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CD8+ T cell metabolism in infection and cancer

Miguel Reina-Campos[1,2], **Nicole E. Scharping**[1,2], **Ananda W. Goldrath**[1,✉]

[1]Division of Biological Sciences, Section of Molecular Biology, University of California, San Diego, La Jolla, CA, USA.

Abstract

Cytotoxic $CD8^+$ T cells play a key role in the elimination of intracellular infections and malignant cells and can provide long-term protective immunity. In the response to infection, $CD8^+$ T cell metabolism is coupled to transcriptional, translational and epigenetic changes that are driven by extracellular metabolites and immunological signals. These programmes facilitate the adaptation of $CD8^+$ T cells to the diverse and dynamic metabolic environments encountered in the circulation and in the tissues. In the setting of disease, both cell-intrinsic and cell-extrinsic metabolic cues contribute to $CD8^+$ T cell dysfunction. In addition, changes in whole-body metabolism, whether through voluntary or disease-induced dietary alterations, can influence $CD8^+$ T cell-mediated immunity. Defining the metabolic adaptations of $CD8^+$ T cells in specific tissue environments informs our understanding of how these cells protect against pathogens and tumours and maintain tissue health at barrier sites. Here, we highlight recent findings revealing how metabolic networks enforce specific $CD8^+$ T cell programmes and discuss how metabolism is integrated with $CD8^+$ T cell differentiation and function and determined by environmental cues.

$CD8^+$ T cells contribute to organismal homeostasis. Cytolytic effector $CD8^+$ T cells help to eliminate pathogens through cytokine secretion and direct killing of infected cells, while long-lived memory $CD8^+$ T cell populations provide enhanced protection from reinfection. The differentiation of naive $CD8^+$ T cells into memory $CD8^+$ T cells through the initial activation, expansion and contraction phases of the immune response is tightly regulated by cell-surface receptors, soluble factors and transcriptional programmes coupled to profound metabolic reprogramming[1]. While surveying the body for antigens, $CD8^+$ T cells are exposed to a myriad of metabolic environments that influence their differentiation and function. These extrinsic cues are imposed by the heterogeneity of healthy tissue microenvironments[2] as well as by the perturbed tissue environments that result from malignancies[3] and infections[4], and the overall metabolic profile of the host[4] (FIG. 1). Combined, cell-intrinsic and cell-extrinsic metabolic milieus impact the energetic and biosynthetic capacity of $CD8^+$ T cells, including the synthesis of cofactors and substrates that are used to fuel and epigenetically control differentiation programmes[5,6]. Recent studies

✉ agoldrath@ucsd.edu .

Author contributions

The authors contributed equally to all aspects of the article.

[2]These authors contributed equally: Miguel Reina-Campos, Nicole E. Scharping.

Competing Interests

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suggest that the environmental adaptations of $CD8^+$ T cells are intertwined with their developmental trajectories, and ultimately determine the ability of $CD8^+$ T cells to provide potent effector responses and long-term tissue immunity[6]. These observations add to the emerging notion that $CD8^+$ T cell metabolism can no longer be considered an accessory to $CD8^+$ T cell biology, but rather should be considered a driving force in protecting us from infection and cancer.

In this Review, we describe the metabolic processes engaged upon $CD8^+$ T cell activation and over the course of differentiation into effector and memory T cell populations in the context of viral pathogens and tumorigenesis. We highlight how $CD8^+$ T cells adapt their metabolism to enable tissue residency, discuss the metabolic alterations that underlie dysfunctional $CD8^+$ T cell estates during their response to solid tumours and bring together emerging evidence illuminating how reciprocal regulation of T cell immunity and whole-body metabolism impacts the ability to fight infection and cancer. Finally, we discuss unanswered questions in the field, and propose possible new approaches to understand and harness the full therapeutic potential of $CD8^+$ T cell immunity.

Metabolism of $CD8^+$ T cell activation

Naive $CD8^+$ T cells show metabolic plasticity, or the ability to rapidly utilize a range of metabolic programmes on demand, allowing flexibility to accommodate a range of environments. Upon T cell receptor (TCR) recognition of a cognate antigen, naive $CD8^+$ T cells become activated and engage transcriptional, translational and metabolic programmes necessary for rapid population expansion and differentiation to effector and memory $CD8^+$ T cell states. Expanding upon the foundational studies on immune metabolism[7–15], recent work has brought increased complexity and more nuanced models to explain how TCR signalling, $CD8^+$ T cell metabolism and the metabolic crosstalk between cells and the environment determine $CD8^+$ T cell activation and effector differentiation.

Early activation demands rapid energy production

Within minutes of activation via TCRs, $CD8^+$ T cells rapidly engage the metabolic machinery that is necessary to fuel their proliferation and differentiation[16,17]. Pyruvate dehydrogenase kinase 1 (PDHK1) becomes activated and forms a complex with ZAP70, LCK and LAT, which results in inhibition of pyruvate dehydrogenase in the mitochondrial membrane[16]. This prevents pyruvate from entering the mitochondria as acetyl-CoA, forcing its cytosolic oxidation to lactate instead, a metabolic route that computational and biochemical modelling has shown produces less ATP per molecule of glucose but that generates ATP almost 100 times faster than oxidative phosphorylation[18]. This initial switch occurs in the absence of increased glucose uptake[16,19] but is associated with decreasing ATP levels[19], which highlights how rapid production of energy is more important to initial T cell activation than glucose efficiency. Enhanced glycolysis is further maintained by PI3K–AKT signalling downstream of CD28 co-stimulation[15,16,20] (FIG. 2a). Over the course of the next few hours and days following activation, $CD8^+$ T cells begin rapid cellular growth and division and initiate transcriptional programmes to enable the nutrient uptake, environmental sensing and carbon anabolism needed for their differentiation and function.

ATP

The molecule of energy currency inside of cells; it is dephosphorylated to AMP and adenosine by CD73 and CD39, respectively, when in the extracellular space to suppress immune function.

Oxidative phosphorylation

Metabolic process in which mitochondrial processes of the electron transport chain make ATP by consuming oxygen, making carbon dioxide and water as metabolic by-products.

Glycolysis

Metabolic process of breaking down glucose for energy, with lactic acid as a metabolic by-product.

Carbon anabolism

Metabolic biosynthetic process building larger molecules from smaller molecules.

Nutrient uptake and environmental sensing

To satisfy the nutrient demands of proliferation and differentiation, activated $CD8^+$ T cells increase expression or translocation of solute carrier (SLC) transporters to the cell membrane, which boosts nutrient uptake and helps maintain the ion gradient and efflux of metabolic end products[21]. Only a handful of the more than 400 SLC members have been well characterized in immune cells. Among these, SLC2A1, better known as glucose transporter GLUT1, is upregulated within 24 hours of TCR- and CD28-induced AKT activation to meet the increased demands of aerobic glycolysis[16,20] (FIG. 2a). Autocrine and paracrine IL-2 and IL-7 signalling, as well as insulin and the adipokine leptin, can also act on $CD8^+$ T cells to upregulate GLUT1 expression and increase glucose uptake via activation of AKT[22–25]. Increased activity of the transcription factors HIF1α and NFAT1 also enforces glycolytic metabolism through upregulation of GLUT1 and enzymes of the glycolytic pathway, which supports effector T cell functions, such as cytokine production[26,27] (FIG. 2b). Glutamine uptake via SLC38A1 and SLC38A2 is amplified upon T cell activation in a CD28-dependent manner to boost anaplerosis — the process of replenishing carbons in the tricarboxylic acid (TCA) cycle — to match the increased biosynthetic and energy demands[28]. Glutamine usage is also fuelled by the transcription factor MYC, which is required to boost nucleotide synthesis necessary for $CD8^+$ T cell growth and proliferation[26] (FIG. 2b). In addition, the system L-amino acid transporter, which comprises SLC3 and SLC7 subfamily members, mediates the uptake of large neutral amino acids to sustain protein synthesis in activated $CD8^+$ T cells[29,30]. Similarly, the large neutral amino acid transporter SLC7A5 is upregulated by activated T cells to import methionine to sustain *S*-adenosylmethionine (SAM) synthesis[31]. SAM is the universal substrate for

methyltransferase reactions, including DNA and histone methylations, which imprint epigenetic programmes of $CD8^+$ T cell activation and differentiation[31] (FIG. 2b). Finally, monocarboxylate transporters of the SLC16 family are involved in the uptake and secretion of lactate, the end product of glycolysis, with complex immunomodulatory effects[32,33]. To synchronize nutrient availability with demand, cells use metabolic checkpoints such as the mechanistic target of rapamycin (mTOR), a nutrient-sensitive signalling complex that couples the energetic and nutritional status of the cell to its anabolic output, effectively controlling cell growth, proliferation, metabolic programming and survival[34]. Thus, availability of glucose, glutamine and amino acids, modulated either by transport or by micropinocytosis[35], is key to supply building blocks to rapidly dividing effector cells and to maintain mTOR complex 1 (mTORC1) engaged to orchestrate these metabolic programmes.

Anaplerosis

Metabolic reactions whose products replenish tricarboxylic acid cycle intermediates.

Tricarboxylic acid (TCA) cycle

Also called the Krebs cycle and the citric acid cycle, a metabolic process of breaking down acetyl-CoA to carbon intermediates for energy and for synthesizing new metabolic products.

mTOR complex 1

(mTORC1). A protein complex that integrates signals from oxygen, energy, nutrients, stress and growth factors to control the anabolic output of the cell by stimulating protein synthesis and anabolic metabolism and restraining autophagy under conditions of nutrient abundance.

