Key Points

1. Naïve, effector and memory T cells have distinct metabolic profiles that are critical for maintenance and function.
2. A complex network of immune cell specific transcription factors, cytokines and canonical regulators of cellular metabolism regulate the diversity of effector and memory T cells.
3. T cell receptor dependent transcription factors IRF4 and c-Myc coordinately induce gene networks required for metabolic reprogramming and for sustaining aerobic glycolysis during periods of rapid clonal expansion.
4. Clonal competition and the selection of highest affinity T cell clones during a primary immune response are driven by metabolic fitness.
5. Common γ-chain cytokines modulate metabolic profiles during effector and memory T cell differentiation and maintenance.
6. Immune specific transcription factors sense and integrate metabolic signals with immunological cues to appropriately regulate adaptive immune responses.

Synchronizing transcriptional control of T cell metabolism and function

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During an immune response cytokines and transcription factors regulate the differentiation and function of effector and memory T cells. Concurrently, T cell metabolism undergoes dynamic and differentiation stage-specific changes that are required for initial activation, rapid proliferation and the acquisition of effector function. Similarly, during the resolution of an immune response, metabolic regulation is critical for restraining inflammatory responses and promoting peripheral tolerance, and is required for the long-term maintenance of memory T cells. T cell receptor induced transcription factors, in particular c-Myc and IRF4, cooperate with canonical nutrient sensing pathways to integrate antigen-specific and metabolic signals to appropriately modulate adaptive immune responses. In this review we will focus on the emerging evidence that cellular differentiation and metabolism are stringently linked and synchronized by immune cell specific cytokines and transcription factors that are induced by antigen-receptor signals.

Introduction

CD8+ cytotoxic T cells are critically important for mediating pathogen clearance during a variety of bacterial and viral infections. Full activation and differentiation of CD8+ T cells requires the help of CD4+ T cells and the appropriate cytokine environment to mediate responses to specific types of infections. CD4+ T cells are also essential for providing help to B cells to undergo immunoglobulin class switching and to differentiate into plasma cells producing high affinity antibodies. Following the clearance of antigen, memory T cells of both lineages with an enhanced ability to respond to their cognate antigen remain and mediate superior protective immunity.

T cell activation induces rapid proliferation and acquisition of effector function. This process is tightly controlled by the coordinated activity of immune cell specific cytokine receptors and transcription factors. In addition, activation of T cells is characterized by the transition from the catabolic state of naïve cells to the anabolic state of active effector cells. Nutrient sensing and canonical signalling pathways such as those mediated by PI(3)K-AKT, AMPK and mechanistic target of rapamycin (mTOR) contribute to T cell differentiation and function, and comprehensive reviews that focus on this research area were published[1-6]. Recently, however, it became evident that cytokines and transcription factors originally thought to be specific to the regulation of immune cell differentiation and function are in fact closely linked to the metabolic programming of lymphocytes. Thus, we will discuss here the complex and highly regulated interplay between immune specific transcriptional programs and metabolic pathways during T cell responses.

Diversity of effector and memory T cells.

Following antigen recognition via the T-cell receptor (TCR), naïve T cells undergo a process of rapid clonal expansion and give rise to different types of effector and memory T cells. This process is particularly well understood in the context of CD4+ T helper (Th) cell differentiation where specific cytokines or combinations of cytokines induce 'lineage' specific transcription factors that ultimately guide the development of functionally distinct Th cell subsets. This includes Th1, Th2, Th17, follicular helper T cells (Tfh), and peripherally induced regulatory T (iTreg) cells that require the transcription factors T-bet, Gata3, Rorgt, Bcl6 and Foxp3, respectively, for their development (reviewed in detail in refs [7,8]). Similarly, antigen-activated CD8+ T cells can differentiate into a diverse array of effector and memory subsets that differ according to phenotype, function, survival potential and anatomical location. This includes short-lived effector and memory precursor cells, which give rise to recirculating memory cells, such as effector memory T cells (T_{EM}) and central memory T cells (T_{CM}), and non-circulating tissue-resident memory T cells (T_{RM})[9-11]. As in CD4+ T cells, cytokine signals and transcription factors play critical roles in guiding the development of CD8+ effector and memory precursor cells. Major regulators include T-bet, B-lymphocyte induced maturation protein 1 (Blimp1) and

inhibitor of DNA binding protein 2 (Id2), which coordinate the differentiation of effector cells, while Eomes, Tcf7, Bcl6 and Id3 counteract effector differentiation and promote memory cell development (reviewed in [12,13]).

