^+$ T cells versus 'memory-competent' control $CD8^+$ T cells revealed that in addition to fatty acid oxidation, amino acid catabolism may also be involved in $CD8^+$ T-cell memory development (98). Previous studies have shown that antigen-presenting cells can regulate amino acid availability to T cells (113–115), which may influence T-cell development during an immune response. Furthermore, autophagy is an important survival pathway when cells are faced with metabolic stress (116). If T cells experience metabolic stress during an immune response, autophagy could be induced, and this presumably would include the breakdown of amino acids for fuel in addition to lipid oxidation. However, as fats are an efficient energy store, yielding more than twice the energy per gram than either carbohydrate or protein, it is logical that lipids would be the preferential fuel source for long-lived quiescent T cells. More work will need to be done in the area of substrate utilization in T cells after infection to reveal whether there is any role for amino acid catabolism fueling the development of memory T cells.

The question of metabolic conversion, promotion, or maintenance

It is still unclear whether T cells that are destined to become memory cells fully commit to using Warburg metabolism in the effector phase and subsequently switch to a quiescent metabolism later, or whether memory cell precursors never fully switch to Warburg metabolism and promote or simply maintain a significant amount of mitochondrial respiratory capacity throughout activation. Although our data as well as those from other laboratories indicate that the population of $CD8^+$ effector T cells generated *in vivo* or after activation *in vitro* actively use both glycolysis and OXPHOS (21, 97, 104), it is not possible to determine whether these measurements are reflecting heterogeneity in the cell populations, where more terminally differentiated effectors T cells are mostly using glycolysis and less differentiated effectors T cells are using more oxidative metabolism, or whether individual cells actually use both metabolic programs simultaneously. There does seem to be a high degree in uniformity in the effector cell populations generated *in vitro*, to where activation marker expression and effector function are adopted evenly across the population, which might suggest that metabolic phenotypes are also evenly distributed across the population and that all cells are using both glycolysis and OXPHOS. However, the real answers to these questions will have to wait until it becomes possible to measure metabolism on a single cell level. While it now has been shown that activated T cells use OXPHOS in addition to glycolysis (21, 97, 104), it has yet to be determined which one or if both of these energy-generating pathways are actually required for the survival and proliferation of effector T cells.

Our research has indicated that memory T cells have more mitochondrial mass than effector T cells and that IL-15 induces mitochondrial biogenesis (104), suggesting that exposure to IL-15 or perhaps other common-γ cytokines, such as IL-7 or IL-4, is critical to ensure that mitochondrial mass is maintained and long-lived memory T cells can develop/persist (Fig. 3). Whether effector T cells will receive these cytokine signals can be controlled at many levels, including cytokine and/or cytokine receptor expression, trans-presentation of the cytokine to T cells by other cells, or T-cell localization to niches where cytokine is expressed (117, 118) (Fig. 3). Most likely a combination of all these factors will determine whether a T cell receives a cytokine signal *in vivo*. Our unpublished preliminary data indicate that lymph node homing receptors are upregulated quickly after IL-2 withdrawal *in vitro*, suggesting that once infection-associated signals disappear, T cells become competent to traffic to the lymph nodes where growth factor cytokines like IL-15 are expressed. A recent study has shown that $CD8^+$ DCs constitutively express higher levels of IL-15 when compared with other DC subsets (119). In addition to the role of $CD8^+$ DC in cross-priming $CD8^+$ T cells (120), perhaps these cells also support mitochondrial maintenance in $CD8^+$ T cells that are destined to become long-lived memory T cells via IL-15 trans-presentation during priming.

Memory T-cell development *in vivo*

The question remains how memory cell formation is organized *in vivo*. One possibility is that effector T cells at the lowest end of the growth factor gradient will be the

first to sense growth factor deprivation and in response will upregulate molecules like CD62L and migrate to the lymph nodes where IL-15 is presented, making these cells more likely to survive contraction. We speculate that the antigen-specific T cells that continue to rapidly proliferate in response to infection will eventually outpace their mitochondrial biogenesis, resulting in lower mitochondrial mass and respiratory capacity, and thus a higher dependency on glycolysis (*Fig.* 4). Alternatively, there may be heterogeneity in CD62L expression on antigen-stimulated T cells already in the early phase of the immune response as a result of variation in strength of T-cell activation. In response to TCR triggering or cytokines, AKT is activated and FOXO transcription factors are sequestered in the cytoplasm (121) (*Fig.* 3) and thus are unable to drive KLF2 expression (76). In the case of inadequate AKT activity, FOXO transcription factors are not retained in the cytoplasm and can still drive the expression of KLF2 (75) and thereby maintain the expression of several adhesion and chemokine receptors, such as CD62L, CCR7, and $S1P_1$, which control T-cell migration (37, 74). Weak antigen-stimulation results in suboptimal activation of the AKT-mTOR pathway, that is sufficient to drive T-cell growth and proliferation but not to suppress KLF2 expression (73, 76). Thus, T cells that are activated by a weaker signal will proliferate but also maintain higher expression of CD62L and maintain the ability to migrate to lymph nodes and gain access to growth factor cytokines like IL-15.