Carbon anabolism

After initial TCR engagement, $CD8^+$ T cells utilize a variety of fuels and metabolic pathways to support differentiation and to mediate pathogen clearance. Recent work showed that in vivo activated $CD8^+$ T cells rely on both glycolysis and oxidative phosphorylation to break down glucose for nucleotide and serine biosynthesis and to upregulate DNA and RNA biosynthesis, DNA replication, and ribosome and mitochondrial biogenesis[36–39]. Ribosomal biogenesis and mitochondrial biogenesis occur within hours of $CD8^+$ T cell activation, with mTOR acting through MYC to initiate protein translation programmes and mitochondrial biogenesis occurring through MAPK1 activation downstream of ATF2–PGC1α signalling[38,40] (FIG. 2b). It has been observed that following T cell activation but before the first cell division, metabolism tracks with the fate of the first daughter cells through asymmetric cell division[41,42]. Daughter cells generated from the T cell proximal to the antigen-presenting cell contain more MYC and mTORC1, show increased glycolytic metabolism and are biased to becoming short-lived effector $CD8^+$ T cells. By

contrast, daughter cells distal to TCR engagement express more antiapoptotic proteins, show increased lipid metabolism and display enhanced survival after infection. This highlights the early bifurcation in metabolism as $CD8^+$ T cells respond to pathogens[41,42].

Several metabolic pathways supply the substrates needed to imprint epigenetic programmes of differentiation during early $CD8^+$ T cell differentiation. SAM synthesized through the serine, glycine and one-carbon pathway and the TCA cycle intermediate α-ketoglutarate, together with oxygen, are used as substrates and cofactors of epigenetic enzymes (extensively reviewed in REFS[6,43]). Fatty acid synthesis through the activity of acetyl-CoA carboxylase 1 (ACC1) is required for $CD8^+$ T cells to proliferate and survive[44,45]. In addition, activated $CD8^+$ T cells funnel acetyl-CoA produced by aerobic glycolysis into the mevalonate biosynthetic pathway, which provides critical intermediates for the synthesis of sterols and ubiquinone, and substrates for protein isoprenylation[46]. These lipid biosynthetic programmes are driven by sterol regulatory element-binding proteins, which are required for $CD8^+$ T cell proliferation and effector functions upon viral infection[47].

Fatty acid synthesis

Metabolic process of building fatty acids from acetyl-CoA in the cell cytoplasm.

Sterol regulatory element-binding proteins

Transcription factors from the basic helix–loop–helix leucine zipper family that translocate to the nucleus when cellular sterol concentration is low to upregulate enzymes involved in sterol biosynthesis.

Metabolism of cytotoxic function

Glycolytic enzymes have also been shown to control the effector T cell response by moonlighting as RNA-binding proteins for several cytokine mRNAs[48]. The AU-rich element of the 3′ untranslated region of mRNAs encoding interferon-γ (IFNγ), TNF, IL-2 and other cytokines can be bound by the glycolytic enzyme lactate dehydrogenase (LDH) while $CD8^+$ T cells are in a quiescent, resting state[16]. Upon increased aerobic glycol-lytic demand, such as following TCR engagement, these glycolytic enzymes release their target mRNAs, allowing mRNA translation and cytokine production. This metabolic regulation of effector cytokine mRNAs was first described in $CD4^+$ T cells, in a process controlled by the glycolytic enzyme GAPDH[10]. Of note, granzyme B mRNA does not contain an AU-rich element in its 3′ untranslated region, and therefore it is not regulated by aerobic glycolysis[16]. Instead, cytolytic granules are polarized to microtubule-organizing centres upon TCR engagement and AMPK activation, which mediates perforin and granzyme B release at the immunological synapse[49]. TCR engagement has also been shown to induce reactive oxygen species (ROS) from the mitochondria, and ROS are essential not only for full T cell activation but also for IL-2 production via NFAT[49,50]. Consequently, loss of UQCRFS1, an electron transport chain (ETC) complex III component, led to diminished mitochondrial ROS production and halted expansion and effector function in T cells in vivo[51]. Thus, beyond the supply of anabolic products by metabolic processes, effector

functions of CD8$^+$ T cells are modulated by post-translational regulatory mechanisms that control the synthesis of cytokines.

Electron transport chain

(ETC). series of protein complexes in the mitochondria that uses proton gradients to drive ATP production.

The multiple programmes engaged upon CD8$^+$ T cell activation coordinate the uptake of environmental nutrients and route them into anabolic reactions, essentially turning CD8$^+$ T cells into biosynthetic factories for nucleotides, amino acids and lipids, which are necessary to rapidly generate daughter cells and sustain differentiation programmes into effector and memory CD8$^+$ T cell populations.

Memory CD8$^+$ T cell differentiation

After pathogen clearance, only a few T cells persist and differentiate into long-lived memory CD8$^+$ T cells[1]. These cells reprogramme their metabolism to meet the new metabolic demands imposed by their long-term survival and poised effector functions (BOX 1; FIG. 3a).

Energy production

On the basis of their extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) — which are proxies for glycolysis and oxidative phosphorylation, respectively (TABLE 1) — CD8$^+$ T cells that have been activated in vitro by sequential IL-2 and IL-15 treatment appear to have lower glycolysis than effector CD8$^+$ T cells and a higher reliance on oxidative phosphorylation than naive CD8$^+$ T cells (FIG. 3a); these cells are used as a proxy for memory T cells[11] and show a larger potential capacity to consume oxygen and produce energy in the mitochondria, also known as spare respiratory capacity[11] (TABLE 1). Studies using this in vitro system suggested that memory CD8$^+$ T cells preferentially use long-chain fatty acids instead of glucose to generate ATP. Paradoxically, circulating memory CD8$^+$ T cells were shown to take up less lipid (namely, oleate and low-density lipoprotein (LDL)) in vivo than effector CD8$^+$ T cells[52]. This could be explained by the observation that effector CD8$^+$ T cells generated intracellular deposits of fatty acids in vitro, while memory-like CD8$^+$ T cells burned fatty acids through fatty acid oxidation to provide acetyl-CoA to the TCA cycle, which can then enhance traits of memory CD8$^+$ T cells in vitro[11,52–55]. Experiments done in vitro supported the idea that circulating memory CD8$^+$ T cells turn glucose into fatty acids, which are immediately rerouted to the mitochondria for fatty acid oxidation[52]. This process can be sustained by the uptake of glycerol by AQP9, a membrane transporter highly induced by IL-7 and IL-15 and required for their actions in supporting memory differentiation[52,54]. However, making fatty acids from glucose to fuel fatty acid oxidation appears to be a futile cycle that does not efficiently produce ATP[52]. Thus, it is possible that other lipid sources could fuel circulating memory CD8$^+$ T cells in vivo.

Extracellular acidification rate

(ECAR). Real-time measurement of medium acidification, used to interpret cellular glycolysis.

Oxygen consumption rate

(OCR). Real-time measurement of oxygen in media, used to interpret mitochondrial and cellular respiration.

Spare respiratory capacity

The maximum capacity of a cell to consume oxygen once the mitochondrial membrane has been uncoupled.

Fatty acid oxidation

Mitochondrial process of breaking down fatty acids into acetyl-CoA derivatives for energy.

Fatty acid

A carboxylic acid with an unsaturated or a saturated aliphatic tail.

The limiting step for fatty acid oxidation in lymphocytes is the transport of fatty acids into the mitochondria by carnitine palmitoyltransferase 1α (CPT1α), which is upregulated by IL-15 (REF.[11]) and peroxisome proliferator-activated receptor (PPAR) agonists[56], and correlates well with the ability of $CD8^+$ T cells to become memory cells[57] and to show increased persistence[56]. However, several genetic experiments question the idea that CPT1α and mitochondrial oxidation are essential for memory differentiation. First, genetic deletion of CPT1α in $CD8^+$ T cells does not prevent memory cell formation[58]. Second, suppressing mitochondrial oxidation in $CD8^+$ T cells did not impair the formation or functions of circulating memory $CD8^+$ T cell populations[59]. On the contrary, it slightly increased effector memory $CD8^+$ T cell differentiation in vivo[59]. Finally, enhanced HIF activity, mimicking hypoxic environments such as those present in pathological conditions, forces glycolytic metabolism and can promote differentiation of effector memory $CD8^+$ T cells[60]. Thus, while fatty acid oxidation might be increased in certain memory $CD8^+$ T cell subsets, these studies indicate that $CD8^+$ T cells can adapt to different metabolic constraints, and this flexibility in metabolism may facilitate their differentiation or survival in diverse tissue environments in vivo (FIG. 3b).

NADPH/NADP$^+$ ratios

Nicotinamide adenine dinucleotide phosphate in its oxidized (NADP$^+$) and reduced forms (NADPH). A cofactor used by all forms of cellular life, it has a key role in central antioxidant systems and anabolism.

Reducing power

Additional metabolic pathways provide the cofactors and reductive energy necessary for biosynthetic processes in memory CD8$^+$ T cells. For example, fatty acid synthesis has an elevated requirement for reductive energy in the form of NADPH. Memory CD8$^+$ T cells upregulate phosphoenolpyruvate carboxykinase 1 to increase glycogenesis via gluconeogenesis[61]. The resulting glycogen is then rerouted to the pentose phosphate pathway for nucleotide synthesis to maintain NADPH pools in memory CD8$^+$ T cells[61] (FIG. 3b). Of note, among several other primary cell types, CD8$^+$ T cells are especially reliant on the pentose phosphate pathway to maintain NADPH pools[62], However, it is not clear how memory CD8$^+$ T cells can both support synthesis of fatty acids from glucose[55] and gluconeogenesis for the pentose phosphate pathway[61]. In addition, NADPH serves both as an important source of reducing power to maintain reduced glutathione levels to limit accumulation of ROS and as a substrate for ROS-producing NADPH oxidases[63] (FIG. 3b). Whether NADPH pools are partitioned and how they are coordinated to control proinflammatory ROS signalling in CD8$^+$ T cell memory populations is not known.