Another group of transcription factors is induced directly by antigen-receptor and cytokine signaling and appears to act upstream of subsequent effector and memory differentiation. This is exemplified by the activities of IRF4, which is required for the development of various CD4+ T helper subsets, the differentiation of FoxP3+ effector regulatory T cells as well as CD8+ T cell responses[14-20]. Thus, an astonishing diversity of effector and memory T cells is regulated by the complex interplay between immune specific cytokine and transcription factors.

Distinct metabolic profiles of naïve, effector and memory T cells.

During T cell activation and differentiation, cellular metabolism undergoes dynamic and differentiation stage-specific changes. Naïve T cells exhibit basal levels of nutrient uptake and primarily utilise mitochondrial oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) for the production of energy in the form of adenosine triphosphate (ATP)[1,4]. Antigen sensing by the TCR triggers an increase in the uptake of nutrients and activation of multiple metabolic pathways to meet the increased biosynthetic demands during clonal expansion and effector function[3,6]. In particular, T cell activation leads to upregulation of facilitated glucose transport and a marked increase in aerobic glycolysis, thereby providing intermediate substrates to fuel *de novo* nucleotide and fatty acid synthesis[21]. Furthermore, activation results in increased facilitated amino acid transport[22,23] and augmented glutaminolytic activity important for polyamine synthesis[3,4]. Concurrently, effector T cells require intact OXPHOS for initial activation[24]. This pathway, along with glutaminolysis, may also be used to maintain cellular survival and ATP synthesis, especially under conditions of nutrient limitation and metabolic stress, demonstrating a remarkable degree of metabolic plasticity of activated T cells[24,25].

During memory development, T cells downregulate glycolytic pathways and revert back to catabolic metabolism, utilizing mainly OXPHOS and FAO. They also upregulate mitochondrial biogenesis pathways resulting in increased mitochondrial

spare respiratory capacity to promote slow homeostatic turnover and long-term survival (details in Box 1)[26-28]. Consistent with this observation, blunting glycolysis with a glucose analogue resulted in greater mRNA expression of memory specific transcription factors Bcl6, Lef1 and Tcf7 and improved memory T cell development[29].

i) Metabolic signals and immunological cues act together during T helper cell differentiation and function

Metabolic signals and nutrient sensing pathways are known to modulate the differentiation and function of Th cells. mTOR, a evolutionarily conserved serine/threonine kinase that senses and integrates signals relating to glucose levels[30], amino acid availability[23,31-34], adenylate levels[35-37] and oxygen tension[38-40] is particularly important in this process. This is exemplified by the reciprocal regulation of Th17 and iTreg cell differentiation by factors that influence the balance between glycolysis and oxidative lipid metabolism, and modulate the activities of the lineage transcription factors Rorγt and FoxP3[41-45]. In response to STAT3 signalling, $CD4^+$ T cells activated under Th17 skewing conditions upregulate hypoxia-inducible factor 1 alpha (Hif1α) expression and adopt a Hif1α dependent program of glycolysis[42,43,46]. Consequently, Th17 cells demonstrate increased glycolytic activity and reserve compared to Th1 and iTreg cells[41,42]. Evidence for the engagement of glycolysis as a critical determinant of Th17 fate arises from studies during which glycolytic flux is inhibited by pharmacologically diverting pyruvate to oxidative metabolism, or by inhibition of Raptor-mTORC1 signalling[41,42,45]. Expression of Hif1α is mTORC1 dependent, and treatment with the mTORC1 inhibitor rapamycin or the glucose analogue 2-deoxy-D-glucose impaired Th17 differentiation, leading to a reciprocal increase in iTreg generation[41]. Th17 cells also rely upon acetyl-CoA carboxylase 1 mediated *de novo* fatty acid synthesis[47], and glutaminolysis to feed OXPHOS for ATP production under conditions of energy stress and glucose limitation[25]. Interestingly, Hif1α and Pparγ are directly regulated by metabolites and environmentally derived signals. For example, Hif1α is induced by low oxygen tension and has well described roles in mediating responses to this condition, which is especially prevalent in non-lymphoid tissues (reviewed in [48,49]). Hif1α expression is also stabilized by the tricarboxylic acid cycle intermediate succinate and the glycolysis product lactic

acid[50,51]. PPARγ and related nuclear hormone receptors exert their transcriptional affects by binding endogenous unsaturated fatty acids, eicosanoids and lipoprotein ligands[52-56], leading to the repression of inflammatory gene expression[57-60]. Although these pathways have been demonstrated only for myeloid cells, similar mechanisms may be active in T cells. Recently, oxysterols and its derivatives where found to be the natural agonists of Rorγt[61]. Consequently, the transcriptional activity of Rorγt could be modulated using small-molecule inhibitors that mimicked oxysterol binding and resulted in changes to DNA occupancy by Rorγt and target gene expression[62]. Thus, the regulation of lymphocyte differentiation and function by transcription factors is closely linked to metabolic regulation and influenced by both environmental and immunological signals.