Another possibility is that heterogeneity in the effector pool as a result of asymmetric cell division may contribute to the generation of T cells that are better equipped for memory development than others (122). Asymmetric division could result in unequal distribution of several critical factors, including cytokine receptors, lymph node homing receptors, transcription factors, and mitochondria. Accordingly, it has been shown that cells generated during the peak of infection that have not terminally differentiated, express IL-7R and persist as long-lived memory T cells (123). It is possible that mitochondria are also unequally distributed during the first divisions after activation, so that terminally differentiated effector T cells acquire fewer mitochondria, while memory precursor T cells acquire more.

Bioenergetics and recall responses

In addition to bioenergetic differences between memory $CD8^+$ T cells and effector $CD8^+$ T cells, we have also shown that naive $CD8^+$ T cells have less SRC and mitochondrial mass than memory $CD8^+$ T cells (104). We speculate that during an immune response, signals from IL-15, as well as other growth factor cytokines, support mitochondrial biogenesis and thus endow memory cells with greater mitochondrial mass compared with naive T cells. In addition to the simply enhanced mitochondrial content contributing to bioenergetic differences, qualitative differences in mitochondria may also exist between these cell types, i.e. differences in the expression of ETC complexes or other mitochondrial proteins could result in intrinsic differences in respiratory capacity. Regardless of the variation in the mitochondria between cell types, we suspect that greater mitochondrial mass would indicate that memory T cells have more readily available ATP-generating capacity than naive T cells. If this were the case, it may explain why antigen-specific $CD8^+$ memory T cells respond faster to re-infection on a per cell basis when compared with their antigen-specific naive $CD8^+$ T-cell counterparts. Investigating cell-intrinsic bioenergetics differences between naive and memory T cells and how this disparity impacts their functionality will be a focus of our future studies.

STAT3

Two recent studies demonstrated that STAT3 has a critical role in the development and maintenance of memory T cells (124, 125). Siegel *et al.* (125) showed that patients with dominant-negative STAT3 mutations have a T-cell intrinsic defect resulting in decreased numbers of central memory T cells. Naive T cells isolated from these patients and cultured in IL-7 or IL-15 exhibited impaired differentiation into central memory T cells. The impaired capacity to form central memory T cells was associated with decreased expression of Bcl6 and SOCS3, two STAT3 responsive transcription factors important in the development of memory T cells (126–128). Cui *et al.* (124) demonstrated the intrinsic requirement of STAT3 in $CD8^+$ T cells for memory differentiation and maturation in a mouse model of lymphocytic choriomeningitis virus (LCMV) infection. STAT3-deficient T cells displayed an effector phenotype, had a shortened lifespan, had reduced self-renewal capacity, and showed poor protective immunity upon secondary infection. In addition, Bcl6 and SOCS3-deficient $CD8^+$ T cells also demonstrated impaired memory T-cell development. Interestingly, it was recently shown that STAT3 is not only present in the cytosol but also in mitochondria, where it regulates the function of

ETC complexes I and II (129, 130). STAT3-deficient cells exhibited defects in ETC function and ATP generation but maintained normal mitochondrial gene expression and content (129). Importantly, it was suggested that STAT3 activity in mitochondria is unrelated to its actions as a transcription factor. Although Cui's study suggests at least a partial role for STAT3-mediated transcriptional effects on memory T-cell formation, it is tempting to speculate that mitochondrial STAT3 plays a role in memory T-cell formation as well. As the importance of fatty acid oxidation and spare respiratory capacity for memory $CD8^+$ T-cell development has recently become clear, the impaired ETC function and ATP-generating capacity evident in the absence of STAT3 suggests that mitochondrial STAT3 may have a key role in this process. Furthermore, in agreement with the idea that maintaining mitochondrial capacity to utilize fats for energy while not depending solely on glucose and IL-2 is a critical feature for $CD8^+$ T cells to persist as long-lived memory T cells, it was suggested that the T-cell memory defect in STAT3-deficient patients was potentially resulting from increased reliance on IL-2 (125).

Diet and the immune response

Malnutrition and immunity

There is abundant epidemiological evidence which suggests that malnutrition impairs vaccine efficacy and increases susceptibility to infections; however, the underlying mechanism is poorly understood (131, 132). As the maintenance of long-lived memory T cells requires homeostatic proliferation and thus availability of building blocks to support the production of new daughter cells, dietary components can be critical to this process. A recent study from Iyer *et al.* (133) demonstrated that mice that were infected with LCMV on a normal diet but then switched to a low protein diet after memory T cells were established developed twofold lower numbers of antigen-specific T cells. Also, acute and basal homeostatic proliferation of $CD44^{hi}$ $CD8^+$ T cells was impaired in mice on the low protein diet, which was not due to lower IL-7R or IL-15R expression. In addition, the memory $CD8^+$ T cells in mice fed the low protein diet showed decreased proliferation upon challenge with LCMV clone 13, resulting in impaired viral clearance. Importantly, supplementation of appropriate protein levels in mice fed the low protein diet rescued the quantitative reduction of memory $CD8^+$ T cells. This study demonstrates the importance of dietary amino acids for optimal maintenance of memory $CD8^+$ T cells and an adequate secondary effector response.

