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Metabolic orchestration of T lineage differentiation and function

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T cells are stimulated by the engagement of antigen, cytokine, pathogen, and hormone receptors. While research performed over many years has focused on deciphering the molecular components of these pathways, recent data underscore the importance of the metabolic environment in conditioning responses to receptor engagement. The ability of T cells to undergo a massive proliferation and cytokine secretion in response to receptor signals requires alterations to their bioenergetic homeostasis, allowing them to meet new energetic and biosynthetic demands. The metabolic reprogramming of activated T cells is regulated not only by changes in intracellular nutrient uptake and utilization but also by nutrient and oxygen concentrations in the extracellular environment. Notably, the extracellular environment can be profoundly altered by pathological conditions such as infections and tumors, thereby perturbing the metabolism and function of antigen-specific T lymphocytes. This review highlights the interplay between diverse metabolic networks and the transcriptional/epigenetic states that condition T-cell differentiation, comparing the metabolic features of T lymphocytes with other immune cells. We further address recent discoveries in the metabolic pathways that govern T-cell function in physiological and pathological conditions.

Keywords: differentiation; effector functions; epigenetics; immune function; metabolism; nutrients; oxygen; T cells; transcription factors

The potential of immune cells to respond to infections, foreign antigens, and even auto-antigens requires the induction of metabolic pathways that support their proliferation and activation. Immune cell responsiveness depends on the generation of energy in the form of ATP but recent work has also highlighted the importance of metabolite pathways in bioenergetic-independent processes including: (a) nucleic acid, amino acid, and phospholipid synthesis; (b) *de novo* lipogenesis; and (c) production of reducing equivalents to maintain the redox state of the cell. Moreover, we have learned that a wide range of diverse fuels as well as their utilization in different pathways govern immune cell function as well as plasticity; for example, metabolic alterations regulate the 'choice' between effector and regulatory T (Treg) cell fates as well as between IFNγ-secreting M1 and IL4-secreting M2 macrophages (reviewed in ref. [1–4]). The metabolism of a cell conditions its response to receptor and cytokine signals, controlling transcription factor networks

Abbreviations
αKG, alpha-ketoglutarate; AMPK, AMP-activated protein kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GCN2, general control nonrepressed 2; GlcNAc, *N*-acetylglucosamine; HDACs, histone deacetylases; HIF1α, hypoxia-inducible factor 1α; IDO, indoleamine 2,3-dioxygenase 1; Kyn, kynurenine; mTOR, mammalian target of rapamycin; OXPHOS, oxidative phosphorylation; R-2HG, R-2-hydroxyglutarate; TCA, tricarboxylic acid; Teff, T effector; Treg, T regulatory; Trp, tryptophan.

and the epigenetic landscape. In this review, we focus on recent discoveries revealing the regulation of T lymphocytes in the context of different metabolite resources and activation of metabolic pathways, specifically addressing the metabolic regulation of transcriptional pathways and epigenetic modifications (Fig. 1). The composition of the extracellular environment also fluctuates in pathological conditions as a consequence of metabolite, electrolyte, proton, and oxygen concentrations, among others. Here, we highlight the importance of pathological extracellular environments, generated as a response to tumor growth or infection, in the regulation of T-cell plasticity and function.

Interplay between nutrients and downstream effectors in T-cell differentiation and function

The metabolic needs of activated immune cells are generally secured by augmented nutrient entry and changes in the utilization of those nutrients. Cell surface upregulation of metabolite transporters is a rate-limiting step in nutrient entry and indeed, the upregulation of glucose, glutamine, and neutral amino acid transporters are a sine qua non for optimal T-cell proliferation and effector function [5–13]. The phenotypic alterations that occur in activated T lymphocytes are conditioned by the range of accessible metabolites and the means in which they are metabolized.

Upon TCR stimulation, naive T cells undergo a metabolic reprogramming favoring aerobic glycolysis, wherein glucose is converted to lactate, as compared to glucose catabolism *via* the mitochondrial tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS). Glycolysis is less efficient than OXPHOS in energy production (2 net ATPs as compared to 32–34 ATPs), but it is more efficient in producing the carbons and electrons (NADH/NADPH) that are required for production of macromolecular precursors; these include acetyl co-A for fatty acids, glycolytic intermediates for amino acids and ribose for nucleotides, promoting a rapid growth and proliferation of immune cells [2,4]. Furthermore, under conditions of high glucose flux, the shunting of glucose through the pentose phosphate pathway, leading to the synthesis of five-carbon sugars used for nucleotide

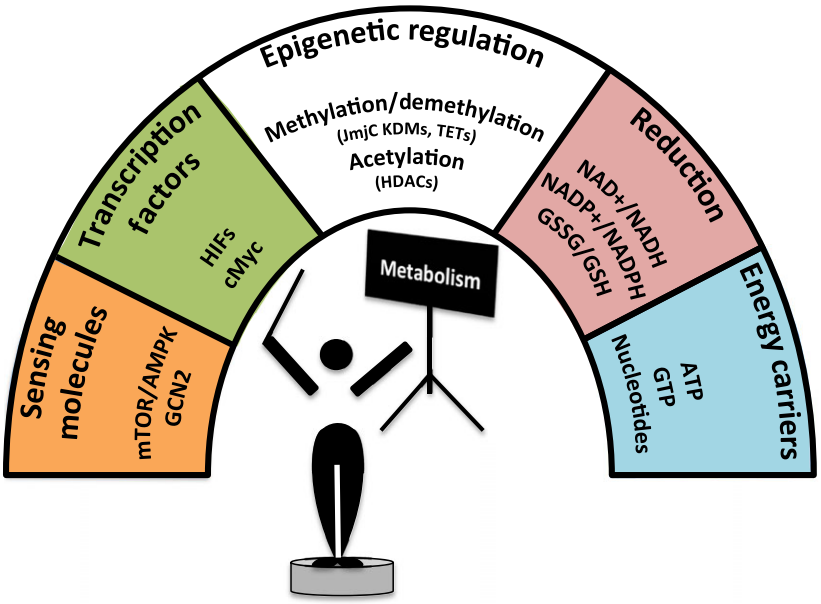

Fig. 1. Metabolic orchestration of immune cell function. A schematic representation of the multiple pathways that are orchestrated by metabolism. The effects of the metabolic environment are conducted in the cell *via* different sensors. These inputs are then transcribed into changes in the transcriptional, epigenetic, redox, and energy status of a cell. The major players in these processes are indicated here: (a) Molecules such as mTOR integrate nutrient-sensing pathways while AMPK and GCN2 respond to the energy homeostasis of the cell and integrate stress responses; (b) Transcription factors such as HIF and Myc not only confer metabolic advantages to cancer cells but also regulate T cell effector function; (c) Epigenetic-metabolomic crosstalk governs T-cell fate *via* changes in methylation, acetylation, and succinylation among others; production of NAPDH through the pentose phosphate pathway favors macromolecular biosynthesis, the ratio of oxidized (GSSG) to reduced glutathione (GSH) serves as a marker of the redox homeostasis of the cell and NAD+ regeneration through mitochondrial respiration contributes to multiple levels of regulation in T lymphocytes including the control of T-cell inflammation [4,15]. Energy transfer within the cell is mediated by nucleotides with the most important being ATP and GTP.

biosynthesis, results in an increased generation of the key intracellular reductant NADPH [14] (Fig. 1).

While T cell activation results in a massive upregulation of glycolysis, OXPHOS is also critical for T cell function. Indeed, NAD+ regeneration through mitochondrial respiration improves T-cell lysosomal function and reduces inflammation [15]. The level of OXPHOS in T lymphocytes is modulated by the presence of amino acids. For example, arginine uptake results in a shift in metabolism from a glycolytic to OXPHOS program and this change has functional consequences, increasing the generation of central memory cells [10]. Furthermore, as outlined below, specific lymphocyte subsets exhibit differences in the utilization of nutrients as energy sources [16]. Glucose and glutamine are also utilized in the hexosamine pathway, producing uridine diphosphate *N*-acetylglucosamine (GlcNAc), a substrate for glycosyltransferases that catalyze post-translational *O*-GlcNAcylation. Notably, this glycosylation of serine/threonine residues by *O*-GlcNAC is required for T-cell activation [17–19] while a high-fat diet increases *O*-GlcNaC, priming CD4+ effector cells and potentially increasing susceptibility to autoimmune diseases [20].