In contrast to Th17 cells, iTreg cells exhibit low glycolytic flux and their generation is inhibited by Hif1α activity[42,43]. Furthermore, iTreg cells show increased reliance upon fatty acid oxidation fuelled by exogenous uptake of fatty acids and the glycolytic-lipogenic pathway[47]. Although iTreg cells exhibit low glycolytic activity and their generation is favoured by low mTORC1 activity[63-66], Raptor-mTORC1 activity is critical for homeostasis and function of thymus-derived natural Treg cells. Specifically, Raptor-mTORC1 integrates TCR and IL-2 signals with the induction of cholesterol and lipid metabolism, which is indispensable for the expression of molecules required for regulatory function such as ICOS and CTLA4[67]. These data are consistent with the notion that similar to conventional T cells, Treg cells undergo an effector differentiation program triggered by TCR signals and cytokines in order to acquire full suppressive function[17,68]. However, further work is required to understand the metabolic regulation of peripherally induced and thymic derived Treg cells *in vivo*.

ii) Metabolic regulation controls memory potential and function of T cells

During the differentiation of cytotoxic CD8+ T cells, the balance between aerobic glycolysis and lipid oxidative metabolism is a critical determinant of effector and memory T cell differentiation[29,69]. Effector CD8+ T cells exhibit high glycolytic flux and mTOR activity required for rapid proliferation and clonal expansion. Additionally, effector T cells upregulate genes involved in lipid synthesis in an mTOR dependent

manner via the activity of SREBP-1 and SREBP2 transcription factors[70]. High mTOR activity favours T-bet transcription via an undetermined mechanism, and also results in the nuclear exclusion and inactivation of the forkhead transcription factor Foxo1. This in turn leads to impaired expression of Eomes and Tcf7, two transcription factors critical for memory T cell development and function. Consequently, signalling via mTORC1 directly impacts on the balance between CD8+ effector and memory T cell differentiation[71-76]. In support of this model, genetic ablation of either tuberous sclerosis complex (Tsc) 1 or Tsc2, which act as a activators for the GTPase Rheb, itself a negative regulator of mTORC1 signalling, did not affect effector differentiation, however, resulted in impaired memory differentiation and function[77,78]. Importantly, metabolic activity impacts on T cell function not only by modulating the expression of the key transcription factors that control differentiation, but also directly by regulating expression of effector molecules. High glycolytic flux maximises cytokine production by diverting enzymes that bind to the 3'UTR of the *Ifnγ* and *Il2* loci and inhibit translation[24]. Similarly, glycolysis has been shown to promote IL-4 production and the expression of IL4Rα and IL2Rα by Th2 cells, affecting cytokine responsiveness and the ability to signal via STAT5 and STAT6[79]. Thus, different effector and memory T cell populations have distinct metabolic requirements that are closely linked to the transcriptional programs required for their differentiation and function.

Clonal expansion and competition as a function of metabolic fitness.

Effective immune responses require pronounced clonal population expansion of antigen-specific T cells. During expansion, through a process of clonal competition, the overall TCR affinity for the antigen increases while the TCR diversity becomes increasingly restricted[80-83]. This 'focusing' of the T cell repertoire is due to the preferential expansion of T cell clones displaying TCR's with a high affinity for cognate antigen at the expense of low-affinity cells. This 'loss' of low-affinity T cell clones is mediated at least in part by apoptosis, proliferation cessation and by premature development of memory T cells[84-88]. However, as discussed below, metabolic 'fitness' and competition for nutrients appears to be the major driver TCR repertoire focusing.

i) Activation induced metabolic reprogramming by T cell receptor dependent transcription factors

TCR signalling induces the transcription factor c-Myc, which initiates cell division as well as metabolic reprogramming. c-Myc is strongly upregulated during T cell blastogenesis and is responsible for activation-induced glycolysis and glutaminolysis[89]. Expression of c-Myc is also triggered by nutrient sensing pathways, including amino acid transport and mTOR signalling[23,90]. While c-Myc is critical in setting up the metabolic program required for T cell proliferation and differentiation, another TCR-induced transcription factor, IRF4, is required for sustaining the metabolic activity of activated T cells[18]. T cells that lack IRF4, although initially proliferating normally, abort clonal population expansion early[18-20]. As we have shown, IRF4 regulates the expression of multiple genes required for aerobic glycolysis and modulates the expression of Foxo1 and Hif1α[18]. Hif1α sustains expression of glycolytic genes during proliferation by relaying signals derived from mTORC1 and IL-2[91,92]. mTORC1 activity also controls the expression of IRF4[19], indicating a feedback mechanism between antigen-receptor and nutrient sensing signals. Thereby, IRF4 tightly integrates TCR and mTORC1 signals with the metabolic reprogramming of T cells.