Mitochondrial dynamics

Mitochondrial morphology impacts the formation of ETC complexes, and thus the ability of CD8$^+$ T cells to couple oxidative phosphorylation with the generation of ATP[55]. In vitro-generated memory CD8$^+$ T cells as well as ex vivo central memory and effector memory CD8$^+$ T cell populations were shown to contain more elongated and networked mitochondria than effector T cells[55,64,65], which correlated with their ability to use long-chain fatty acid β-oxidation to sustain their elevated spare respiratory capacity in vitro[55,66]. Deletion of OPA1, which helps maintain mitochondrial cristae conformation and ETC function[67], impairs the development of central memory CD8$^+$ T cells, but not effector T cells, while overexpression of OPA1 skews T cells towards a memory phenotype[55] (FIG. 3b). Loss of the mitochondrial fission protein dynamin-related protein 1 (DRP1) also promoted elongated fused mitochondria concurrent to a memory CD8$^+$ T cell phenotype, but impaired extravasation of these T cells[66] (FIG. 3b). In addition, the intracellular location of mitochondria and the DRP1-dependent fission of these organelles at the immunological synapse was required for optimal CD8$^+$ T cell activation[68]. Loss of the methylation-controlled J protein (MCJ), which blocks the formation of ETC supercomplexes, led to increased mitochondrial metabolism and mitochondrial ATP, in the absence of increased mitochondrial mass[69] (FIG. 3b). This increase in ETC activity was accompanied by an upregulation of IFNγ secretion, independent of transcriptional changes, and increased capacity of CD8$^+$ T cells to protect mice from influenza virus[69]. However, despite the increase in oxidative phosphorylation activity, MCJ deficiency did not promote memory CD8$^+$ T cell formation[69], suggesting that additional signals couple this activity with

differentiation. Together, these studies show that memory differentiation programmes are modulated by molecules that influence the inner structure of the mitochondria and its ability to couple the products of the TCA cycle with the generation of ATP at the ETC.

Metabolic checkpoints

Metabolic checkpoints, such as mTORC1 and mTORC2, coordinate cellular functions on the basis of the nutritional status and the particular needs of the cell. mTORC1 function is crucial during memory $CD8^+$ T cell formation: mTORC1 inhibition with rapamycin at the time of an acute viral infection, or in vitro[70], increased accumulation of virus-specific memory $CD8^+$ T cells in lymphoid and non-lymphoid tissues by promoting memory precursors, and thus decreasing the magnitude of the contraction phase[71]. This is partly because mTORC1 inhibition with rapamycin counteracts the effects of IL-12 and CD80-mediated co-stimulation during $CD8^+$ T cell activation[72]. Thus, switching off mTORC1 allows memory precursors to reduce the overall glycolytic flux, and engages mitochondrial oxidative phosphorylation by allowing pyruvate entry into the mitochondria, a more energetically efficient route that reduces their overall glucose consumption[73].

mTORC2

A protein complex that senses growth factors to control survival, proliferation and cytoskeletal rearrangements.

mTOR kinase exists in two different complexes, mTORC1 and mTORC2, which differ in their composition of regulatory proteins, upstream sensors and effector targets[74]. Both mTORCs have been implicated in controlling memory differentiation by dictating metabolic reprogramming after the initial $CD8^+$ T cell expansion. Through control of mTORC activity, TSC1–TSC2 promotes $CD8^+$ T cell survival and controls the quiescent and memory states[75–78]. Genetic deletion of TSC1 activates mTORC1, and slightly decreases mTORC2 activity, while TSC2 deficiency increases mTORC1, but does not affect mTORC2 activity in $CD8^+$ T cells[57,76]. Enforcing high mTORC1 activation under nutrient abundance sustains potent effector responses as long as mTORC2-dependent control of survival pathways remains functional, but it impairs memory formation, potentially because it prevents the metabolic reprogramming needed to support this differentiation. Under moderate mTORC1 activation, downregulation of mTORC2 is not only tolerated but surprisingly leads to increased protective immunity[57,79]. This is partly explained by an increase in mitochondrial activity, and the induction of transcriptional programmes due to the stabilization and nuclear translocation of the memory-promoting factor FOXO1 (REF.[79]) (FIG. 3b). In addition to TSC1–TSC2, AMPK is triggered as a result of declining ATP/AMP ratios to restrict mTORC1 activity (FIG. 3b). Although ATP/AMP ratios are tightly controlled, they can drop owing to nutrient scarcity, extracellular secretion of ATP through pannexin channels[80,81] or the use of chemical agonists[53]. Thus, AMPK activation has been associated with increased memory $CD8^+$ T cell differentiation in the context of bacterial and viral infections, in agreement with its mTORC1 inhibitory function[80].

Upstream regulation of mTORC1 by metabolites is coupled to co-inhibitory receptor signalling, a safety mechanism that curtails the cytotoxic $CD8^+$ T cell effector response to avoid immunopathology[82]. mTORC1 inhibition combined with CTLA4 blockade before $CD8^+$ T cell priming cooperates to promote memory differentiation associated with enhanced vaccine responses[83]. Conversely, sustained mTORC1 activation, such as that observed in patients lacking the key enzyme in sphingolipid biosynthesis (SPTLC2), or hyperactivating mutations in PI3Kδ subunits (which cause activated PI3Kδ syndrome), experience recurrent infections and poor immune recall responses[84,85]. Interestingly, signalling through PD1, another co-inhibitory receptor, on activated $CD8^+$ T cells decreased glycolysis and increased fatty acid oxidation of endogenous lipids via upregulation of CPT1α[86]. Thus, PD1-dependent inhibition of the effector response may involve enforcing a metabolic programme that sustains memory $CD8^+$ T cell formation. Together, these studies have placed mTORC1 and mTORC2 as key metabolic checkpoints that control effector and memory responses by synchronizing metabolic supply with the biosynthetic requirements through metabolic reprogramming. However, the mechanisms by which mTORC activity is relayed to metabolic changes in $CD8^+$ T cells are still not fully understood.

Metabolic control of epigenetic programmes

How metabolic changes are coupled to epigenetic programmes of memory $CD8^+$ T cell differentiation is not well understood, but multiple lines of study have highlighted the intersection of metabolite generation and regulation of gene expression. The fate of pyruvate at the crossroads of mitochondrial entry or lactate conversion through LDH appears to be an important regulatory node. Inhibition of LDH blocks the effector-inducing properties of IL-2 and synergizes with IL-21 to skew $CD8^+$ T cells towards a memory phenotype in vitro[64]. Alteration of NADH/NAD^+ ratios as a result of modifying the flux of glucose through LDH into lactate could potentially enforce epigenetic programmes by modulation of NAD-dependent epigenetic enzymes, including histone deacetylases and poly(ADP-ribose) polymerases[87], although formal proof is lacking. Lactate itself can also be used as a substrate for epigenetic alterations through histone lactylation, although a role for this modification in epigenetic programmes of memory $CD8^+$ T cell differentiation is not known[88]. Similarly, LDHA-derived (*S*)-2-hydroxyglutarate accumulation as a result of constitutive HIF1α signalling, or TCR stimulation, regulates $CD8^+$ T cell fate, at least in part, through modulating 2-oxoglutarate-dependent dioxygenases, such as TET2, that in turn regulate DNA methylation[89]. Thus, the metabolites that converge at the entry of pyruvate into the mitochondria can potentially influence the transcriptional programmes of $CD8^+$ T cell fate. However, how $CD8^+$ T cells relay changes in metabolite concentrations into epigenetic marks at defined loci, such as CD62L or TCF1, and what factors confer that specificity are still not known.

NADH/NAD^+ ratios

Nicotinamide adenine dinucleotide in its oxidized (NAD^+) and reduced (NADH) forms. A cofactor found in all living cells that is involved in redox reactions for the generation of ATP.

These studies highlight how multiple metabolic routes can sustain memory CD8$^+$ T cell differentiation as long as the cell can procure enough energetic and biosynthetic capacity to comply with the metabolic checkpoints that engage cell differentiation and functional programmes, including cofactors and substrates that build the epigenetic landscape.

Tissue-resident memory CD8$^+$ T cell metabolism

Tissue-resident memory CD8$^+$ T cells are one of the most abundant memory populations in the body[90]. They become embedded in lymphoid and non-lymphoid peripheral tissues during acute infection and provide a rapid and potent first line of defence against reinfection[90–93]. Below we highlight some of the metabolic changes that enable tissue-resident memory CD8$^+$ T cells to adapt to the specific tissue context.

Sensing the tissue environment

BHLHE40 is a stress-responsive transcription factor upregulated in tissue-resident memory CD8$^+$ T cells but not in circulating memory CD8$^+$ T cells[94]. Accordingly, deletion of *Bhlhe40* profoundly reduced lung tissue-resident memory CD8$^+$ T cell populations but not circulating effector or memory populations after influenza virus infection. Mechanistically, BHLHE40 was shown to support the expression of constituents of mitochondria, whose activity in generating acetyl-CoA from the TCA cycle supports the activity of histone acetyltransferases in regulating the epigenetic imprinting necessary for tissue-residency functions[94] (FIG. 4a).