The importance of mitochondrial function in T-cell stimulation is underlined by the finding that within 15 min after TCR stimulation, mitochondrial ROS are generated, creating a positive feedback loop for TCR signaling [21,22]. Indeed, fueling mitochondrial ROS production is a prerequisite for antigen-specific T-cell expansion [23] and mitochondria contribute to T-cell activation [23–25] as well as to the maintenance of memory and Treg cell subsets [25–29]. Moreover, distinct T-cell subsets exhibit different requirements for mitochondrial activity; CD8+ T cells are significantly more sensitive than CD4+ lymphocytes to complex IV dysfunction (regulated by cytochrome *c* oxidase) with increased mitochondrial mass in CD8 memory cells resulting in a bioenergetic advantage for rapid recall while Tregs are more resistant than T effector (Teff) subsets to mitochondrial failure [30]. Mitochondrial function is linked to its morphology [3,31] and mitochondrial fusion–fission dynamics have been revealed to regulate immune function; mitofusin-mediated fusion is required for lymphocyte differentiation of hematopoietic stem cells [32] while memory/effector CD8 T cell generation is balanced by fusion–fission, respectively [33].

The data presented above reinforce the postulate that immune cell populations exhibit distinct bioenergetic requirements. Indeed, T lymphocyte subsets exhibit disparate metabolic profiles; Teff cells are highly glycolytic, and sometimes lipogenic [34,35], while suppressive Treg cells display a mixed metabolism with augmented lipid oxidation [13,35–37]. Conversely, effector T-cell secretion of IFNγ is directly dependent on the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [38,39]. Th17 cells also strongly depend on glycolysis, as demonstrated by the massive induction of glycolytic enzymes and upregulation of glycolytic activity [40]. Although the mechanisms that contribute to regulating glycolysis are complex, the mammalian target of rapamycin (mTOR) signaling pathways plays a critical role. mTOR is a serine/threonine protein kinase that integrates environmental cues from nutrients, growth factors, and stress signals into an 'optimal' cellular response that is mediated *via* two complexes, mTORC1 and mTORC2 [13,41–43]. Indeed, mTOR signaling complexes show specificity in immune cell regulation; in the absence of mTORC1 but not mTORC2, Th17 cell differentiation is abrogated [44]. Furthermore, mTORC1 is required for Th1 and Th17 differentiation as well as CD8 cytolytic activity while mTORC2 promotes Th2 differentiation [44–48].

Mammalian target of rapamycin activity is directly regulated by the AMP-activated protein kinase (AMPK) complex, a sensor of nutrient stress, in cooperation with the stress-activated kinase general control nonrepressed 2 (GCN2) (reviewed in ref. [49,50]. AMPK is transiently upregulated on mature T cells by antigen receptor engagement [51] and regulates glycolysis and cytokine secretion [52]. Under conditions of glucose deprivation, AMPK directly inhibits mTOR signaling, resulting in changes in nutrient utilization (toward glutamine) and long-term memory CD8 T-cell generation and survival [53,54]. Moreover, AMPK activity in memory CD8 T cells (at the expense of mTOR activation) promotes the mitochondrial uptake of fatty acids and their oxidation [28,29]. Thus, AMPK activity results in a decreased dependence on glucose metabolism, contrasting with the glycolytic metabolism of effector cells.

Mammalian target of rapamycin signaling is mediated by numerous transcription factors but among the myriad of factors, c-Myc and hypoxia-inducible factor 1α (HIF1α) are probably the most critical [55–57] (Fig. 1). c-Myc directly regulates glutamine utilization within the cell by upregulating the expression of the rate-limiting glutaminase I enzyme that catalyzes the conversion of glutamine to glutamate [13,58,59]. HIF1α regulates Th1/Th17 effector differentiation and B-cell antibody production *via* the induction of the Glut1 glucose transporter as well as glycolytic enzymes [35,37,40,44,60]. It can also bind directly to the *IFNG* promoter, enhancing its expression [61]. Intriguingly,

HIF1α has been reported to both positively [62,63] and negatively [37,61,64] impact Foxp3 expression in Tregs. In support of a negative role for Foxp3 in regulating an mTOR-HIF1α-cMyc axis, it is notable that Foxp3 can suppress Myc expression and downstream glycolysis in Tregs, allowing them to function in low-glucose environments [65]. Furthermore, we and others have shown that Treg, but not Teff, generation is maintained under conditions where glucose, glutamine, and leucine transport are limiting [9,12,66–68]. Decreased nutrient entry inhibits mTOR activity and indeed, genetic abrogation of mTOR signaling promotes the generation and function of Tregs while blocking the generation of Teff [69–71]. Conversely, activation of mTOR blocks Treg differentiation and function [72–74] (Fig. 2). The importance of suppressed mTOR function in inhibiting inflammation is also revealed in the macrophage system; inhibition of mTOR enhances the polarization of anti-inflammatory M2 polarization and decreases IFNγ secretion by LPS-stimulated M1 macrophages [75–77]. Thus, under physiological conditions, immune cell function is critically regulated by mTOR signaling pathways with limiting nutrient resources levels fostering an anti-inflammatory environment mediated by increased Treg and M2 macrophage function.

Regulation of T-cell function by metabolite-mediated epigenetic changes

Epigenetic modifications, including DNA methylation and acetylation as well as histone marks, have been shown to play an important role in immune cell differentiation and function. Deletion of the JMJD2 and UTX histone H3 lysine 27 trimethylation (H3K27Me3) dimethylases inhibit terminal thymocyte development [78], while JMJD3 deletion attenuates Th1 and Treg differentiation, promoting polarization to a Th2 or Th17 fate and decreasing the expression of inflammatory genes in LPS-stimulated macrophages [79–81]. The JMJD2 enzyme has also been linked with IL17 production [82]. Furthermore, TET family deoxygenases play multiple roles in T-cell effector function—increasing IL17/IFNγ, stabilizing the Foxp3 transcription factor in Treg cells, and promoting expansion of iNKT cells [83–87]. While these types of postnatal epigenetic modifications in immune cells were once thought to be completely independent of the cell's metabolic status, it is now clear that these two processes are intricately related. Within the innate immune system, trained immunity, the long-term memory that is associated with an epigenetic reprogramming at the level of histone H3 methylation, has recently been shown to be regulated by mTOR/HIF1α-stimulated glycolysis [88]. Furthermore, expression of the JHDM1D histone demethylase increases markedly upon long-term nutrient starvation [89] and both JMJD and TET demethylases require the alpha-ketoglutarate (αKG) metabolite as a cofactor [90,91]. Thus, alterations in citric acid cycle enzymes that result in either the accumulation of succinate or fumarate or reduction of αKG to R-2-hydroxyglutarate (R-2HG)—inhibiting αKG-dependent demethylases—will alter the cell's epigenetic state [92–96]. Succinate can also independently alter the epigenetic state of a cell *via* succinylation [97], exacerbating the effects of R-2HG [94].

In M1 monocytes, metabolic constraints that increase succinate levels result in an inhibition of the activity of αKG-dependent prolyl hydroxylases that target HIF1α for proteosomal degradation [98,99]. Thus, the presence of succinate leads to a stabilization of HIF and this has significant functional consequences for the M1 macrophage, enhancing IL1b production [100,101]. Conversely, αKG promotes M2 macrophage differentiation [102] as well as Th1 differentiation in glutamine-deprived conditions [66]. Furthermore, the αKG/succinate ratio modulates stem cell differentiation potential [103,104]. Another enantiomer of 2-HG, S-2-hydroxyglutarate, accumulates in T lymphocytes following physiological TCR engagement and its effects on demethylation and stabilization of HIF1α have been shown to enhance CD8 T-cell proliferation and antitumor function [105]. Thus, further research will undoubtedly uncover additional epigenetic networks *via* which the balance between succinate, fumarate, αKG, and 2-HG regulate immune cell function. The changes in the cell that occur as a result of these metabolites extend far beyond alterations in ATP production, altering the cell's epigenetic landscape.

Histone acetylation is a critical epigenetic modification that regulates immune cell function. Acetylation is regulated by multiple metabolites including acetyl-coA and acetate, produced *via* glycolysis and the fermentation of carbohydrates in the intestine by gut microbiota (reviewed in ref. [106]). Notably, decreased glycolysis has recently been shown to lead to lower levels of acetyl-coA and subsequent histone acetylation, resulting in a loss of stem cell pluripotency [107] as well as decreased IFNγ transcription in CD4 T cells [108]. Conversely, hyperacetylation in CD8 T cells is associated with an active state of the *IFNG* promoter [109]. There is also a complex interplay between glycolysis, IFNγ and expression of HIF1α; the glycolytic enzyme GAPDH not only inhibits IFNγ production by binding to the 3′ UTR [38] but similarly negatively