Interestingly, affinity signals induce graded amounts of IRF4 that determine the capacity of T cells to sustain proliferation[18,93]. Strong or high-affinity TCR stimulation in an IRF4-dependent manner results in greater glucose uptake and engagement of aerobic glycolysis compared to weakly or low-affinity TCR stimulated T cells[18]. Thus, IRF4 mediates TCR-signal dependent competition for nutrients favoring clonal expansion and effector differentiation of high-affinity T cells. High-affinity signals also induce robust expression of CD25 (*Il2ra*), thereby directly promoting IL-2 sensitivity and sustained proliferation of responding T cells[85,88]. Importantly, cytokines modulate IRF4 transcriptional activity by promoting its binding to target genes in a signal transducer and activator of transcription (STAT) dependent manner[16]. IRF4 acts in concert with the AP-1 family transcription factor BATF to control gene expression in CD8+ [18,94], Th17[95-97] and Treg cells[98]. IRF4 therefore integrates multiple extracellular cues, including antigen-receptor and

cytokine signals as well as nutrient sensing signals, to sustain a metabolic program that promote T cell survival and proliferation.

As outlined above, both cMyc and IRF4 are required for the regulation of glycolytic pathways in activated T cells. However, although c-Myc and IRF4 have overlapping functions in regulating genes involved in metabolic reprogramming they appear to operate during temporally discrete periods of proliferation and early differentiation. Whereas c-Myc protein expression peaks during the cellular growth phase before entry into cell division and is extinguished within days after initial antigen encounter, expression of IRF4 protein reaches a maximum during clonal expansion[18,99]. Another transcription factor that plays an important role in this process is AP-4, a basic helix-loop-helix protein directly induced by c-Myc and found to contribute to the maintenance of glycolysis and biosynthetic pathways during CD8+ T cell responses[99]. In summary, TCR stimulation induces graded amounts of IRF4, which is a central component of a transcription factor network that includes c-Myc, BATF, AP4 and Hif1α that coordinately regulate the metabolic program of activated CD8+ T cells. IRF4 thereby promotes clonal population expansion and effector differentiation of high-affinity T cells.

ii) Crosstalk between cellular metabolism and apoptotic pathways

Metabolic reprogramming and the engagement of aerobic glycolysis are critical not only for clonal expansion but also for the survival of activated T cells by antagonizing the activity of several pro-apoptotic BH3-only proteins. Multiple mechanisms have been evoked for this observation. One example of the crosstalk between regulation of apoptosis and metabolism stems from the interaction between glycolytic enzyme hexokinase II (*Hk2*) and voltage-dependent anion channels (VDAC1) in the mitochondria. This interaction prevents cytochrome c release by antagonising the cleavage and activation of pro-apoptotic effector molecule BID[100,101]. Sufficient glycolysis and intracellular glucose levels are also required to prevent the phosphorylation and activation of p53, thereby restraining the transcription of pro-apoptotic BH3-only proteins PUMA and BIM[102-104] and the activity of pro-apoptotic BAX[105,106]. Furthermore, glucose is needed for T cells to inhibit degradation of pro-survival molecule Mcl-1, which neutralizes NOXA, a mediator of mitochondrial cytochrome c release and apoptosis[107]. Interestingly, high levels of glycolysis and

high affinity TCR signals act synergistically to stabilize the expression of Mcl-1, thus, enhancing the survival of Treg cells and effector T cells with high affinity TCRs for their cognate antigen[84,108]. These observations provide an additional mechanism that links clonal competition and preferential expansion of T cells of high affinity with metabolic fitness and high glycolytic activity.