Expression of the purinergic receptor P2RX7 (which can sense extracellular ATP and NAD$^+$ released by dying cells and intestinal microbial communities[95]) was also shown to be required for the differentiation of tissue-resident memory and central memory CD8$^+$ T cells (but not other circulating memory CD8$^+$ T cell populations) during acute lymphocytic choriomeningitis virus (LCMV) infection in mice (FIG. 4a). This requirement can vary depending on the mouse genetic background, the intestinal microbiota composition, expression of other genes affecting P2RX7 signalling and the timing after infection[81,96,97]. Such variability may be explained by the dual mode of action of this receptor: stimulation with submicromolar ATP concentrations engages a small-amplitude current, while stimulation with micromolar concentrations of ATP causes the formation of cytolytic membrane pores and cell death[98]. Mechanistically, non-lethal P2XR7 signalling has been suggested to increase AMPK activation to maintain mitochondrial homeostasis and TGFβ-dependent programmes of tissue residency in mice[81,97]. This is because in response to an initial stimulus, CD8$^+$ T cells further maintain P2RX7 signalling by autocrine secretion of ATP through pannexin channels, which lowers intracellular ATP concentrations and activates AMPK[81]. In addition to directly binding ATP, activation of P2RX7 can be triggered by its covalent ADP-ribosylation by the ecto-ADP-ribosyltransferase ARTC2.2, which is highly expressed in tissue-resident memory CD8$^+$ T cells, in the presence of NAD$^+$ (REF.[99]). ARTC2.2 can be blocked by small antibodies to prevent P2RX7-mediated cell death of liver tissue-resident memory CD8$^+$ T cells during isolation from the tissue[100]. Although ARTC2.2 is not expressed in humans, studying ex vivo mouse tissue-resident memory CD8$^+$ T cells is relevant due to ARTC2.2's ability to induce cell death[100], and

future work will address whether a similar mechanism is conserved in humans. Together, these data have revealed a complex, yet robust system for tissue-resident memory $CD8^+$ T cells to sense their environment and adapt to it.

Epithelial tissues and barrier sites

Memory $CD8^+$ T cells usually downmodulate effector programmes. However, intestinal tissue-resident memory $CD8^+$ T cells[101–103] contain more cytotoxic molecules and higher expression of degranulation markers, suggesting a basal state of heightened activation[101,104,105]. Similarly, skin tissue-resident memory $CD8^+$ T cells express genes associated with cytotoxic function and long-lived memory $CD8^+$ T cells[101,106]. At the cell-intrinsic level, it has been suggested that intestinal tissue-resident memory $CD8^+$ T cells maintain their heightened basal activation state by altering the cardiolipin composition of the inner mitochondrial membrane[104] (FIG. 4b). Cardiolipins are diphosphatidylglycerols that constitute up to 20% of inner mitochondrial membrane lipid, and they are required for the correct assembly of ETC components, protein transport, mitochondrial morphology and metabolism[104]. Despite having elevated basal OCR and increased mitochondrial content, intestinal tissue-resident memory $CD8^+$ T cells shift to increased ECAR-to-OCR ratios and low spare respiratory capacity compared with other memory populations in vitro[104]. These metabolic characteristics correlate with cardiolipin composition, as loss of tafazzin, a key enzyme in the synthesis of cardiolipins, impairs the ability of intestinal tissue-resident memory $CD8^+$ T cells to fend off the small intestinal parasite *Eimeria vermiformis* and correlates with reduced mitochondrial numbers and function in mice[104]. These data suggest that intestinal tissue-resident memory $CD8^+$ T cells have an elevated requirement for lipid production to maintain the levels of cardiolipin required for proper mitochondrial function[104]. Early studies reported that intestinal γδ T cells have increased transcriptional activation of lipid biosynthetic routes[107]. However, the alternative functions of lipid intermediates and how much of these is provided by scavenging lipids versus intracellular synthesis are not known.

Skin and intestinal tissue-resident memory $CD8^+$ T cells generated in response to an acute viral infection induce transcription of enzymes involved in lipid metabolism, have increased uptake of palmitate in vitro, have increased oxidative metabolism when exposed to extracellular fatty acids and show increased accumulation of lipid droplets, unlike circulating memory populations[52,104,108] (FIG. 3b). Together, these data have been used to argue that tissue-resident memory $CD8^+$ T cells heavily rely on lipids as a source of energy and biomass (FIG. 4b). However, the increased reliance on lipid metabolism imposes logistical challenges. To reduce fatty acid hydrophobicity, facilitate transport of fatty acids between cellular compartments and prevent unwanted signalling through lipid receptors, cells utilize fatty acid-binding proteins (FABPs) (FIG. 4b). FABPs show organ-specific and, despite some overlap, mutually exclusive patterns of expression[109,110]. Tissue-resident memory $CD8^+$ T cells share the same FABP expression profile as their tissue of residence and other co-residing immune cells[110]. This includes high expression of FABP4 and FABP5 for skin tissue-resident memory $CD8^+$ T cells[108,110], and FABP1 in liver tissue-resident memory $CD8^+$ T cells[109,110]. Accordingly, loss of FABP4 and FABP5 impaired tissue-resident memory T cell formation in the skin[108], while loss of FABP1 impaired liver

tissue-resident memory T cell formation[110]. Upon migration to new microenvironments, ex-liver-tissue-resident memory CD8$^+$ T cells adopt the FABP expression profile of their new location[110]. This suggests that these cells metabolically adapt to their niche through still unknown tissue-specific cues. Local microenvironment signals have been shown to transcriptionally regulate specific FABP isoforms[109]. How the distinct characteristics of each FABP isoform mechanistically contribute to tissue-specific metabolic adaptation of tissue-resident memory CD8$^+$ T cells remains to be determined.

Fatty acid-binding proteins

(FABPs). A family composed of 12 different isoforms with distinct stoichiometries, affinities and specificities for fatty acids and heterogenous expression among CD8$^+$ T cell subsets.

One potential consequence of tissue-resident memory CD8$^+$ T cells adopting the same FABP profile as their surrounding tissues would include competition for the same nutrient pool. However, it is not known whether additional fatty acid transporters and lipid scavengers, such as the LDL receptor or CD36 (REF.[111]), offer alternative strategies to warrant increased uptake of exogenous lipids by tissue-resident memory CD8$^+$ T cells[108]. The wide range of FABP substrates, such as eicosanoids, and phospholipid-rich membranes, and the activity of enzymes that mediate transfer of lipid ligands to proteins, such as CPT1α, make the study of FABP isoform functions in tissue-resident memory CD8$^+$ T cell metabolism an important emerging area of research.

Non-epithelial tissue sites

Liver tissue-resident memory CD8$^+$ T cells patrol the hepatic sinusoids, where they establish an extensive population that protects from parasitic[112] and viral infections[113]. Due to the irrigation from the intestine through the portal vein, liver tissue-resident memory CD8+ T cells are exposed to potentially toxic products. Human liver CD69$^+$ and CXCR6$^+$ tissue-resident memory CD8$^+$ T cells have increased levels of basal macroautophagy, which serves as a detoxification mechanism to preserve healthy mitochondria and maintain metabolic fitness[114] (FIG. 4c). It is not yet known whether other tissue-resident memory CD8$^+$ T cells share an increase in basal macroautophagy, although this is likely since autophagy has a critical role in peripheral T cell survival[115].

The white adipose tissue is a reservoir for memory T cell populations, including effector memory CD8$^+$ T cells and tissue-resident memory CD8$^+$ T cells[90,116]. Fat tissue-resident memory CD8$^+$ T cells appear to possess a distinct functional and metabolic profile, with increased homeostatic proliferation compared with other tissue CD8$^+$ T cells[116] (FIG. 4c). Importantly, transcriptional analysis of effector memory CD8$^+$ T cells and tissue-resident memory CD8$^+$ T cells from fat revealed few differentially expressed genes but a strikingly different transcriptional profile when compared with intestinal tissue-resident memory CD8$^+$ T cells and spleen effector memory CD8$^+$ T cells[116]. In addition, both fat effector CD8$^+$ T cells and tissue-resident memory CD8$^+$ T cells had the highest uptake of palmitate, and similarly elevated mitochondrial mass ex vivo[116] (FIG. 3a). These data suggest that the

imprinting of the adipose tissue environmental milieu onto immune populations in that tissue can supersede cell-fate transcriptional determinants of different CD8$^+$ T cell subsets. Thus, this study and others[117] reveal the importance of comparing cell types from similar environments to truly parse cell type-specific versus environmentally driven phenotypes.

Metabolism of CD8$^+$ T cell dysfunction

Ongoing TCR and inflammatory signalling in disease states such as cancer or chronic viral infection drives CD8$^+$ T cells into dysfunctional states[118,119]. In the setting of cancer, the antitumour CD8$^+$ T cell response is further repressed by an immunosuppressive environment, preventing control of tumour growth[3,120] (FIG. 5a). Pan-cancer meta-analysis of transcriptional and metabolic data has offered an emerging landscape of how metabolites are altered in cancer, both in the tumour microenvironment (TME) and in the circulation[121–124]. Despite the heterogeneity seen in CD8$^+$ T cell phenotype and function[124], these studies have found conserved metabolic alterations across different tumours, highlighting potential targets for therapeutic intervention.