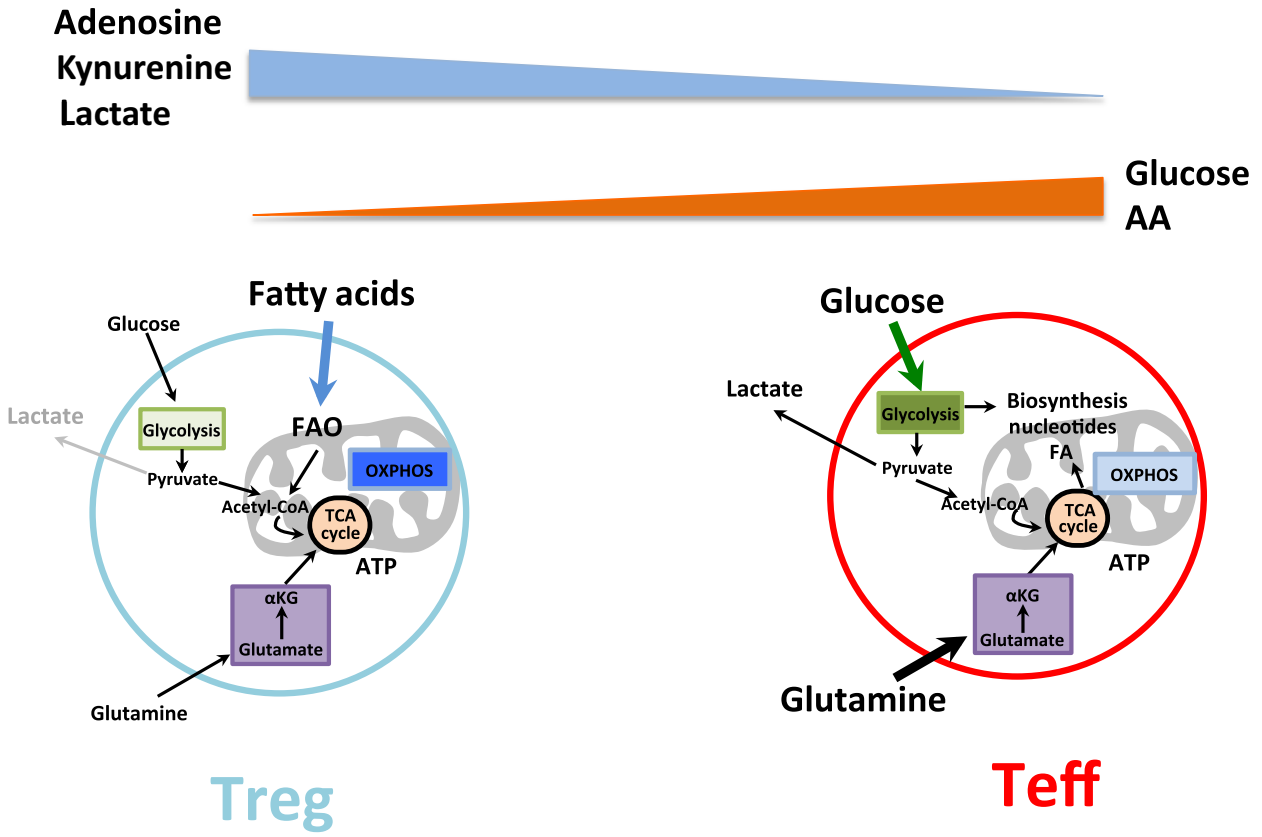

Fig. 2. Metabolic pathways condition the differentiation of regulatory and effector T cells. Multiple metabolic pathways regulate the differentiation of naïve T cells to regulatory (Treg) and effector (Teff) fates. While we still have much to learn regarding the relative importance of different metabolites, polarization is biased to a Treg fate in the presence of high levels of adenosine, Kyn, and lactate while glucose and amino acids (AA) favor a Teff fate [9,10,12,13,65,66,136,155,156]. In Tregs, there is an important contribution of fatty acid oxidation and OXPHOS while Teffs exhibit an enhanced utilization of glucose *via* glycolysis, with acetyl-CoA promoting histone acetylation. Teffs also utilize both glucose and glutamine for the formation of αKG, an intermediate in the mitochondrial TCA cycle and required for αKG-dependent enzymatic reactions. The pathways indicated here are only a simplistic schematic but they highlight the significant complexity of metabolite utilization in T-cell differentiation and function.

regulates HIF1α expression [110]. Thus, it will be important for future studies to determine the relative contributions of glycolysis-linked acetylation of the *IFNG* promoter and GAPDH-mediated binding to *HIF1α* and *IFNγ* RNAs in the regulation of IFNγ expression.

The level of histone acetylation is also regulated by histone deacetylases (HDACs) with the family of sirtuin HDACs (Sirt) playing a critical role. Sirts are themselves directly regulated by the metabolic status of the cell; their activity is directly dependent on intracellular NAD+ levels [111]. Studies on the effects of Sirt1 in T lymphocytes have already revealed an impressive complexity; the absence of Sirt1 attenuates T-cell signaling and effector function [112–116] while deacetylation of RORγt and Foxp3 promote Th17 differentiation [53] at the expense of Treg generation [117–119], respectively. However, pharmacological Sirt1 activation inhibits Th17 differentiation [120] but increases IFNγ secretion [121]. Irrespective of the precise effects of Sirt1 in T lymphocytes, the ensemble of these studies point to the importance of metabolite-regulated deacetylation in Teff differentiation and function.

Metabolic alterations in pathological conditions: effects on lymphocyte responsiveness

When individuals are affronted by a tumor or infection, the initiation of an immune response is generally extremely advantageous. However, the ability of immune cells to optimally respond to these insults is often negatively modulated by their metabolite environment, an environment that is conditioned by nutrient composition, 'waste' products, oxygen concentration, pH, and physical forces, among others. The

dysregulated growth of cancer cells can directly influence the extracellular environment and multiple studies now strongly suggest that the metabolic phenotype of the tumor governs the ensuing immune response (reviewed in ref. [122,123]). In the context of several solid tumors including hepatocellular carcinomas and stomach/colon tumors, quantitative metabolome profiling has revealed decreased intratumoral concentrations of glucose and glutamine [124,125]. Nutrient deprivation is associated with mitochondrial fusion and inhibition of autophagy [126], processes that will alter the function of intratumoral T lymphocytes [33]. Moreover, tumor-infiltrating T cells demonstrate a loss of mitochondrial function due to decreased expression of PPAR-gamma coactivator 1α (PGC1α), a key factor in mitochondrial biogenesis [127]. Similarly, following CMV infection, decreased PGC1α in CD8 T cells results in mitochondrial dysfunction and exhaustion [128]. Notably, the competitiveness of T cells within the tumor environment may be enhanced by low mitochondrial function; adoptively transferred T cells with low mitochondrial membrane potential allow the identification of cells with optimal *in vivo* persistence and antitumor activity [129].

Competition of T cells and tumor cells for limiting amounts of glucose can lead to a decreased glycolysis in the former, resulting in a subsequent inhibition of T-cell cytolytic and effector function [38,67,130]. A metabolic checkpoint in glycolysis, phosphoenolpyruvate, has been identified as a key factor in the regulation of calcium flux and NFAT (nuclear factor of activated T cells) activity in T lymphocytes [131]. Furthermore, aerobic glycolysis promotes Th17 generation by starving the hexosamine pathway; when glycolysis is attenuated, reduced levels of GlcNAc promote a switch from a Th17 to an iTreg fate [65]. Bacteria and viruses such as *Staphylococcus aureus* and Hepatitis C, respectively, also generate glucose-limiting conditions, promoting adaptation *via* a metabolic reprogramming [132,133]. Indeed, in the context of influenza infection, nutrient supplementation, and specifically glucose utilization, is necessary to protect against mortality [134]. Nevertheless, the competition for nutrients in the 'fight' against cancer and infections is clearly complex; nutrient restriction has been found to sensitize cancer cells to cytotoxic therapies and protect mice from the deleterious effects of bacterial sepsis [134,135]. Thus, future research will be necessary to optimally define the role of diet and nutrients in regulating the body's responses to specific types of cancers, infections, and autoimmune conditions.

Competition for amino acids is also critical for T-cell function; decreases in glutamine, arginine, and leucine, among others, severely impact on the potential of naïve T cells to differentiate into Teff cells [5–13,66,136]. In the absence of glucose and glutamine, T lymphocytes are biased toward a Treg cell fate [66,136]. Unlike Teff cells, Foxp3+ Treg cells are capable of surviving in a low-glucose environment [35,65]. Amino acid uptake by macrophages or their production of arginase can also serve to decrease amino acid availability for T lymphocytes [137,138].

'Waste' products within a tumor site or infection will also modulate immune responsiveness. The major metabolite, initially thought to function solely as a 'waste' product, is lactate. Lactate produced by aerobic glycolysis in tumor cells can be transported into T cells *via* monocarboxylate transporters [139]. Glycolysis and lactate production are regulated by pH as both phosphofructokinase and lactate dehydrogenase activities increase at alkaline pH, due at least in part to post-translational deprotonation (reviewed in ref. [140]). Lactate has been found to decrease cytolytic CD8 T-cell function and T-cell motility [141–143]. Furthermore, lactate decreases NK cell cytotoxic function [144,145] as well as the antigen presentation potential and differentiation of dendritic cells [146]. Lactate also functions to actively promote anti-inflammatory responses; unlike Teff cells, Tregs are relatively resistant to lactate. The Foxp3 transcription factor was found to mediate this resistance by inhibiting Myc activity and glycolysis [65] (Fig. 2). Furthermore, lactate promotes the polarization and growth of immunosuppressive cells including M2 macrophages and myeloid-derived suppressor cells [137,144,145]. In light of these data, it is interesting to note that high lactate dehydrogenase levels correlate with a poor prognosis in several different types of tumors [147–150].