Interestingly, in addition to its role in regulating cellular metabolism, IRF4 also promotes survival of activated T cells[18-20]. This may be partially due to directly repressing transcription of pro-apoptotic BIM[19]; however, blocking the intrinsic apoptosis pathway by overexpressing pro-survival Bcl2 or deleting BIM did not rescue the curtailed population expansion of IRF4-deficient T cells[18]. IRF4 was also shown to directly bind to several genes encoding cyclin-dependent kinase (CDK) inhibitors[19] suggesting that IRF4 may directly promote cell cycle progression of activated T cells. Together these results demonstrate the critical importance and non-redundant nature of the molecular networks regulated by IRF4, which promotes TCR repertoire focusing and the preferential expansion of high affinity clones through several interconnected mechanisms, including maintenance of efficient aerobic glycolysis, promotion of survival and cell cycle progression. Thus, IRF4 is a molecular determinant for the 'fitness' of T cells and is at the centre of a transcriptional network that promotes the enrichment of high-affinity T cell clones during the course of the immune response (Figure 1).

Immune cell specific cytokine signals shaping metabolic function

Cytokines, in particular those signaling through the common γ-chain, including IL-2, IL-7, IL-15 and IL-21, are critical for the differentiation, proliferation and survival of T cells. They signal via Janus-kinase (JAK) and STAT factors to regulate the dynamic expression of transcription factors that control effector and memory differentiation[109,110].

IL-2 is secreted early after TCR stimulation and is critical for sustained proliferation and survival as well as effector differentiation and memory programming of CD8+ T cells[111-114]. As discussed previously, strong TCR signals are required for robust expression of CD25 (*Il2ra*), thereby directly influencing IL-2 sensitivity of responding T cells[85,88]. Prolonged IL-2 signalling and the presence of inflammatory

cytokines, in particular IL-12, promote differentiation of effector cells. In contrast, limiting IL-2 and inflammation allows for memory precursor development[112-116]. While some of the activities of IL-2 are due to its direct roles in the modulation of the expression of immune-specific transcription factors such as Blimp1, Bcl6 or Eomes, it also regulates metabolic features of T cells. IL-2 transduces signals via PDK1, Akt1 and mTORC1 to sustain glucose and amino acid uptake as well as expression of genes involved in protein and lipid biosynthesis[117] (Figure 2). It also promotes expression of c-Myc and Hif1α both of which required for metabolic reprogramming and sustained glycolysis[91,99] as discussed above. Thus, by stimulating aerobic glycolysis, IL-2 signalling supports clonal expansion and effector differentiation and is inversely correlated with the memory potential of activated T cells. Acting in conjunction with IL-2, inflammatory IL-12 signals via STAT4 to amplify clonal expansion of T cells by sustaining mTOR-dependent protein synthesis and metabolic processes[74]. Thus, IL-2 and inflammatory signals impact on T cell biology at multiple levels during effector and memory differentiation of T cells.

In the absence of antigenic stimulation, IL-7 and IL-15 are critical for the homeostasis and maintenance of memory T cells[118-120]. IL-15 is required for upregulating mitochondrial biogenesis and FAO[27,121]. It also promotes the expression of mitochondrial transcription factor A (TFAM) and Cpt1α, an acetyltransferase that catalyses fatty acid transport into the mitochondrial matrix and is required for the increased mitochondrial spare respiratory capacity of memory T cells[121]. Furthermore, IL-15 signalling results in the upregulation of lysosomal hydrolase (LAL), an essential enzyme that mobilizes free fatty acids stored in acidic lysosomes for utilisation via FAO, thus coupling fatty acid synthesis (FAS) and FAO in memory T cells[28,122]. IL-7 supports glycolytic activity by promoting Glut1 trafficking and glucose uptake in an Akt dependent manner, and thereby may support *de novo* fatty acid synthesis[123,124] (Figures 2 and 3). Additionally, IL-7 was recently found to be required for triacylglycerol (TAG) synthesis and glycerol transport through the upregulation of aquaporin9 (*Aqp9*) solute channels and TAG synthases[125]. The synthesis of triglycerols from the esterification of glycerol and free fatty acids provides an important source of neutral lipids that are subsequently utilised during fatty acid oxidation to produce ATP, thereby promoting CD8+ memory T cell survival. Thus, IL-7 dependent regulation of TAG synthesis and glycerol transport provides a complementary mechanism that fuels FAO in concert with IL-15 regulated

processes.

Finally, cytokines such as IL-10 and IL-21 that induce phosphorylation and DNA-binding of STAT3 contribute to the development of memory T and Tfh cells[126,127]. This may partially be due to the role of STAT3 in promoting OXPHOS and activity of complex I and II of the electron transport chain, thus modulating mitochondrial function and memory T cell metabolism[128,129].

Together these data indicate that immune specific cytokines not only promote expression of transcription factors that control peripheral T cell differentiation but also modulate cellular metabolism.