Metabolic insufficiency in tumour environments

The activity of malignant and accessory cells within a tumour can impact the trafficking, differentiation, function and persistence of CD8$^+$ T cells by restraining their metabolic potential[3] (FIG. 5a). A clear example of nutrient competition is the depletion of glucose, which correlates with reduced infiltration and antitumour function of CD8$^+$ T cells[125–127]. Interestingly, CD8$^+$ T cell subsets have distinct responses to low-glucose environments[128]. In particular, central memory T cells and naive T cells, but not effector memory CD8$^+$ T cells, engaged fatty acid synthesis, oxidative phosphorylation and reductive glutaminolysis in low-glucose environments, which increases the levels of fatty acid species and limits effector T cell programmes[16,128]. Thus, at low glucose concentrations, only effector memory CD8$^+$ T cells were able to maintain high expression of IFNγ[128]. However, the IFNγ expression capacity of all memory T cell types was similarly blunted by increase of the concentrations of exogenous lipids in the media[128]. In the context of the TME, CD8$^+$ T cells devoid of autophagic capacity had increased antitumour activity resulting from an upregulation of glucose usage and greater production of effector cytokines, including IFNγ[129]. Tumour cells were shown to outcompete immune cells in methionine uptake by upregulating the transporter SLC43A2, which impaired CD8$^+$ T cell antitumour immunity by lowering the levels of SAM needed to epigenetically sustain STAT5 expression[130]. Together, these metabolic adaptions imposed by the TME on CD8$^+$ T cells dictate their antitumour potential by impinging on the cytokine production capacity of tumour-infiltrating lymphocytes (TILs).

Glutaminolysis

Metabolic process to break down glutamine for energy and for carbon products such as α-ketoglutarate.

Lactic acid is perhaps the best documented and most recurrent example of an immunosuppressive metabolite that accumulates as a result of tumour metabolism[33,122,123].

Pathophysiological concentrations of lactic acid were able to directly inhibit NFAT-dependent T cell activation and lower the expression of IFNγ[33] (FIG. 5a). In addition, lactate accumulation increased NADH/NAD^+ ratios, blunting glycolysis and the serine, glycine and one-carbon pathway, and limited the proliferation of effector $CD4^+$ T cells[131]. In contrast, lactate has been shown to be an important carbon source for anaplerosis in certain cell types[132–134], which suggests that lactate can metabolically reprogramme central carbon metabolism in some T cell subsets, skewing their function and differentiation. Thus, emerging evidence suggests a more complex and nuanced role for this important end product of glycolysis.

Other recurrent immunosuppressive metabolites include kynurenine and other tryptophan-derivate metabolites. Kynurenine can be imported into $CD8^+$ T cells to activate the aryl hydrocarbon receptor (AHR) to promote PD1 expression[122,135–137]. Kynurenine feeds the de novo synthesis of NAD^+, an important anabolic process specifying immune function of macrophages[138], but of unknown relevance in $CD8^+$ T cells.

Adenosine accumulates in hypoxic tumour environments[139], and its binding to adenosine receptor A_{2A} on $CD8^+$ T cells[140] impacts their cytokine-producing activity and upregulates inhibitory checkpoint molecules such as PD1 (REFS[141,142]) (FIG. 5a). Tumour-secreted metabolites can also affect TIL antitumour activity through indirect mechanisms such as by facilitating the recruitment and accumulation of immunosuppressive cells. These mechanisms include, for example, the transfer of the toxic metabolite methylglyoxal from myeloid-derived suppressor cells to T cells, blocking their activity[143] (FIG. 5a). In addition to metabolites, high tumour interstitial levels of potassium have been correlated with impaired nutrient uptake by $CD8^+$ T cells, dampening their antitumour capacity and skewing their differentiation potential[144] (FIG. 5a). Also, secretion of certain enzymes, such as arginase 1, which can be embedded in extracellular vesicles secreted by tumour cells, can suppress the proliferation of $CD8^+$ T cells by limiting the amount of available arginine, which is critical for their antitumour function[145,146] (FIG. 5a).

Multiple lines of evidence suggest that direct and indirect mechanisms are likely to induce $CD8^+$ T cell dysfunction in the TME. While a number of these mechanisms appear to be conserved across several tumour types, many show tumour-specific effects that reflect the specific tissue context or a defined set of mutations[122,123]. The intratumoural metabolic heterogeneity present in some tumours[147] offers a convenient tool to study $CD8^+$ T cell adaptation to diverse metabolic environments within the same tumour context. A systematic characterization of how these metabolic alterations affect $CD8^+$ T cell function in distinct cancer types will complement the emerging models of how tumours instruct antitumour immunity and will guide new approaches to target the antitumour function of $CD8^+$ T cells.

Metabolic insufficiency and $CD8^+$ T cell exhaustion

$CD8^+$ T cell exhaustion is a hyporesponsive cell state originally defined in the setting of chronic viral infection[118,119]. Due to chronic TCR stimulation, exhausted $CD8^+$ T cells progressively lose polyfunctional cytokine secretion and the ability to productively proliferate, causing them to depend on chronic TCR stimulation for survival and compromising their ability to fully control chronic viral infection or tumour growth (FIG.

5b). Ongoing exposure to pathogen-derived or tumour-derived antigens drives $CD8^+$ T cells to transition from a more functional progenitor state to a terminally exhausted and dysfunctional state, and this progression can be profiled by co-inhibitory marker expression and unique epigenetic and transcriptional modules[119]. The definition of T cell exhaustion continues to be refined as research continues[148], but it is now clear that metabolic alterations can drive T cell exhaustion rather than simply characterizing it, and as $CD8^+$ T cells show extensive metabolic plasticity, targeted relief in T cell metabolic suppression can have beneficial effects on $CD8^+$ T cell function.

Metabolite uptake and sensing defects

Exhausted T cells have diminished glucose uptake due to decreased GLUT1 surface expression[125,126,149–152]. Not only is glucose uptake altered in TILs but upstream glycolytic enzyme expression can be reduced as exhaustion develops[151–153]. The glycolytic enzyme ENO1 is suppressed in TILs, but bypassing aerobic glycolysis enzymes and feeding cells pyruvate directly improves TIL cytokine function in vitro[151]. T cells can utilize alternatives to glucose such as inosine, which can be converted into phosphorylated ribose and fed into the TCA cycle; in conjunction with immunotherapy, inosine treatment decreased tumour burden and increased survival[154]. Acetate was used as an alternative to glucose anaplerosis, and allowed $CD8^+$ T cells to increase global histone acetylation and chromatin accessibility, and when supplemented, increased TIL cytokine production in vivo[155] (FIG. 5b).

Glutamine uptake and metabolism has recently been shown to be a promising immunometabolic target in cancer. Pharmacological inhibition with the small-molecule glutaminase antagonist JHU083 has differential effects on tumour cells and $CD8^+$ TILs[156]. Pharmacological glutaminase inhibition negatively affected tumour cell metabolism, leading to decreased number TCA cycle intermediates, but in the same environment $CD8^+$ T cells exhibited metabolic plasticity and upregulated glucose anaplerosis, giving them an advantage in the TME and leading to decreases in tumour burden that were $CD8^+$ T cell dependent. Uptake of other metabolites by TILs is also being explored as an immunotherapeutic strategy, as TILs can import creatine via SLC6A8, and loss of this transporter negatively affected TIL function[157] (FIG. 5b). Diet-supplemented creatine plus checkpoint blockade therapy greatly increased antitumour efficacy[157], highlighting that the metabolite-poor TME can have a negative impact on TIL function.

Adequate oxygen supply is key for T cell function as the ultimate electron acceptor in the ETC during ATP synthesis. Hypoxia in isolation does not inhibit $CD8^+$ T cell function; in contrast, it has been shown to improve effector T cell differentiation, increase proliferation and increase cytokine production[60,158,159]. But in the context of the TME, hypoxia can create a barrier to TIL function in both transcriptionally mediated responses to hypoxia via HIF1α and through the impact of low oxygen concentration on mitochondrial respiration[160–162]. The TME's plethora of inhibitory signals, as well as suppression of glucose uptake and mitochondria, contribute to metabolic inelasticity and hypoxia's negative effect in the TME[125,126,152,163]. Interestingly, hypoxia can affect electron flow in the mitochondria, causing electrons to move from complex V to complex I, which generates ROS superoxide, a phenomenon shown to be a driver of TIL exhaustion[164–167] (FIG. 5b).

Genetically targeting the ETC in tumour cells or pharmacologically inhibiting tumour cell respiration with mitochondrial complex I inhibitor metformin can aid in reoxygenating the TME, which can lead to increased TIL infiltration, decreased exhaustion, increased cytokine production and reduction in tumour burden when used in combination with checkpoint blockade immunotherapy[160,161]. Hypoxia also elicits a transcriptional response in T cells, mediated by HIF1α. At tissue normoxia, HIF1α is targeted for degradation by the E3 ubiquitin ligase VHL (von Hippel–Lindau disease tumour suppressor). Low-oxygen environments prevent VHL-mediated degradation of HIF1α, allowing HIF1α–HIF1β heterodimers to bind hypoxia-responsive element DNA enhancer regions, initiating transcriptional responses to low oxygen levels that result in the expression of glycolytic enzymes and glucose transporters in nearly all cells and the induction of tissue-specific targets such as vascular endothelial growth factor (VEGF) and erythropoietin[168]. In chronic viral infection and cancer, deletion of VHL, which leads to accumulation of HIF1α, leads to $CD8^+$ T cells with increased co-inhibitory receptor expression and an altered transcriptional profile but increased cytokine expression and improved control of chronic infection and tumour burden[159]. Thus, low metabolite availability in the TME can negatively impact TIL function by affecting multiple metabolic pathways.