The degradation of tryptophan (Trp) results in the generation of a key metabolite in T-cell function, kynurenine (Kyn). This process is catalyzed by indoleamine 2,3-dioxygenase 1 (IDO), an enzyme that is upregulated in many cancers and correlates with a poor prognosis [151–154]. High IDO levels result in the depletion of Trp in the tumor microenvironment and furthermore, Kyn promotes Treg polarization by activation of its downstream effector, the metabolic stress-sensing protein kinase GCN2 [155,156]. In this regard, it is notable that Kyn is a ligand for the aryl hydrocarbon receptor that promotes Treg generation (for a review see [157]). Trp depletion also attenuates Th1 proliferation but does not affect Th2 or Th17 subsets, highlighting metabolic differences between these subsets [158] (Fig. 2). Finally, from a clinical perspective, IDO inhibitors show promising results in preclinical tumor models [155,159,160] and most significantly,

the combined utilization of the IDO inhibitor indoximod with a PD-1 checkpoint inhibitor (pembrolizumab) has just been reported to result in a remarkable 52% overall response rate in patients with advanced melanoma [161].

The immune response is also regulated by electrolytes such as sodium chloride, increasing T follicular helper and Th17 responses while decreasing Treg suppression and inflammation [162–164]. In contrast, extracellular potassium, increased by tumor necrosis, attenuates mTOR signaling and Teff function [165]. On another level, the antimicrobial metabolite itaconate, synthesized by the product of the immunoresponsive gene 1 (*Irg1*), cis-aconitate decarboxylase, regulates cytokine secretion by proinflammatory macrophages. Briefly, TCA cycle-generated citrate is used for the synthesis of itaconate which then acts as an inhibitor of succinate dehydrogenase, resulting in succinate accumulation and enhanced M1 immune reactivity [166–168]. Extracellular ATP also regulates the immune response upon its metabolism to adenosine *via* the CD39/CD73 ectoenzymes. Activation of the CD39/adenosine axis correlates with a higher Treg suppressor activity and lower CD4+ counts in HIV-1+ patients [169,170] (Fig. 2).

Finally, it is important to note that the extracellular environment is not solely defined by its composition in metabolites, electrolytes, protons or oxygen but also by physical parameters such as hydrostatic pressure, shear stress, and tension forces [171]. Tumors generally exhibit an increased stiffness [172] and a recent study has elegantly shown the critical nature of stiffness in the cell cycle progression, metabolism, and cytokine secretion profile of TCR-stimulated T cells [173]. Thus, the ensemble of these data point to the importance of the global extracellular environment in regulating the metabolic status of immune cells in both physiological and pathological conditions.

Conclusion

The integration of data emerging from research on metabolite-regulated transcriptional, epigenetic, and energy networks will bring novel insights to our understanding of T lineage differentiation and function. This is particularly crucial for furthering our understanding of pathological situations where stresses such as infections and tumors can alter the metabolite levels that are available for infiltrating immune cells. While much recent research has focused on the importance of nutrients—including amino acids—in effector function, future studies will allow us to ascertain the role(s) of electrolytes, minerals, and pH in immune regulation. Evaluation of the metabolic crosstalk between immune cells and their complex microenvironment remains a challenge and the development of novel tools that allow *in vivo* real time metabolic imaging and carbon flux analyses will promote innovative biological interventions and clinical evaluations. The identification and exploitation of the metabolic pathways regulating the function of T lymphocytes as well as other immune subsets will be critical for the development of new therapeutic strategies targeting tumors, infections, and autoimmune diseases.

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References

1 Beckermann KE, Dudzinski SO and Rathmell JC (2017) Dysfunctional T cell metabolism in the tumor microenvironment. *Cytokine Growth Factor Rev* **35**, 7–14.
2 Buck MD, Sowell RT, Kaech SM and Pearce EL (2017) Metabolic instruction of immunity. *Cell* **169**, 570–586.
3 Chao T, Wang H and Ho PC (2017) Mitochondrial control and guidance of cellular activities of T cells. *Front Immunol* **8**, 473.
4 Loftus RM and Finlay DK (2016) Immunometabolism: cellular metabolism turns immune regulator. *J Biol Chem* **291**, 1–10.
5 Kinet S, Swainson L, Lavanya M, Mongellaz C, Montel-Hagen A, Craveiro M, Manel N, Battini JL, Sitbon M and Taylor N (2007) Isolated receptor binding domains of HTLV-1 and HTLV-2 envelopes bind Glut-1 on activated CD4+ and CD8+ T cells. *Retrovirology* **4**, 31.
6 Manel N, Kinet S, Battini JL, Kim FJ, Taylor N and Sitbon M (2003) The HTLV receptor is an early T-cell activation marker whose expression requires de novo protein synthesis. *Blood* **101**, 1913–1918.
7 Carr EL, Kelman A, Wu GS, Gopaul R, Senkevitch E, Aghvanyan A, Turay AM and Frauwirth KA (2010) Glutamine uptake and metabolism are coordinately regulated by ERK/MAPK during T lymphocyte activation. *J Immunol* **185**, 1037–1044.
8 Hayashi K, Jutabha P, Endou H, Sagara H and Anzai N (2013) LAT1 is a critical transporter of essential amino acids for immune reactions in activated human T cells. *J Immunol* **191**, 4080–4085.
9 Sinclair LV, Rolf J, Emslie E, Shi YB, Taylor PM and Cantrell DA (2013) Control of amino-acid transport by antigen receptors coordinates the metabolic

reprogramming essential for T cell differentiation. *Nat Immunol* **14**, 500–508.
10 Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, Kogadeeva M, Picotti P, Meissner F, Mann M *et al.* (2016) L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* **167**, 829–842 e13.
11 Hukelmann JL, Anderson KE, Sinclair LV, Grzes KM, Murillo AB, Hawkins PT, Stephens LR, Lamond AI and Cantrell DA (2016) The cytotoxic T cell proteome and its shaping by the kinase mTOR. *Nat Immunol* **17**, 104–112.
12 Nakaya M, Xiao Y, Zhou X, Chang JH, Chang M, Cheng X, Blonska M, Lin X and Sun SC (2014) Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. *Immunity* **40**, 692–705.
13 Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, McCormick LL, Fitzgerald P, Chi H, Munger J *et al.* (2011) The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* **35**, 871–882.
14 Yang Z, Fujii H, Mohan SV, Goronzy JJ and Weyand CM (2013) Phosphofructokinase deficiency impairs ATP generation, autophagy, and redox balance in rheumatoid arthritis T cells. *J Exp Med* **210**, 2119–2134.
15 Baixauli F, Acin-Perez R, Villarroya-Beltri C, Mazzeo C, Nunez-Andrade N, Gabande-Rodriguez E, Ledesma MD, Blazquez A, Martin MA, Falcon-Perez JM *et al.* (2015) Mitochondrial respiration controls lysosomal function during inflammatory T cell responses. *Cell Metab* **22**, 485–498.
16 Cao Y, Rathmell JC and Macintyre AN (2014) Metabolic reprogramming towards aerobic glycolysis correlates with greater proliferative ability and resistance to metabolic inhibition in CD8 versus CD4 T cells. *PLoS One* **9**, e104104.
17 Golks A, Tran TT, Goetschy JF and Guerini D (2007) Requirement for O-linked N-acetylglucosaminyltransferase in lymphocytes activation. *EMBO J* **26**, 4368–4379.
18 Lund PJ, Elias JE and Davis MM (2016) Global analysis of O-GlcNAc glycoproteins in activated human T cells. *J Immunol* **197**, 3086–3098.
19 Swamy M, Pathak S, Grzes KM, Damerow S, Sinclair LV, van Aalten DM and Cantrell DA (2016) Glucose and glutamine fuel protein O-GlcNAcylation to control T cell self-renewal and malignancy. *Nat Immunol* **17**, 712–720.
20 Machacek M, Li J, Li T, Slawson C and Fields P (2016) O-GlcNAc regulates CD4+ T cell differentiation. *J Immunol* **196**(Suppl 1), 133.22.
21 Devadas S, Zaritskaya L, Rhee SG, Oberley L and Williams MS (2002) Discrete generation of superoxide and hydrogen peroxide by T cell receptor stimulation: selective regulation of mitogen-activated protein kinase activation and fas ligand expression. *J Exp Med* **195**, 59–70.
22 Kwon J, Shatynski KE, Chen H, Morand S, de Deken X, Miot F, Leto TL and Williams MS (2010) The nonphagocytic NADPH oxidase Duox1 mediates a positive feedback loop during T cell receptor signaling. *Sci Signal* **3**, ra59.
23 Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, Wang CR, Schumacker PT, Licht JD, Perlman H *et al.* (2013) Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* **38**, 225–236.
24 Ron-Harel N, Santos D, Ghergurovich JM, Sage PT, Reddy A, Lovitch SB, Dephoure N, Satterstrom FK, Sheffer M, Spinelli JB *et al.* (2016) Mitochondrial biogenesis and proteome remodeling promote one-carbon metabolism for T cell activation. *Cell Metab* **24**, 104–117.
25 van der Windt GJ, O'Sullivan D, Everts B, Huang SC, Buck MD, Curtis JD, Chang CH, Smith AM, Ai T, Faubert B *et al.* (2013) CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. *Proc Natl Acad Sci USA* **110**, 14336–14341.
26 Buck MD, O'Sullivan D and Pearce EL (2015) T cell metabolism drives immunity. *J Exp Med* **212**, 1345–1360.
27 Pearce EL and Pearce EJ (2013) Metabolic pathways in immune cell activation and quiescence. *Immunity* **38**, 633–643.
28 Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, Jones RG and Choi Y (2009) Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* **460**, 103–107.
29 van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, Pearce EJ and Pearce EL (2012) Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity* **36**, 68–78.
30 Tarasenko TN, Pacheco SE, Koenig MK, Gomez-Rodriguez J, Kapnick SM, Diaz F, Zerfas PM, Barca E, Sudderth J, DeBerardinis RJ *et al.* (2017) Cytochrome c oxidase activity is a metabolic checkpoint that regulates cell fate decisions during T cell activation and differentiation. *Cell Metab* **25**, 1254–1268 e7.
31 Wai T and Langer T (2016) Mitochondrial dynamics and metabolic regulation. *Trends Endocrinol Metab* **27**, 105–117.
32 Luchsinger LL, de Almeida MJ, Corrigan DJ, Mumau M and Snoeck HW (2016) Mitofusin 2 maintains haematopoietic stem cells with extensive lymphoid potential. *Nature* **529**, 528–531.
33 Buck MD, O'Sullivan D, Klein Geltink RI, Curtis JD, Chang CH, Sanin DE, Qiu J, Kretz O, Braas D, van