Immune cell specific transcription factors integrate effector and memory differentiation with distinct metabolic pathways.

As outlined above, metabolic reprogramming and the maintenance of anabolic metabolism is an actively regulated process that involves the coordinated activity of canonical regulators of cellular metabolism as well as immune-specific cytokines and transcription factors. Environmental signals, nutrient availability and immunological cues are integrated by a variety of sensors and signaling pathways that influence the activity of transcription factors and affect the differentiation and function of cytotoxic CD8+ T cells and CD4+ T helper cells. In particular, the mTOR pathway plays a central role in this process and is known to impact on expression and activity of many important transcription factors (reviewed in [2,5,130,131]).

The TCR-dependent transcription factors IRF4 and BATF exemplify the close link between metabolism and differentiation. In addition to their critical role in orchestrating cellular metabolism, IRF4 and BATF also establish a program of effector differentiation by inducing the expression of cytokine receptors and key transcription factors that further reinforce the lineage specification and differentiation of peripheral T cells. This is particularly well understood in the context of the transcriptional network that drives Th17 cell differentiation[96]. As mentioned previously, IRF4 and BATF cooperatively regulate the expression of Hif1α. Th17 cells utilise a Hif1α-dependent program of glycolysis and demonstrate high glycolytic flux important for stabilising the activity of lineage specifying transcriptional

regulator RORγt. IRF4 and BATF also directly induce expression of *Pparγ*, which is involved in regulating lipid metabolism and required for differentiation of adipose tissue resident Treg cells[98,132]. Importantly, PPARγ activity inhibits Th1 and Th2 cytokine production[133,134] as well as Th17 differentiation[44,135], suggesting that tight control of lipid metabolism is required for the differentiation of T helper subsets.

During CD8+ T cell responses, IRF4 and BATF are required for the induction of a transcriptional network that supports effector differentiation[18,19,94]. The coordinate binding of both factors induces expression of Blimp1 and Runx3. At the same time, possibly through indirect mechanisms, IRF4 and BATF support high expression of T-bet while repressing Eomes. Interestingly, while induction of effector molecules such as IFNγ and granzyme B are IRF4 independent, sustained expression of these molecules depends on IRF4[18]. This may be attributed to a direct role for IRF4 in regulating the epigenetic state of the *Ifng* and *GzmB* gene loci or to the requirement for IRF4 to maintain glycolysis, which effector T cells require to sustain efficient effector function[24,79]. Thus, in addition to their function during clonal population expansion, IRF4 and BATF enforce a transcriptional program that directly induces and sustains differentiation of cytotoxic effector cells.

Although induction and maintenance of glycolytic activity is critical for population expansion and effector differentiation of T cells, impaired negative regulation of glycolysis can also contribute to immune pathology. Specifically, increased activity of Hif1α due to the loss of Von Hippel-Lindau (VHL) tumour suppressor resulted in unrestrained cytokine production and cytotoxicity during situations of chronic antigen stimulation[92]. Thus, tight regulation of cellular metabolism and effector function by the coordinated activity of immune-specific and canonical transcription factors is required for the progression of the immune response and the prevention of collateral damage.

While the transcriptional networks engaged during the early stages of T cell activation and effector differentiation are now relatively well understood, less is known about memory T cell development. Memory T cell formation is accompanied by dampening of glycolysis and concomitant upregulation of OXPHOS and FAO (Figure 3). Although this could simply be a passive process due to the clearance of antigen and inflammation, it now emerges that several transcription factors that are indispensable for memory T cell differentiation, actively repress glycolysis and upregulate pathways

important for memory T cell metabolism. For example, Bcl6, which is critically important for Tfh and central memory T cell development[136,137], represses many genes involved in aerobic glycolysis, while T-bet promotes their expression[138]. Other factors such as Eomes may impact on memory T cell development by regulating the expression of the IL-15 receptor[139], which is critical for stimulating mitochondrial function. However, more work is required to understand the relative importance of these factors in regulating cellular metabolism.