Mitochondrial defects

In both mouse and human tumours, exhausted $CD8^+$ T cells exhibit decreased mitochondrial mass compared with effector or memory $CD8^+$ T cells, which correlates with diminished oxygen consumption, depolarized mitochondrial membrane potential, punctate mitochondrial morphology and loss of cristae ultrastructure[152,160,163,165,169]. Similar mitochondrial defects have been seen in exhausted $CD8^+$ T cells during chronic viral infection[149,150,170,171]. The effector dysfunction associated with exhaustion is due in part to chronic T cell activation in hypoxic conditions, mediating repression of PGC1α, the master regulator of mitochondrial programming[163,172]. Repression of PGC1α can occur through multiple mechanisms, including AKT and BLIMP1, and enforced expression of PGC1α in TILs can rescue mitochondrial function[163,172] (FIG. 5a,b). In solid tumours and chronic viral infection, treatment in vivo with an AKT inhibitor rescued PGC1α expression and increased mitochondrial mass, suggesting repression of PGC1α in $CD8^+$ T cells is mediated in part by AKT and mTOR[163,170]. Defects in mitochondrial morphology also limit $CD8^+$ T cell function in tumours, with loss of the GTPase DRP1 — which is important for mitochondrial fission (FIG. 3b) — shown to negatively affect TIL function by accelerating exhaustion[66] (FIG. 5b).

Exhausted T cells not only lose mitochondrial mass; the remaining mitochondria become dysfunctional, producing excessive mitochondrial ROS, a phenomenon exacerbated specifically in the TME due to increased hypoxia[149,165,166,169,172,173]. ROS alone have been shown to be sufficient to drive an exhaustion phenotype and can contribute to chronic TCR signalling by acting as a phosphatase inhibitor, contributing to a feedforward cycle in the TCR tyrosine kinase signalling cascade[172]. Targeting TIL intracellular ROS either via PGC1α or glutathione peroxidase 1 (GPX1) overexpression, or by decreasing tumour hypoxia by tumour cell ETC component *NDUFS4*-knockout or by pharmacological intervention, mitigates the negative effect of ROS on TIL function[161,172]. Reducing the

levels of ROS in TILs via overexpression of the antioxidant thioredoxin can also improve TIL cytokine function and reduce tumour burdens via increased TCF7 expression[174]. Deletion of GCH1, the first and rate-limiting enzyme in tetrahydrobiopterin (BH4) biosynthesis, impaired T cell responses and ameliorated autoimmunity in mice, while BH4 supplementation enhanced antitumour activity of T cells against mammary tumours in mice by balancing iron metabolism and mitochondrial respiration[175] (FIG. 5b).

Co-receptor signalling in $CD8^+$ T cells can also impact gene programmes used to enforce metabolic states. During chronic LCMV infection, PD1 signalling contributed to loss of mitochondrial fitness, as virus-specific *Pdcd1*-knockout T cells or T cells from mice treated with anti-PDL1 had increase mitochondrial mass and improved mitochondrial function compared with controls[170]. 4-1BB signalling by $CD8^+$ T cells, acting through p38 MAPK, can increase ATF2 expression, which in turn increased PGC1α and mitofusin expression, generating T cells with more networked mitochondria, leading to increased oxidative phosphorylation, spare respiratory capacity, and superior TIL function[40,176] (FIG. 5a,b). Thus, dysfunctional mitochondria can drive T cell exhaustion, and therapeutic increases of mitochondrial mass or therapeutic improvement of mitochondrial morphology can improve TIL function.

Lipid processing and accumulation defects

Lipid accumulation is an immunosuppressive characteristic of some solid tumour environments[177,178]. Intrapancreatic $CD8^+$ T cells accumulate specific long-chain fatty acids, which dampen their antitumour function by decreasing their mitochondrial activity and triggering transcriptional changes that reduce their ability to extract energy from lipids via fatty acid oxidation[179]. Some $CD8^+$ TILs are able to metabolically adapt to a lipid-rich TME by increasing the catabolism of fatty acids, thus preventing their intracellular accumulation[177]. This is particularly relevant in low-glucose environments, where lipids can contribute as an important source of biomass for ATP production. Further increasing fatty acid catabolism with pharmacological agonists of PPAR contributes to increasing the antitumour ability of TILs and synergizes with PD1 blockade therapy to delay tumour growth[56,177,180,181]. Intriguingly, ligation of co-inhibitory receptors can also lead to changes in TIL metabolism, although this might apply only to $CD4^+$ T cells and not $CD8^+$ T cells[86,177]. Other lipid species, such as cholesterol, also accumulate in the TME and correlate with increased $CD8^+$ T cell exhaustion[182] (FIG. 5a). However, cholesterol is important to improve TCR clustering and formation of immunological synapses. Preventing cholesterol esterification by genetic deletion or pharmacological blockade of ACAT1 (an acetyl-CoA acetyltransferase) in $CD8^+$ T cells increased cytokine production and reduced tumour burden in vivo[183]. Further research is needed to clarify how cholesterol uptake, synthesis and storage contribute to the different aspects of $CD8^+$ T cell antitumour immunity.

With numerous metabolic defects or alterations evident in exhausted T cells, future research will need to elucidate how either chronic viral infection or the TME can cause or exacerbate impairments in metabolic pathways. It will also be important to determine how improving exhausted T cell metabolism can be applied broadly to any patient with exhausted T cells or

whether a more personalized medicine approach will need to be applied to T cell exhaustion, depending on the disease context or underlying patient genetics shaping environmental or T cell metabolism.

Host metabolism and CD8+ T cell function

Dietary restrictions and CD8+ T cell responses

Alterations in nutrition can influence disease outcomes in the context of infection and cancer (FIG. 1). Limiting overall food intake and other dietary modifications may constrain the tumour's access to nutrients[184], but the impact on the antitumour immune response is not well understood. Fasting in combination with chemotherapy can promote $CD8^+$ T cell-mediated control of breast cancer and melanoma in mice[185]. Similarly, caloric restriction enhanced $CD8^+$ T cell-dependent antitumour immunity in combination with chemotherapy in mutant KRAS-induced lung cancer in mice[186]. The T cell-intrinsic mechanisms involved here are not yet clearly delineated from the pleiotropic effects of these dietary modifications.

In healthy humans and mice, dietary restriction without compromising balanced nutrition depleted immune cell populations, including B cells, natural killer cells and T cells, from the periphery and increased their presence in the bone marrow[187,188]. Paradoxically, sustained dietary restriction increased T cell recall responses against lethal bacterial rechallenge, but decreased OCR, spare respiratory capacity and mTORC1 activation of central memory T cells in both the spleen and the bone marrow[188]. It is not known how the bone marrow protects mature $CD8^+$ T cells during periods of food scarcity, but it could shield T cells from exposure to immunosuppressive glucocorticoids and other factors induced by dietary restriction[188] or it could supply prosurvival signals[188]. Whether dietary restriction can force the egress of tissue-resident memory $CD8^+$ T cells, as infections do[102], and compromise tissue memory responses remains to be explored.

Infection and cancer directly impinge on the eating habits of the host, and this is referred to as 'sickness-induced anorexia' (SIA)[189]. In contrast to controlled dietary restriction, SIA impairs $CD8^+$ T cell recruitment and activation, and is detrimental for immune checkpoint blockade therapy in a mouse model of pancreatic ductal adenocarcinoma[190]. Similarly, in the context of a chronic viral infection, SIA promotes blooms of an intestinal commensal, which, upon oral inoculation, reduced splenic $CD8^+$ T cell expression of T-bet and granzyme B after acute or chronic LCMV infection in association with delayed viral clearance of the former[191]. In the context of acute infection with LCMV, $CD8^+$ T cell-intrinsic farnesoid X receptor (FXR) activity limited metabolic flexibility, as its loss prevented the starvation-induced reduction in $CD8^+$ T cell numbers, presumably by allowing them to switch from the limiting glucose to other fuel sources such as glutamine and fatty acids[192]. Why limiting host metabolism through controlled dietary restriction versus SIA has such different outcomes on $CD8^+$ T cell metabolism and function is not known.

Within isocaloric diets, fluctuations in the intake of specific dietary components, such as fibre, protein and fat, have distinct effects on $CD8^+$ T cell responses[193]. Diets containing fibre support fibre-degrading microbial communities in the gut[194]. In turn, fermentation of dietary fibre by the microbiota produces short-chain fatty acids, which have direct

immunomodulatory properties[195] beyond intestinal tissue barriers[196,197]. Short-chain fatty acids can be interconverted to acetate to support immune recall responses[198], or to acetyl-CoA to replenish intermediates of the TCA cycle[199] and fuel fatty acid oxidation to support increased mitochondrial function and glycolysis, which enhances $CD8^+$ T cell functionality against influenza virus infection in mice[195,200]. Another short-chain fatty acid, pentanoate, increases glucose oxidation in lymphocytes, fuels the acetyl-CoA pools that sustain histone acetyltransferases and enhances mTOR activity, overall promoting effector-like metabolism, although it decreases the numbers of IL-17- and IFNγ-producing cells, reducing the number of infiltrating $CD8^+$ T cells in the central nervous system in a mouse model of multiple sclerosis[201].

Short-chain fatty acids

Carboxylic acids with a small hydrocarbon chain, usually derived from the diet or bacterial fermentation.

Immune recall

The ability of memory lymphocytes to mount an immune response to a previously encountered antigen. A diet in which the most energy is derived from fat, rather than sugar or protein.