der Windt GJ *et al.* (2016) Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell* **166**, 63–76.
34 O'Sullivan D, van der Windt GJ, Huang SC, Curtis JD, Chang CH, Buck MD, Qiu J, Smith AM, Lam WY, DiPlato LM *et al.* (2014) Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity* **41**, 75–88.
35 Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, Sullivan SA, Nichols AG and Rathmell JC (2011) Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol* **186**, 3299–3303.
36 Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, Sandouk A, Hesse C, Castro CN, Bahre H *et al.* (2014) De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med* **20**, 1327–1333.
37 Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR and Chi H (2000) HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med* **208**, 1367–1376.
38 Chang CH, Curtis JD, Maggi LB Jr, Faubert B, Villarino AV, O'Sullivan D, Huang SC, van der Windt GJ, Blagih J, Qiu J *et al.* (2013) Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* **153**, 1239–1251.
39 Gubser PM, Bantug GR, Razik L, Fischer M, Dimeloe S, Hoenger G, Durovic B, Jauch A and Hess C (2013) Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. *Nat Immunol* **14**, 1064–1072.
40 Gerriets VA, Kishton RJ, Nichols AG, Macintyre AN, Inoue M, Ilkayeva O, Winter PS, Liu X, Priyadharshini B, Slawinska ME *et al.* (2015) Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. *J Clin Invest* **125**, 194–207.
41 Laplante M and Sabatini DM (2009) An emerging role of mTOR in lipid biosynthesis. *Curr Biol* **19**, R1046–R1052.
42 Laplante M and Sabatini DM (2009) mTOR signaling at a glance. *J Cell Sci* **122**, 3589–3594.
43 Sengupta S, Peterson TR and Sabatini DM (2010) Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell* **40**, 310–322.
44 Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR, Xiao B, Worley PF and Powell JD (2011) The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat Immunol* **12**, 295–303.
45 Finlay D and Cantrell DA (2011) Metabolism, migration and memory in cytotoxic T cells. *Nat Rev Immunol* **11**, 109–117.
46 Lee K, Gudapati P, Dragovic S, Spencer C, Joyce S, Killeen N, Magnuson MA and Boothby M (2010) Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. *Immunity* **32**, 743–753.
47 Li Q, Rao RR, Araki K, Pollizzi K, Odunsi K, Powell JD and Shrikant PA (2011) A central role for mTOR kinase in homeostatic proliferation induced CD8+ T cell memory and tumor immunity. *Immunity* **34**, 541–553.
48 Rao RR, Li Q, Odunsi K and Shrikant PA (2010) The mTOR kinase determines effector versus memory CD8+ T cell fate by regulating the expression of transcription factors T-bet and eomesodermin. *Immunity* **32**, 67–78.
49 MacIver NJ, Michalek RD and Rathmell JC (2013) Metabolic regulation of T lymphocytes. *Annu Rev Immunol* **31**, 259–283.
50 Ma EH, Poffenberger MC, Wong AH and Jones RG (2017) The role of AMPK in T cell metabolism and function. *Curr Opin Immunol* **46**, 45–52.
51 Tamas P, Hawley SA, Clarke RG, Mustard KJ, Green K, Hardie DG and Cantrell DA (2006) Regulation of the energy sensor AMP-activated protein kinase by antigen receptor and Ca2+ in T lymphocytes. *J Exp Med* **203**, 1665–1670.
52 MacIver NJ, Blagih J, Saucillo DC, Tonelli L, Griss T, Rathmell JC and Jones RG (2011) The liver kinase B1 is a central regulator of T cell development, activation, and metabolism. *J Immunol* **187**, 4187–4198.
53 Blagih J, Coulombe F, Vincent EE, Dupuy F, Galicia-Vazquez G, Yurchenko E, Raissi TC, van der Windt GJ, Viollet B, Pearce EL *et al.* (2015) The energy sensor AMPK regulates T cell metabolic adaptation and effector responses in vivo. *Immunity* **42**, 41–54.
54 Rolf J, Zarrouk M, Finlay DK, Foretz M, Viollet B and Cantrell DA (2013) AMPKalpha1: a glucose sensor that controls CD8 T-cell memory. *Eur J Immunol* **43**, 889–896.
55 Linke M, Fritsch SD, Sukhbaatar N, Hengstschlager M and Weichhart T (2017) mTORC1 and mTORC2 as regulators of cell metabolism in immunity. *FEBS Lett*, https://doi.org/10.1002/1873-3468.12711.
56 Wei J, Raynor J, Nguyen TL and Chi H (2017) Nutrient and metabolic sensing in T cell responses. *Front Immunol* **8**, 247.
57 Zeng H and Chi H (2017) mTOR signaling in the differentiation and function of regulatory and effector T cells. *Curr Opin Immunol* **46**, 103–111.
58 Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT *et al.* (2009) c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature* **458**, 762–765.