Foxo1 and Foxo3a are another two transcription factors that are essential for memory T cell development and function[73,140]. Although this has not directly been shown, microarray data from CD8+ memory precursor T cells suggest that Foxo1 is required for the expression of enzymes involved in FAO (*Acoxl*), transporters involved in shuttling TCA intermediates (*Slc13a3*) and mitochondrial solute carriers (*Slc25a33, Slc25a51*), suggesting that Foxo1 may regulate mitochondrial homeostasis[73]. During chronic viral infection, Foxo1 expression is increased and is preferentially retained in the nucleus, where it is thought to dampen anabolic metabolism and maintain the exhausted state of antiviral T cells[141]. IRF4 and BATF modulate expression of both Foxo1 and Foxo3a, and Foxo1 itself has been implicated in the regulation of IRF4 expression[142], suggesting an unexplored feedback mechanism between both molecules. This notion is supported by transcriptional profiling data that implicate IRF4 and BATF not only in anabolic metabolism but also in the reversion to the catabolic state during memory development[18,94]. In summary, these studies show that transcriptional regulators link effector and memory differentiation with metabolic pathways appropriate for the differentiation state.

Perspectives

The generation of near infinite numbers of antigen-specificities in the repertoire of naïve lymphocyte by random combination of elements of the antigen-receptor is key to the adaptive immune system. Such a system necessitates the antigen-driven selection and rapid expansion of specific clonotypes best suited for the control of an invading pathogen and adaptation to a broad range of environmental conditions. This process requires dramatic quantitative and qualitative changes to the metabolic features of the responding cells to meet the increased demand for the synthesis of

macromolecules during proliferation. At the same time, proliferating antigen-specific cells need to acquire effector function to control the infection. Antigen-receptor regulated transcription factors such as c-Myc, IRF4, Foxo1, Hif1α and PPARγ appear to play key roles in the coordination of cellular metabolism and differentiation. Interestingly, the same transcription factors are implicated in the regulation of organismal metabolism by regulating responses to feeding and fasting, thermogenesis and maintenance of adipose tissue homeostasis[32,142-145]. In addition, there is some evidence that Bcl6 induces expression of genes involved in lipid metabolism[146], while Foxo transcription factors are involved in gluconeogenesis and responses to metabolic stress[147]. These studies suggest that transcription factors that regulate immune cell differentiation have ancestral roles in regulating cellular metabolism and energy expenditure. This supports a model in which during the evolution of the adaptive immune system, transcriptional regulators of metabolic pathways have been coopted for the regulation of effector and memory differentiation.

The molecular networks that affect dynamic changes to lymphocyte metabolism following antigen recognition and effector differentiation are beginning to be elucidated. However, there is a lack of knowledge of transcriptional networks that coordinate cellular metabolism permissive for memory T cell differentiation. Similarly, metabolic programs that promote alternative differentiation fates such as T cell 'exhaustion' during situations of chronic antigen stimulation are only partially known. Therefore, for the development of therapeutic strategies to target tumor cell metabolism, it is imperative to understand the impact of such therapies on immune cell differentiation and function. Gaining a detailed picture of the factors that control cellular metabolism in lymphocytes is critically important for uncovering ways to modulate immune responses, which could be especially valuable in the context of reversing functional impairments observed during exhaustion or in the design of vaccine strategies to bolster memory T cell responses.

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Boxes

Box 1. Antigen driven metabolic programming in T cells (plus figure).

Naïve T cells rely on oxidative phosphorylation (OXPHOS) during which pyruvate produced through aerobic glycolysis enters the mitochondria and is oxidatively decarboxylated through the tricarboxylic acid cycle (TCA). Antigen recognition via the T cell receptor (TCR) induces broad metabolic reprogramming, which is instructed by signalling via the PI(3)K-Akt-mTORC1 pathways and the activity of the transcription factors c-Myc and Hif1α[89,99]. This leads to upregulation of amino acid and glucose transport to fuel glutaminolysis and aerobic glycolysis during which pyruvate is converted to lactate in a process that generates each two molecules of NADH and ATP, and regenerates NAD for later use in glycolysis[148]. Intermediates from glycolysis feed into the pentose phosphate and serine biosynthesis pathways required for nucleotide synthesis[1]. Furthermore, genes related to increased *de novo* synthesis of fatty acids and cholesterol are upregulated[28]. During clonal expansion

and effector differentiation the coordinated activity of several transcription factors, including IRF4, AP-4, T-bet and Hif1α, is required to maintain glycolysis and anabolic metabolism.