Protein energy malnutrition is associated with increased risk of infections and low vaccine efficacy[202]. Low protein diets compromise immunity against LCMV infection by impairing the homeostatic proliferation, memory formation and recall responses of $CD8^+$ T cell populations[203,204]. It is not yet known which amino acids are most impacted by protein energy malnutrition, but a decrease in available serine or alanine would impact $CD8^+$ T cell effector responses[36,205]. How specific amino acid availability, through the diet or local environmental metabolism, translates to $CD8^+$ T cell-intrinsic changes is a pending question in the field.

Dietary excesses and $CD8^+$ T cell responses

Obesity has been linked to impaired memory $CD8^+$ T cell responses to influenza virus with increased OCR-to-ECAR ratios, which cannot be restored by weight loss[206]. The exact mechanism by which increased adiposity affects $CD8^+$ T cell immunity is not known, but could be related to increased infiltration of cytotoxic $CD8^+$ T cells into the adipose tissue[207]. Fructose-rich diets can induce non-alcoholic fatty liver disease in a T cell-dependent manner[208], a liver condition that often precedes the emergence of hepatocellular carcinoma. Similarly, a choline-free high-fat diet can promote spontaneous hepatocellular carcinoma through activation of intrahepatic $CD8^+$ T cells that induce inflammation and liver damage[209]. In mice lacking the LDL receptor, an increase in the levels of dietary lipids over the course of 9 weeks increased proliferation of $CD8^+$ T cells and induced reprogramming of their intracellular lipid composition in the absence of obesity or inflammation[210]. Enforcing a high-fat diet on mice to model obesity altered the TME and impaired antitumour $CD8^+$ T cell responses due to metabolic maladaptation and

competition with tumour cells[211]. Lastly, a ketogenic diet, which is high in lipids and low in carbohydrates, promoted enhanced survival in a mouse model of glioblastoma, and this was partly through effects on $CD8^+$ T cells[212].

High-fat diet

A diet in which the most energy is derived from fat, rather than sugar or protein.

Conclusion and perspectives

The emerging picture supports the notion that metabolism is a strong driver of $CD8^+$ T cell differentiation and function. In turn, there is growing evidence that $CD8^+$ T cells can also influence host metabolism and behaviour (BOX 2). It has become apparent that the tissue environment, both during homeostasis and in disease, strongly modulates $CD8^+$ T cell function. A highlight of these newer studies is the remarkable metabolic plasticity that $CD8^+$ T cells use for activation, differentiation and tissue residency, and their untapped potential to overcome the hurdles of particularly challenging environments, such as the TME. While metabolic preferences for specific stages of $CD8^+$ T cell differentiation and function might exist in certain in vivo contexts, genetic studies suggest that $CD8^+$ T cells are not entirely committed to specific metabolic routes, as long as they are able to meet specific energetic and biomass requirements.

Tissue-resident memory $CD8^+$ T cells are defined by a core transcriptional module that drives cell-fate specification[101]. However, it is unlikely that a general metabolic adaptation unique for all tissue-resident memory $CD8^+$ T cells exists[101]. Rather, it is possible that tissue-specific $CD8^+$ T cells express niche-specific transcriptional modules that endow them with the ability to function and become integrated with a given tissue ecosystem. How tissue-specific cues influence regional variations in $CD8^+$ T cell behaviour, and whether or how $CD8^+$ T cells sense these niche-specific factors are important questions for future research.

The metabolic perturbations that occur in tumours and chronic viral infections highlight the relationships between $CD8^+$ T cell dysfunction and exhaustion and intrinsic and extrinsic metabolic cues. Metabolic dysfunction appears to be a driver of $CD8^+$ T cell exhaustion, and targeting specific metabolic pathways has been shown to improve $CD8^+$ T cell function in models of cancer or chronic viral infection. Developing therapies that target the TME metabolism or improve exhausted $CD8^+$ T cell metabolism could improve patient outcomes in multiple disease settings.

At the macroenvironmental scale, physiological perturbations of whole-body metabolism impact T cell responses. The mechanisms by which T cell-intrinsic metabolic states are influenced by these changes are not well described. However, a surprising preliminary conclusion is that our immune system has not evolved to cope with excessive food intake. Fine-tuning our understanding of this maladaptation to specific components of the diet, or interactions with the microbiota, requires further research.

Changes in the levels of intracellular metabolites are coupled to epigenetic regulation of $CD8^+$ T cell fate and function[213,214]. Increasing our understanding of this complexity will surely be needed to tackle the intracellular compartmentalization of metabolites, including those that accumulate in strategic locations, such as the nucleus[215] or mitochondria[69], to exert local effects. In this regard, understanding how metabolites are able to exert targeted or directed epigenetic and transcriptional effects through proteins that are seemingly not DNA sequence specific, will serve us to better understand how metabolism controls cell-fate decisions[6].

Profiling $CD8^+$ T cell metabolism in vivo will undoubtedly expand in vitro observations, although differences in experimental settings might pose challenges for the unification of a universal model. It is clear that substantial differences arise in these contexts, and for the field to move forward an understanding of these differences will be required (BOX 3). Developing a standardized metabolic classification of $CD8^+$ T cell states that contain context-independent measurements would allow comparisons across species, animal models, infections, tumour types and experimental conditions. In keeping with this idea, a potential systems-wide approach could consist of expanding current resources of immune genomics (such as the Immunological Genome Project) to include proteomic and metabolomic analyses and the modelling of enzymatic reactions to infer metabolic determinants of $CD8^+$ T cell function, as well as application of single-cell metabolism and in situ metabolism to further our understanding of immune cell metabolism within tissues. Together, existing and future approaches to immunometabolism will aid in understanding $CD8^+$ T cell metabolism not only across subsets but also within tissues, and will help identify metabolic targets for therapy development and application to human diseases.

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Box 1 |

Metabolic adaptations empower recall responses

The memory CD8$^+$ T cell response upon pathogen re-encounter is faster and more robust than the T cell response observed in a primary immune response. While different T cell receptor signalling thresholds[226] and a more permissive epigenetic landscape contribute to this capacity, a greater metabolic reserve of glycolytic enzymes, mitochondrial fitness, and the disposition of intracellular organelles are key metabolic adaptations that allow memory CD8$^+$ T cells to respond robustly to reactivation[11,17,65]. Upon restimulation, CD8$^+$ T cells undergo a burst of glycolysis, independent of mechanistic target of rapamycin complex 1 (mTORC1), that precedes their proliferation and is required for interferon-γ (IFNγ) production[17]. This activity is driven by the increased activity of acetylated GAPDH, a post-translational modification that augments its enzymatic capacity[17,198]. Acetyl-CoA levels can be limiting to maintain acetylated GAPDH, but systemic bacterial infections can raise acetate levels in the circulation, which enables CD8$^+$ T cells to maintain the pool of cytosolic acetyl-CoA through ATP citrate synthase activity[198]. In addition, unlike naive T cells, effector memory CD8$^+$ T cells upregulate mitochondrial respiration by increasing their mitochondrial mass[11] and establishing mitochondrion–endoplasmic reticulum contact sites, where voltage-dependent anion channel (VDAC) and hexokinase 1 (HK1) can coordinately upregulate the flux of glucose by shunting pyruvate into the tricarboxylic acid (TCA) cycle[65]. In addition to the production of ATP[12], TCA-derived citrate supports the production of acetyl-CoA used by histone acetyltransferases to maintain epigenetic activation of IFNγ[65]. Ageing memory CD8$^+$ T cells displayed paradoxically better recall responses than early memory cells via a series of metabolic adaptations that improved their overall fitness[227]. These included increased glucose utilization, decreased neutral lipid content, reduced fatty acid and low-density lipoprotein (LDL) uptake, and reactivation of mTORC1 activity[227]. It appears that after mTORC1 activity is turned off to allow memory differentiation, these cells gradually reacquired mTORC1 activity, and increased lymphoid tissue homing[227].

Box 2 |

$CD8^+$ T cells influence organismal metabolism

$CD8^+$ T cell activity can influence whole-body metabolism. For example, in mice lacking PD1, augmented $CD8^+$ T cell activation depleted systemic amino acids[223], and the shortage of circulating amino acids, especially tyrosine and tryptophan, limited synthesis of the neurotransmitters serotonin and dopamine in the brain, and induced heightened anxiety-driven behaviour and fear responses[223]. In addition, mice with $CD8^+$ T cells harbouring dysfunctional mitochondria (owing to loss of the transcription factor TFAM, which is responsible for replicating the mitochondrial genome) showed accumulation of proinflammatory cytokines in the circulation, accelerated senescence and premature death[216]. Nicotinamide riboside supplementation and TNF inhibition were able to slow down the progressive signs of ageing in these mice, suggesting that metabolic intervention may be a strategy to regulate premature ageing in some individuals[216].

Chronic viral infections can cause immune-associated cachexia mediated by a mechanism distinct from cancer-associated cachexia[228]. In chronic lymphocytic choriomeningitis virus infection, anorexia is dependent on virus-specific $CD8^+$ T cell activity[191], which also increased lipolysis in adipocytes and remodelled adipose tissue in a type I interferon-mediated manner[228]. Similarly, early after chronic lymphocytic choriomeningitis virus infection, hepatocyte-intrinsic type I interferon signalling induced a reprogramming of the hepatic urea metabolism, which lowered the level of circulating arginine, a key metabolite to sustain $CD8^+$ T cell survival and activity[146,229]. Recall responses by memory $CD8^+$ T cells embedded in white adipose tissue were also shown to reduce systemic adiponectin and cholesterol levels[116]. Further research will be needed to understand how $CD8^+$ T cells are able to shape host metabolism in different immune settings.