59 Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB *et al.* (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci USA* **105**, 18782–18787.
60 Oestreich KJ, Read KA, Gilbertson SE, Hough KP, McDonald PW, Krishnamoorthy V and Weinmann AS (2014) Bcl-6 directly represses the gene program of the glycolysis pathway. *Nat Immunol* **15**, 957–964.
61 Lee JH, Elly C, Park Y and Liu YC (2015) E3 ubiquitin ligase VHL regulates hypoxia-inducible factor-1alpha to maintain regulatory T cell stability and suppressive capacity. *Immunity* **42**, 1062–1074.
62 Ben-Shoshan J, Maysel-Auslender S, Mor A, Keren G and George J (2008) Hypoxia controls CD4+CD25+ regulatory T-cell homeostasis via hypoxia-inducible factor-1alpha. *Eur J Immunol* **38**, 2412–2418.
63 Clambey ET, McNamee EN, Westrich JA, Glover LE, Campbell EL, Jedlicka P, de Zoeten EF, Cambier JC, Stenmark KR, Colgan SP *et al.* (2012) Hypoxia-inducible factor-1 alpha-dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. *Proc Natl Acad Sci USA* **109**, E2784–E2793.
64 Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, Bordman Z, Fu J, Kim Y, Yen HR *et al.* (2000) Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* **146**, 772–784.
65 Angelin A, Gil-de-Gomez L, Dahiya S, Jiao J, Guo L, Levine MH, Wang Z, Quinn WJ III, Kopinski PK, Wang L *et al.* (2017) Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. *Cell Metab* **25**, 1282–1293 e7.
66 Klysz D, Tai X, Robert PA, Craveiro M, Cretenet G, Oburoglu L, Mongellaz C, Floess S, Fritz V, Matias MI *et al.* (2015) Glutamine-dependent alpha-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. *Sci Signal* **8**, ra97.
67 Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, Anderson SM, Abel ED, Chen BJ, Hale LP *et al.* (2014) The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* **20**, 61–72.
68 Sundrud MS, Koralov SB, Feuerer M, Calado DP, Kozhaya AE, Rhule-Smith A, Lefebvre RE, Unutmaz D, Mazitschek R, Waldner H *et al.* (2009) Halofuginone inhibits TH17 cell differentiation by activating the amino acid starvation response. *Science* **324**, 1334–1338.
69 Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, Worley PF, Kozma SC and Powell JD (2009) The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* **30**, 832–844.
70 Powell JD, Pollizzi KN, Heikamp EB and Horton MR (2012) Regulation of immune responses by mTOR. *Annu Rev Immunol* **30**, 39–68.
71 Sauer S, Bruno L, Hertweck A, Finlay D, Leleu M, Spivakov M, Knight ZA, Cobb BS, Cantrell D, O'Connor E *et al.* (2008) T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. *Proc Natl Acad Sci USA* **105**, 7797–7802.
72 Haxhinasto S, Mathis D and Benoist C (2008) The AKT-mTOR axis regulates de novo differentiation of CD4+Foxp3+ cells. *J Exp Med* **205**, 565–574.
73 Liu G, Burns S, Huang G, Boyd K, Proia RL, Flavell RA and Chi H (2009) The receptor S1P1 overrides regulatory T cell-mediated immune suppression through Akt-mTOR. *Nat Immunol* **10**, 769–777.
74 Merkenschlager M and von Boehmer H (2010) PI3 kinase signalling blocks Foxp3 expression by sequestering Foxo factors. *J Exp Med* **207**, 1347–1350.
75 Byles V, Covarrubias AJ, Ben-Sahra I, Lamming DW, Sabatini DM, Manning BD and Horng T (2013) The TSC-mTOR pathway regulates macrophage polarization. *Nat Commun* **4**, 2834.
76 Zhu L, Yang T, Li L, Sun L, Hou Y, Hu X, Zhang L, Tian H, Zhao Q, Peng J *et al.* (2014) TSC1 controls macrophage polarization to prevent inflammatory disease. *Nat Commun* **5**, 4696.
77 Vergadi E, Ieronymaki E, Lyroni K, Vaporidi K and Tsatsanis C (2017) Akt signaling pathway in macrophage activation and M1/M2 polarization. *J Immunol* **198**, 1006–1014.
78 Manna S, Kim JK, Bauge C, Cam M, Zhao Y, Shetty J, Vacchio MS, Castro E, Tran B, Tessarollo L *et al.* (2015) Histone H3 Lysine 27 demethylases Jmjd3 and Utx are required for T-cell differentiation. *Nat Commun* **6**, 8152.
79 De Santa F, Narang V, Yap ZH, Tusi BK, Burgold T, Austenaa L, Bucci G, Caganova M, Notarbartolo S, Casola S *et al.* (2009) Jmjd3 contributes to the control of gene expression in LPS-activated macrophages. *EMBO J* **28**, 3341–3352.
80 Li Y, Reddy MA, Miao F, Shanmugam N, Yee JK, Hawkins D, Ren B and Natarajan R (2008) Role of the histone H3 lysine 4 methyltransferase, SET7/9, in the regulation of NF-kappaB-dependent inflammatory genes. Relevance to diabetes and inflammation. *J Biol Chem* **283**, 26771–26781.
81 Liu Z, Cao W, Xu L, Chen X, Zhan Y, Yang Q, Liu S, Chen P, Jiang Y, Sun X *et al.* (2015) The histone H3 lysine-27 demethylase Jmjd3 plays a critical role in specific regulation of Th17 cell differentiation. *J Mol Cell Biol* **7**, 505–516.
82 Song MH, Nair VS and Oh KI (2017) Vitamin C enhances the expression of IL17 in a Jmjd2-dependent manner. *BMB Rep* **50**, 49–54.

83 Ichiyama K, Chen T, Wang X, Yan X, Kim BS, Tanaka S, Ndiaye-Lobry D, Deng Y, Zou Y, Zheng P *et al.* (2015) The methylcytosine dioxygenase Tet2 promotes DNA demethylation and activation of cytokine gene expression in T cells. *Immunity* **42**, 613–626.
84 Yue X, Trifari S, Aijo T, Tsagaratou A, Pastor WA, Zepeda-Martinez JA, Lio CW, Li X, Huang Y, Vijayanand P *et al.* (2016) Control of Foxp3 stability through modulation of TET activity. *J Exp Med* **213**, 377–397.
85 Tsagaratou A, Gonzalez-Avalos E, Rautio S, Scott-Browne JP, Togher S, Pastor WA, Rothenberg EV, Chavez L, Lahdesmaki H and Rao A (2017) TET proteins regulate the lineage specification and TCR-mediated expansion of iNKT cells. *Nat Immunol* **18**, 45–53.
86 Yang R, Qu C, Zhou Y, Konkel JE, Shi S, Liu Y, Chen C, Liu S, Liu D, Chen Y *et al.* (2015) Hydrogen sulfide promotes Tet1- and Tet2-mediated Foxp3 demethylation to drive regulatory T cell differentiation and maintain immune homeostasis. *Immunity* **43**, 251–263.
87 Sasidharan Nair V, Song MH and Oh KI (2016) Vitamin C facilitates demethylation of the Foxp3 enhancer in a Tet-dependent manner. *J Immunol* **196**, 2119–2131.
88 Cheng SC, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, Giamarellos-Bourboulis EJ, Martens JH, Rao NA, Aghajanirefah A *et al.* (2014) mTOR- and HIF-1alpha-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* **345**, 1250684.
89 Osawa T, Muramatsu M, Wang F, Tsuchida R, Kodama T, Minami T and Shibuya M (2011) Increased expression of histone demethylase JHDM1D under nutrient starvation suppresses tumor growth via down-regulating angiogenesis. *Proc Natl Acad Sci USA* **108**, 20725–20729.
90 Sullivan LB, Gui DY and Vander Heiden MG (2016) Altered metabolite levels in cancer: implications for tumour biology and cancer therapy. *Nat Rev Cancer* **16**, 680–693.
91 Zdzisinska B, Zurek A and Kandefer-Szerszen M (2017) Alpha-ketoglutarate as a molecule with pleiotropic activity: well-known and novel possibilities of therapeutic use. *Arch Immunol Ther Exp (Warsz)* **65**, 21–36.
92 Chowdhury R, Yeoh KK, Tian YM, Hillringhaus L, Bagg EA, Rose NR, Leung IK, Li XS, Woon EC, Yang M *et al.* (2011) The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep* **12**, 463–469.
93 Laukka T, Mariani CJ, Ihantola T, Cao JZ, Hokkanen J, Kaelin WG Jr, Godley LA and Koivunen P (2016) Fumarate and succinate regulate expression of hypoxia-inducible genes via TET enzymes. *J Biol Chem* **291**, 4256–4265.
94 Li F, He X, Ye D, Lin Y, Yu H, Yao C, Huang L, Zhang J, Wang F, Xu S *et al.* (2015) NADP(+)-IDH mutations promote hypersuccinylation that impairs mitochondria respiration and induces apoptosis resistance. *Mol Cell* **60**, 661–675.
95 Xiao M, Yang H, Xu W, Ma S, Lin H, Zhu H, Liu L, Liu Y, Yang C, Xu Y *et al.* (2012) Inhibition of alpha-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. *Genes Dev* **26**, 1326–1338.
96 Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT *et al.* (2011) Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. *Cancer Cell* **19**, 17–30.
97 Zhang Z, Tan M, Xie Z, Dai L, Chen Y and Zhao Y (2011) Identification of lysine succinylation as a new post-translational modification. *Nat Chem Biol* **7**, 58–63.
98 Koivunen P, Hirsila M, Remes AM, Hassinen IE, Kivirikko KI and Myllyharju J (2007) Inhibition of hypoxia-inducible factor (HIF) hydroxylases by citric acid cycle intermediates: possible links between cell metabolism and stabilization of HIF. *J Biol Chem* **282**, 4524–4532.
99 Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB and Gottlieb E (2005) Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell* **7**, 77–85.
100 Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, Chmielewski K, Stewart KM, Ashall J, Everts B *et al.* (2015) Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* **42**, 419–430.
101 Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH *et al.* (2013) Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. *Nature* **496**, 238–242.
102 Liu PS, Wang H, Li X, Chao T, Teav T, Christen S, Di Conza G, Cheng WC, Chou CH, Vavakova M *et al.* (2017) alpha-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. *Nat Immunol* **18**, 985–994.
103 Carey BW, Finley LW, Cross JR, Allis CD and Thompson CB (2015) Intracellular alpha-ketoglutarate maintains the pluripotency of embryonic stem cells. *Nature* **518**, 413–416.
104 TeSlaa T, Chaikovsky AC, Lipchina I, Escobar SL, Hochedlinger K, Huang J, Graeber TG, Braas D and