Following the clearance of antigen and the contraction of the majority of effector T cells, developing memory T cells revert back to catabolic metabolism, resulting in the upregulation of mitochondrial biogenesis, OXPHOS and lipid oxidation pathways. Memory cells upregulate mitochondrial biogenesis pathways and fatty acid oxidation (FAO) metabolism in an IL-7, IL-15 and AMPK-dependent manner[27,28,121]. IL-7 also mediates glycerol transport and triacylglycerol synthesis, contributing to neutral lipid stores utilised for FAO and ATP synthesis[125]. IL-15 stimulates the expression of mitochondrial transcription factor A (TFAM), which promotes mitochondrial biogenesis, and Cpt1α, an acetyltransferase which catalyses fatty acid transport into the mitochondrial matrix and is required for increasing the mitochondrial spare respiratory capacity of memory T cells[121]. In addition, *de novo* fatty acid synthesis (FAS), powered by glucose metabolism, contributes to the maintenance of memory T cells[122]. Fatty acids are stored in acidic lysosomes and upregulation of lysosomal hydrolase (LAL) in an IL-15 dependent manner is essential for mobilizing free fatty acids for utilisation via FAO, thus coupling FAS and FAO in memory T cells[122]. In addition to cytokines, transcriptional regulators Bcl6 and Foxo1 are required for repressing the transcription of genes involved in glycolysis.

NADH, nicotinamide adenine dinucleotide; ATP, adenosine triphosphate; Cpt1α, carnitine palmitoyltransferase I; mTORC1, mechanistic target of rapamycin complex 1; PI(3)K, phosphoinositide 3-kinase; ETC, electron chain transport; PPP, Pentose phosphate pathway.

Figure legends

Figure 1. Clonal expansion and competition during immune responses is determined by IRF4 and the metabolic state of cells.

T cell receptor (TCR) affinity dependent signals induce the expression of transcription factors, cytokine receptors and survival molecules that coordinate effector

differentiation and clonal expansion with metabolic reprogramming. Clones that exhibit the highest affinity for antigen undergo the greatest population expansion, while low-affinity clones undergo limited proliferation before entry into quiescence and apoptosis, or the development of memory T cell characteristics. TCR-affinity signals induce graded levels of the transcription factor IRF4, which regulates the majority of the TCR-affinity driven transcriptional changes, including the level of aerobic glycolysis. Thereby, IRF4 directly regulates the magnitude of clonal expansion. Additionally, clonotypes that exhibit higher affinity for antigen express higher levels of IL-2Rα (CD25) resulting in higher IL-2 sensitivity, and show greater stabilization of pro-survival Mcl-1, contributing to the preferential selection and survival of higher affinity clones during an immune response. Overall, the process of clonal competition results in an increased overall affinity of the effector population for antigen, which is critical for facilitating pathogen control during acute viral or bacterial infection. OXPHOS – oxidative phosphorylation; mTORC1, mechanistic target of rapamycin complex 1.

Figure 2. Immune specific transcriptional regulators cooperate with canonical metabolic regulators during effector and memory differentiation

TCR-dependent transcription factors IRF4 and BATF establish a transcriptional network that facilitates effector differentiation and coordinates cellular metabolism during T cell proliferation. The cooperative binding of IRF4 and BATF is required to induce Blimp1, and indirectly modulates the activity of Eomes, T-bet and Bcl6, thereby initiating terminal effector differentiation and opposing memory specific gene networks. Additionally, IRF4 integrates mTORC1 activity and together with BATF, induces expression of Hif1α required to sustain aerobic glycolysis during clonal expansion. Thus, gene networks that are IRF4 and BATF dependent, act in concert with canonical metabolic regulators, such as c-Myc and AP-4, to coordinate effector differentiation with the metabolic state of proliferating cells.

With the clearance of antigen and the downregulation of mTORC1 signaling, memory T cells revert back to catabolic metabolism. c-Myc, AP-4 and Hif1α are rapidly downregulated, and memory promoting cytokines such as IL-7, IL-15, IL-10 and IL-21 promote OXPHOS and lipid oxidation through multiple mechanisms. Although down-regulated during memory development, IRF4 modulates expression of Foxo1, a

critical regulator of memory T cell development, and for expression of PPARγ, important for regulating lipid metabolism.

OXPHOS, oxidative phosphorylation; FAO, fatty acid oxidation; FAS, fatty acid synthesis; TCA, tricarboxylic acid cycle; ETC, electron chain transport; mTORC1, mechanistic target of rapamycin complex 1.

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124 *Jacobs, S. R. et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. J Immunol **180**, 4476-4486 (2008).*

125 *Cui, G. et al. IL-7-Induced Glycerol Transport and TAG Synthesis Promotes Memory CD8(+) T Cell Longevity. Cell **161**, 750-761 (2015).*

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These papers (refs 142, 143) show that IRF4, typically considered a hematopoietic-specific transcriptional regulator, regulates lipid metabolism in adipocytes and is critical for the thermogenic capacity of brown adipose tissue in response to environmental cold via cooperative transcriptional binding with PGC1a.

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