Caloric restriction and T-cell responses

In contrast with malnutrition, caloric restriction, as defined as a reduction in nutrient availability without malnutrition, has long been known to increase lifespan in a variety of species ranging from yeast to mammals (134). In parallel with the proposed model linking T-cell longevity and maintaining the capacity to use a wide variety of fuels (*Fig.* 4), the capacity of animals to adapt to metabolic stress or harsh environmental conditions may have evolved along with genetic adaptation for longevity. Indeed, the stress resistance of vertebrate cells in culture positively correlates with the lifespan of the species from which they were isolated (135, 136). Most of the work exploring the molecular pathways that control longevity has been performed using worms, yeast, and fruit flies. Studies have shown that insulin/IGF signaling, DAF-16/FOXO transcription factors, and TOR all play crucial roles in the regulation of metabolism and lifespan (137–142). Inhibition of TOR by the AMPK-FOXO pathway has been shown to positively regulate longevity by caloric restriction in *C. elegans* (143). In agreement with these findings, administration of rapamycin or metformin, which inhibit mTOR and activate AMPK, respectively, has been demonstrated to increase mammalian lifespan (144, 145), which corresponds with studies showing that these agents increase T-cell lifespan (98, 99, 146). Furthermore, consistent with our studies showing that increased fatty acid metabolism primes T cells for longevity (98, 104), a positive correlation between fat metabolism and increased lifespan has been observed in *C. elegans* (147).

Mitochondrial function and cellular longevity

As fatty acid oxidation occurs in the mitochondria, it is logical that maintenance of these organelles is critical for the survival of long-lived memory T cells (104). Support for this idea comes from studies that compared the transcriptional profiles of the aging process across humans, mice, flies, and worms, and identified the downregulation of nuclear-encoded genes of the mitochondrial ETC as the only age effect common to all four species (148). The decline in mitochondrial function with aging is opposed by caloric restriction by the induction of nuclear encoded genes that promote mitochondrial function (149). A key component in the transcriptional changes upon caloric restriction is increased expression of the transcriptional coactivator PGC-

1α, a protein that is directly phosphorylated by AMPK (150). Activation of PGC-1α results in induction of mitochondrial biogenesis, increased mitochondrial respiration and fatty acid oxidation, and regulation of ROS detoxification (151, 152). The latter process seems critical to prevent extensive oxidative damage as a result of increased respiration. It is likely that caloric restriction-induced PGC-1α expression results in a wide array of adaptations to provide a positive setting for increased OXPHOS. For instance, expression of uncoupling proteins, which have been associated with decreased ROS-induced damage, is increased in response to caloric restriction (153). In line with this, the *Drosophila* homolog of PGC-1 increases mitochondrial gene expression and activity and extends lifespan (154). Future studies are required to determine whether PGC-1 is also the critical regulator in T cells that links mitochondrial mass to cellular longevity.

It was shown that Tsc1-deficient T cells (discussed in section on naive T cells), which have hyperactive mTOR, have a survival defect and fewer mitochondria than cells with functional Tsc1, fitting with their exit from quiescence and decreased longevity and persistence (35). However, it was shown several years ago that mTOR inhibition decreased the gene expression of the mitochondrial transcriptional regulators PGC-1α, ERR-α, and nuclear respiratory factors in skeletal muscle, resulting in a decrease in mitochondrial gene expression and oxygen consumption in these cells (155). It is not precisely clear at this time why mTOR signaling induces opposing effects on mitochondrial regulation in these settings, but it may reflect tissue-specific differences or temporal events. Since both mTOR and AMPK have been shown to modulate PGC-1α activity (150, 155), the balance of how these factors regulate mitochondrial changes in T cells will need to be explored.

Concluding remarks

In recent years, there has been a renewed focus on immune cell metabolism and the overall importance of particular pathways in defined immunological processes has been established. However, further investigation into when a particular metabolism or bioenergetic process is required for cellular function and survival is still needed, especially concerning how metabolic fluctuations in T cells play out during immune responses *in vivo*. In addition, little is known about the relative contribution of metabolic pathways in T cells to fuel energy production, provide biosynthetic precursors, or generate reducing equivalents to balance the redox status of the cell at a particular time. Defining the metabolic and energetic requirements of T cells during different phases of an immune response is critical for our understanding of T-cell development and for the future design of therapeutics.

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