Teitell MA (2016) Alpha-ketoglutarate accelerates the initial differentiation of primed human pluripotent stem cells. *Cell Metab* **24**, 485–493.
105 Tyrakis PA, Palazon A, Macias D, Lee KL, Phan AT, Velica P, You J, Chia GS, Sim J, Doedens A *et al.* (2016) S-2-hydroxyglutarate regulates CD8+ T-lymphocyte fate. *Nature* **540**, 236–241.
106 Morrison DJ and Preston T (2016) Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* **7**, 189–200.
107 Moussaieff A, Rouleau M, Kitsberg D, Cohen M, Levy G, Barasch D, Nemirovski A, Shen-Orr S, Laevsky I, Amit M *et al.* (2015) Glycolysis-mediated changes in acetyl-CoA and histone acetylation control the early differentiation of embryonic stem cells. *Cell Metab* **21**, 392–402.
108 Peng M, Yin N, Chhangawala S, Xu K, Leslie CS and Li MO (2016) Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. *Science* **354**, 481–484.
109 Fann M, Godlove JM, Catalfamo M, Wood WH III, Chrest FJ, Chun N, Granger L, Wersto R, Madara K, Becker K *et al.* (2006) Histone acetylation is associated with differential gene expression in the rapid and robust memory CD8(+) T-cell response. *Blood* **108**, 3363–3370.
110 Xu Y, Chaudhury A, Zhang M, Savoldo B, Metelitsa LS, Rodgers J, Yustein JT, Neilson JR and Dotti G (2016) Glycolysis determines dichotomous regulation of T cell subsets in hypoxia. *J Clin Invest* **126**, 2678–2688.
111 Canto C, Menzies KJ and Auwerx J (2015) NAD(+) metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. *Cell Metab* **22**, 31–53.
112 Kong S, McBurney MW and Fang D (2012) Sirtuin 1 in immune regulation and autoimmunity. *Immunol Cell Biol* **90**, 6–13.
113 Nimmagadda VK, Bever CT, Vattikunta NR, Talat S, Ahmad V, Nagalla NK, Trisler D, Judge SI, Royal W III, Chandrasekaran K *et al.* (2013) Overexpression of SIRT1 protein in neurons protects against experimental autoimmune encephalomyelitis through activation of multiple SIRT1 targets. *J Immunol* **190**, 4595–4607.
114 Sequeira J, Boily G, Bazinet S, Saliba S, He X, Jardine K, Kennedy C, Staines W, Rousseaux C, Mueller R *et al.* (2008) sirt1-null mice develop an autoimmune-like condition. *Exp Cell Res* **314**, 3069–3074.
115 Wang Y, Bi Y, Chen X, Li C, Li Y, Zhang Z, Wang J, Lu Y, Yu Q, Su H *et al.* (2016) Histone deacetylase SIRT1 negatively regulates the differentiation of interleukin-9-producing CD4(+) T cells. *Immunity* **44**, 1337–1349.
116 Zhang J, Lee SM, Shannon S, Gao B, Chen W, Chen A, Divekar R, McBurney MW, Braley-Mullen H, Zaghouani H *et al.* (2009) The type III histone deacetylase Sirt1 is essential for maintenance of T cell tolerance in mice. *J Clin Invest* **119**, 3048–3058.
117 Beier UH, Wang L, Bhatti TR, Liu Y, Han R, Ge G and Hancock WW (2011) Sirtuin-1 targeting promotes Foxp3+ T-regulatory cell function and prolongs allograft survival. *Mol Cell Biol* **31**, 1022–1029.
118 Kwon HS, Lim HW, Wu J, Schnolzer M, Verdin E and Ott M (2012) Three novel acetylation sites in the Foxp3 transcription factor regulate the suppressive activity of regulatory T cells. *J Immunol* **188**, 2712–2721.
119 van Loosdregt J, Vercoulen Y, Guichelaar T, Gent YY, Beekman JM, van Beekum O, Brenkman AB, Hijnen DJ, Mutis T, Kalkhoven E *et al.* (2010) Regulation of Treg functionality by acetylation-mediated Foxp3 protein stabilization. *Blood* **115**, 965–974.
120 Limagne E, Thibaudin M, Euvrard R, Berger H, Chalons P, Vegan F, Humblin E, Boidot R, Rebe C, Derangere V *et al.* (2017) Sirtuin-1 activation controls tumor growth by impeding Th17 differentiation via STAT3 deacetylation. *Cell Rep* **19**, 746–759.
121 Craveiro M, Cretenet G, Mongellaz C, Matias MI, Caron O, Pedroso de Lima MC, Zimmermann VS, Solary E, Dardalhon V, Dulic V *et al.* (2017) Resveratrol modulates human CD4 T cell metabolism and enhances effector function. *Sci Signal* (in press).
122 Ackerman D and Simon MC (2014) Hypoxia, lipids, and cancer: surviving the harsh tumor microenvironment. *Trends Cell Biol* **24**, 472–478.
123 Mayers JR and Vander Heiden MG (2017) Nature and nurture: what determines tumor metabolic phenotypes? *Cancer Res* **77**, 3131–3134.
124 Bode BP and Souba WW (1999) Glutamine transport and human hepatocellular transformation. *JPEN J Parenter Enteral Nutr* **23**, S33–S37.
125 Hirayama A, Kami K, Sugimoto M, Sugawara M, Toki N, Onozuka H, Kinoshita T, Saito N, Ochiai A, Tomita M *et al.* (2009) Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. *Cancer Res* **69**, 4918–4925.
126 Rambold AS, Kostelecky B, Elia N and Lippincott-Schwartz J (2011) Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation. *Proc Natl Acad Sci USA* **108**, 10190–10195.
127 Scharping NE, Menk AV, Moreci RS, Whetstone RD, Dadey RE, Watkins SC, Ferris RL and Delgoffe GM (2016) The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell

metabolic insufficiency and dysfunction. *Immunity* **45**, 374–388.

128 Bengsch B, Johnson AL, Kurachi M, Odorizzi PM, Pauken KE, Attanasio J, Stelekati E, McLane LM, Paley MA, Delgoffe GM *et al.* (2016) Bioenergetic insufficiencies due to metabolic alterations regulated by the inhibitory receptor PD-1 are an early driver of CD8(+) T cell exhaustion. *Immunity* **45**, 358–373.

129 Sukumar M, Liu J, Mehta GU, Patel SJ, Roychoudhuri R, Crompton JG, Klebanoff CA, Ji Y, Li P, Yu Z *et al.* (2016) Mitochondrial membrane potential identifies cells with enhanced stemness for cellular therapy. *Cell Metab* **23**, 63–76.

130 Cham CM, Driessens G, O'Keefe JP and Gajewski TF (2008) Glucose deprivation inhibits multiple key gene expression events and effector functions in CD8+ T cells. *Eur J Immunol* **38**, 2438–2450.

131 Ho PC, Bihuniak JD, Macintyre AN, Staron M, Liu X, Amezquita R, Tsui YC, Cui G, Micevic G, Perales JC *et al.* (2015) Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell* **162**, 1217–1228.

132 Ripoli M, D'Aprile A, Quarato G, Sarasin-Filipowicz M, Gouttenoire J, Scrima R, Cela O, Boffoli D, Heim MH, Moradpour D *et al.* (2010) Hepatitis C virus-linked mitochondrial dysfunction promotes hypoxia-inducible factor 1 alpha-mediated glycolytic adaptation. *J Virol* **84**, 647–660.

133 Vitko NP, Spahich NA and Richardson AR (2015) Glycolytic dependency of high-level nitric oxide resistance and virulence in *Staphylococcus aureus*. *MBio* **6**, e00045–15.

134 Wang A, Huen SC, Luan HH, Yu S, Zhang C, Gallezot JD, Booth CJ and Medzhitov R (2016) Opposing effects of fasting metabolism on tissue tolerance in bacterial and viral inflammation. *Cell* **166**, 1512–1525 e12.

135 Vernieri C, Casola S, Foiani M, Pietrantonio F, de Braud F and Longo V (2016) Targeting cancer metabolism: dietary and pharmacologic interventions. *Cancer Discov* **6**, 1315–1333.

136 Metzler B, Gfeller P and Guinet E (2016) Restricting glutamine or glutamine-dependent purine and pyrimidine syntheses promotes human T cells with high FOXP3 expression and regulatory properties. *J Immunol.* **196**, 3618–3630.

137 Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM *et al.* (2014) Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* **513**, 559–563.

138 Van de Velde LA, Subramanian C, Smith AM, Barron L, Qualls JE, Neale G, Alfonso-Pecchio A, Jackowski S, Rock CO, Wynn TA *et al.* (2017) T cells encountering myeloid cells programmed for amino acid-dependent immunosuppression use Rictor/mTORC2 protein for proliferative checkpoint decisions. *J Biol Chem* **292**, 15–30.

139 Renner K, Singer K, Koehl GE, Geissler EK, Peter K, Siska PJ and Kreutz M (2017) Metabolic hallmarks of tumor and immune cells in the tumor microenvironment. *Front Immunol* **8**, 248.

140 White KA, Grillo-Hill BK and Barber DL (2017) Cancer cell behaviors mediated by dysregulated pH dynamics at a glance. *J Cell Sci* **130**, 663–669.

141 Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, Matos C, Bruss C, Klobuch S, Peter K *et al.* (2016) LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metab* **24**, 657–671.

142 Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M, Gottfried E, Schwarz S, Rothe G, Hoves S *et al.* (2007) Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood* **109**, 3812–3819.

143 Haas R, Smith J, Rocher-Ros V, Nadkarni S, Montero-Melendez T, D'Acquisto F, Bland EJ, Bombardieri M, Pitzalis C, Perretti M *et al.* (2015) Lactate regulates metabolic and pro-inflammatory circuits in control of T cell migration and effector functions. *PLoS Biol* **13**, e1002202.

144 Husain Z, Huang Y, Seth P and Sukhatme VP (2013) Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. *J Immunol* **191**, 1486–1495.

145 Husain Z, Seth P and Sukhatme VP (2013) Tumor-derived lactate and myeloid-derived suppressor cells: linking metabolism to cancer immunology. *Oncoimmunology* **2**, e26383.

146 Gottfried E, Kunz-Schughart LA, Ebner S, Mueller-Klieser W, Hoves S, Andreesen R, Mackensen A and Kreutz M (2006) Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. *Blood* **107**, 2013–2021.

147 Brown JE, Cook RJ, Lipton A and Coleman RE (2012) Serum lactate dehydrogenase is prognostic for survival in patients with bone metastases from breast cancer: a retrospective analysis in bisphosphonate-treated patients. *Clin Cancer Res* **18**, 6348–6355.

148 Li G, Wang Z, Xu J, Wu H, Cai S and He Y (2016) The prognostic value of lactate dehydrogenase levels in colorectal cancer: a meta-analysis. *BMC Cancer* **16**, 249.

149 Passardi A, Scarpi E, Tamberi S, Cavanna L, Tassinari D, Fontana A, Pini S, Bernardini I, Accettura C, Ulivi P *et al.* (2015) Impact of pre-treatment lactate dehydrogenase levels on prognosis and bevacizumab efficacy in patients with metastatic colorectal cancer. *PLoS One* **10**, e0134732.

150 Walenta S, Wetterling M, Lehrke M, Schwickert G, Sundfor K, Rofstad EK and Mueller-Klieser W (2000) High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers. *Cancer Res* **60**, 916–921.
151 Fukuno K, Hara T, Tsurumi H, Shibata Y, Mabuchi R, Nakamura N, Kitagawa J, Shimizu M, Ito H, Saito K *et al.* (2015) Expression of indoleamine 2,3-dioxygenase in leukemic cells indicates an unfavorable prognosis in acute myeloid leukemia patients with intermediate-risk cytogenetics. *Leuk Lymphoma* **56**, 1398–1405.
152 Maliniemi P, Laukkanen K, Vakeva L, Dettmer K, Lipsanen T, Jeskanen L, Bessede A, Oefner PJ, Kadin ME and Ranki A (2017) Biological and clinical significance of tryptophan-catabolizing enzymes in cutaneous T-cell lymphomas. *Oncoimmunology* **6**, e1273310.
153 Masaki A, Ishida T, Maeda Y, Suzuki S, Ito A, Takino H, Ogura H, Totani H, Yoshida T, Kinoshita S *et al.* (2015) Prognostic significance of tryptophan catabolism in adult T-cell leukemia/lymphoma. *Clin Cancer Res* **21**, 2830–2839.
154 Theate I, van Baren N, Pilotte L, Moulin P, Larrieu P, Renauld JC, Herve C, Gutierrez-Roelens I, Marbaix E, Sempoux C *et al.* (2015) Extensive profiling of the expression of the indoleamine 2,3-dioxygenase 1 protein in normal and tumoral human tissues. *Cancer Immunol Res* **3**, 161–172.
155 Munn DH and Mellor AL (2016) IDO in the tumor microenvironment: inflammation, counter-regulation, and tolerance. *Trends Immunol* **37**, 193–207.
156 Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D and Mellor AL (2005) GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity* **22**, 633–642.
157 Julliard W, Fechner JH and Mezrich JD (2014) The aryl hydrocarbon receptor meets immunology: friend or foe? A little of both. *Front Immunol* **5**, 458.
158 Tykocinski LO, Lauffer AM, Bohnen A, Kaul NC, Krienke S, Tretter T, Adam I, Mohapatra SR, Saikali P, Lohning M *et al.* (2017) Synovial fibroblasts selectively suppress Th1 cell responses through IDO1-mediated tryptophan catabolism. *J Immunol* **198**, 3109–3117.
159 Spranger S, Koblish HK, Horton B, Scherle PA, Newton R and Gajewski TF (2014) Mechanism of tumor rejection with doublets of CTLA-4, PD-1/PD-L1, or IDO blockade involves restored IL-2 production and proliferation of CD8(+) T cells directly within the tumor microenvironment. *J Immunother Cancer* **2**, 3.
160 Wainwright DA, Chang AL, Dey M, Balyasnikova IV, Kim CK, Tobias A, Cheng Y, Kim JW, Qiao J, Zhang L *et al.* (2014) Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4, and PD-L1 in mice with brain tumors. *Clin Cancer Res* **20**, 5290–5301.
161 Zakharia Y, McWilliams R, Shaheen M, Grossmann K, Drabick J, Milhem M, RIxe O, Khleilf S, Lott R, Kennedy E *et al.* (2017) Interim analysis of the phase 2 clinical trial of the IDO pathway inhibitor Indoximod in combination with Pembrolizumad for patients with advanced melanoma. *AACR meeting 2017*.
162 Hernandez AL, Kitz A, Wu C, Lowther DE, Rodriguez DM, Vudattu N, Deng S, Herold KC, Kuchroo VK, Kleinewietfeld M *et al.* (2015) Sodium chloride inhibits the suppressive function of FOXP3+ regulatory T cells. *J Clin Invest* **125**, 4212–4222.
163 Wu C, Yosef N, Thalhamer T, Zhu C, Xiao S, Kishi Y, Regev A and Kuchroo VK (2013) Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. *Nature* **496**, 513–517.
164 Wu H, Huang X, Qiu H, Zhao M, Liao W, Yuan S, Xie Y, Dai Y, Chang C, Yoshimura A *et al.* (2016) High salt promotes autoimmunity by TET2-induced DNA demethylation and driving the differentiation of Tfh cells. *Sci Rep* **6**, 28065.
165 Eil R, Vodnala SK, Clever D, Klebanoff CA, Sukumar M, Pan JH, Palmer DC, Gros A, Yamamoto TN, Patel SJ *et al.* (2016) Ionic immune suppression within the tumour microenvironment limits T cell effector function. *Nature* **537**, 539–543.
166 Cordes T, Wallace M, Michelucci A, Divakaruni AS, Sapcariu SC, Sousa C, Koseki H, Cabrales P, Murphy AN, Hiller K *et al.* (2016) Immunoresponsive gene 1 and itaconate inhibit succinate dehydrogenase to modulate intracellular succinate levels. *J Biol Chem* **291**, 14274–14284.
167 Lampropoulou V, Sergushichev A, Bambouskova M, Nair S, Vincent EE, Loginicheva E, Cervantes-Barragan L, Ma X, Huang SC, Griss T *et al.* (2016) Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. *Cell Metab* **24**, 158–166.
168 Meiser J, Kramer L, Sapcariu SC, Battello N, Ghelfi J, D'Herouel AF, Skupin A and Hiller K (2016) Pro-inflammatory macrophages sustain pyruvate oxidation through pyruvate dehydrogenase for the synthesis of itaconate and to enable cytokine expression. *J Biol Chem* **291**, 3932–3946.
169 Jenabian MA, Seddiki N, Yatim A, Carriere M, Hulin A, Younas M, Ghadimi E, Kok A, Routy JP, Tremblay A *et al.* (2013) Regulatory T cells negatively affect IL-2 production of effector T cells through CD39/adenosine pathway in HIV infection. *PLoS Pathog* **9**, e1003319.
170 Nikolova M, Carriere M, Jenabian MA, Limou S, Younas M, Kok A, Hue S, Seddiki N, Hulin A, Delaneau O *et al.* (2011) CD39/adenosine pathway is

involved in AIDS progression. *PLoS Pathog* **7**, e1002110.

171 Butcher DT, Alliston T and Weaver VM (2009) A tense situation: forcing tumour progression. *Nat Rev Cancer* **9**, 108–122.

172 Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D *et al.* (2005) Tensional homeostasis and the malignant phenotype. *Cancer Cell* **8**, 241–254.

173 Saitakis M, Dogniaux S, Goudot C, Bufi N, Asnacios S, Maurin M, Randriamampita C, Asnacios A and Hivroz C (2017) Different TCR-induced T lymphocyte responses are potentiated by stiffness with variable sensitivity. *Elife* **6**, e23190.