Box 3 |

New approaches to immunometabolism

As we move forward in tackling the next big questions in immunometabolism, we have reflected on key considerations that could guide the approach.

- Probing metabolism in vivo is key to have physiologically relevant insights[37]. However, system-level analysis of organ-specific and bulk tissue metabolic activities lacks granularity about the cell types contributing to such profiles, including important metabolite exchange activities and cell type cooperativity within the organ. Conversely, adding granularity by profiling in vivo or ex vivo populations misses important short-scale metabolic processes, compromises the viability of tissue-resident memory $CD8^+$ T cells[100] and introduces artefacts[230,231]. Emerging technologies aim to add granularity and spatial information, while preserving physiological conditions[232,233].
- Mouse models will likely remain the gold standard for functional genomics, but the use of human samples and humanized mouse models will greatly increase clinical relevance and translation.
- Tissue immunity, including less explored peripheral tissues such as muscle, brain or prostate, will most likely unravel underappreciated functions of tissue-resident immune populations and their crosstalk with other cells of the niche. In keeping with this idea, metabolic profiling will benefit from retaining spatial distribution information, so associations between neighbouring cell types, intratissue location and function can be established.
- Exploring the intracellular compartmentalization of metabolism will provide unprecedented mechanistic insights into how enzymatic complexes organize and channel metabolites[234].
- Profiling immune dynamics in vivo[235], combined with genetically coded biosensors to profile intrinsic metabolic states, could reveal processes that occur on timescales otherwise not appreciable[236].
- The creation of a standardized metabolic classification of individual $CD8^+$ T cell states[233,237] that integrates enzymatic (kinetics and cofactor requirements), transcriptomic and proteomic data into a searchable resource database, will be an important future goal.

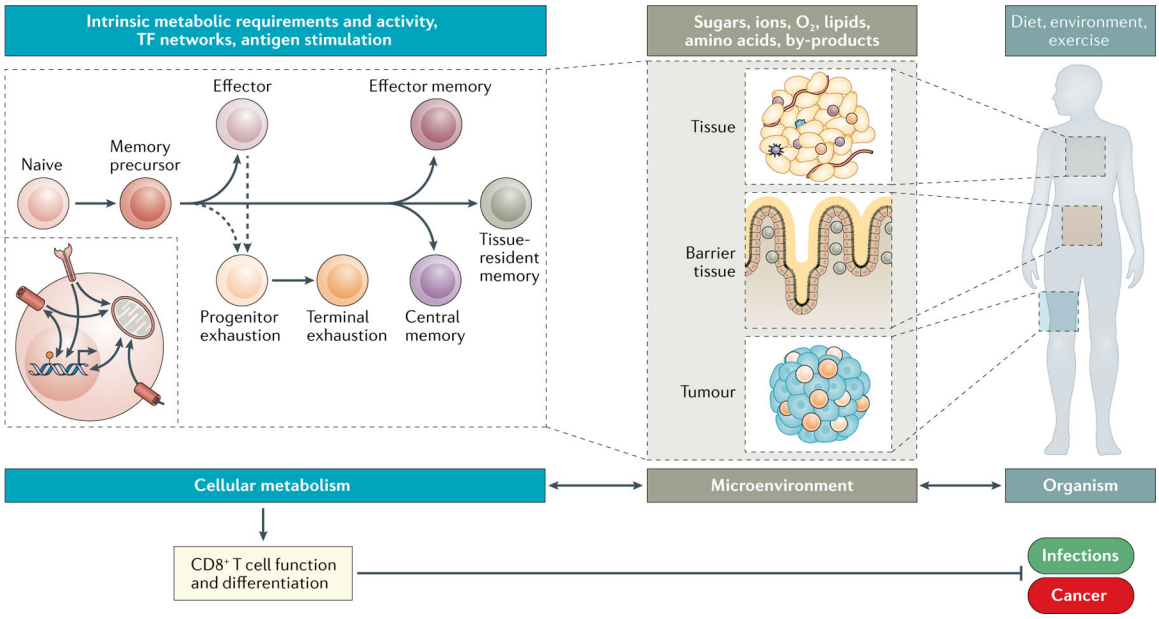

Fig. 1 |. Organismal, niche and intrinsic metabolism dictate CD8$^+$ T cell fate and function. Effector CD8$^+$ T cells spearhead the adaptive branch of the immune system by directly killing infected cells and cancer cells, while long-lived memory CD8$^+$ T cell populations provide protective immunity for the lifetime of the organism. In the setting of chronic viral infection and cancer, the tissue environment helps drive CD8$^+$ T cells to a dysfunctional state known as T cell exhaustion. These functions of T cells are intimately linked to their capacity to obtain nutrients from heterogenous tissue environments (microenvironment), which are in turn influenced by tissue-specific activities and the overall organismal metabolism (the macroenvironment) to further influence the fate of CD8$^+$ T cells. TF, transcription factor.

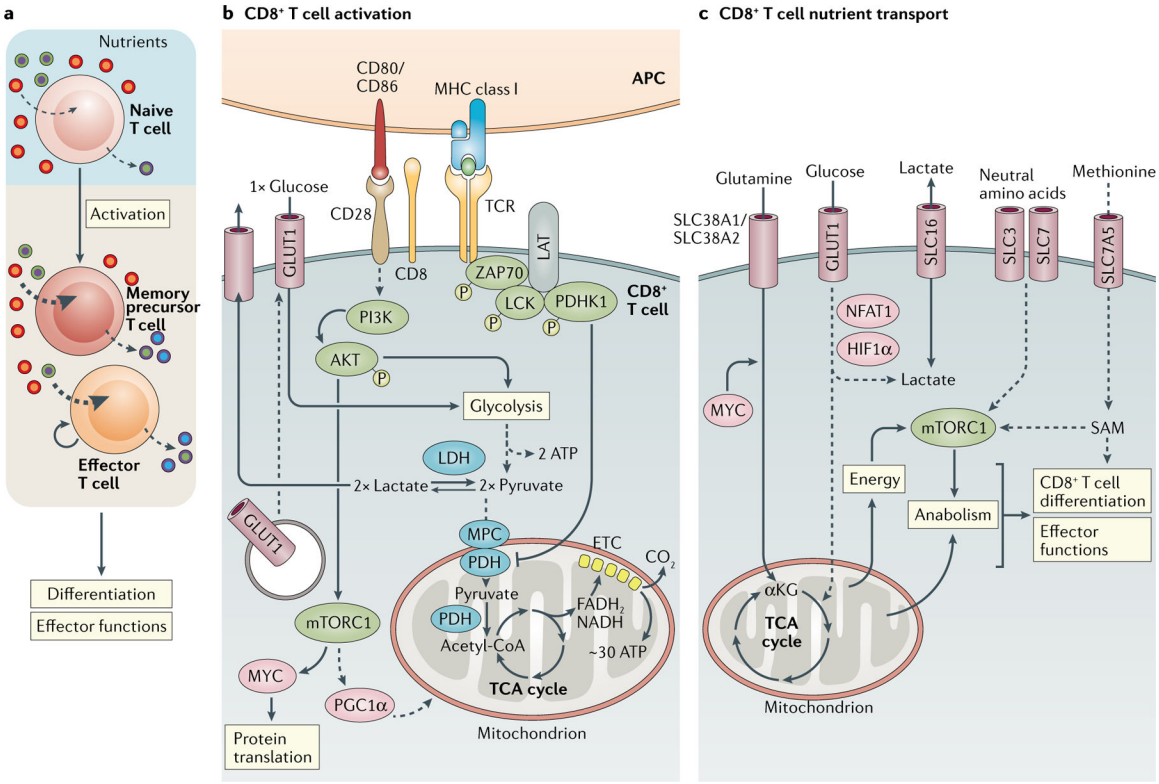

Fig. 2 |. Metabolism of CD8$^+$ T cell activation and effector function.

a | CD8$^+$ T cell activation increases metabolic activity — including nutrient uptake (red circles) and secretion of metabolic by-products (purple circles) — to fuel increased anabolism and proliferation needed to mount effector T cell responses and differentiation into long-lived memory CD8$^+$ T cells. **b** | Immediately after CD8$^+$ T cell activation, glucose-derived carbons are diverted from the TCA cycle into lactate production, a less efficient but faster route of ATP production, without increasing the overall glucose uptake. This is achieved in a CD28-independent fashion by engaging the pyruvate dehydrogenase kinase 1 (PDHK1)-dependent inhibition of pyruvate dehydrogenase (PDH) at the T cell synapse. Shortly after, glucose uptake is boosted by increasing the amount of glucose transporters on the cell surface, and the overall flux through glycolysis is upregulated by PI3K–AKT-dependent signalling downstream of CD28. Kinases are coloured green, metabolic enzymes are coloured blue, transporters are coloured pink and transcription factors are coloured light pink. **c** | CD8$^+$ T cell activation requires increased nutrient consumption from the environment to support differentiation of T cells into effector and memory subsets. αKG, α-ketoglutarate; APC, antigen-presenting cell; ETC, electron transport chain; $FADH_2$, dihydroflavin adenine dinucleotide; LAT, linker for activation of T cells family member 1; LDH, lactate dehydrogenase; MPC, mitchondrial pyruvate carrier; mTORC1, mechanistic target of rapamycin complex 1; NFAT1, nuclear factor of activated T cells 1; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PI3K, phosphoinositide 3-kinase; SAM, *S*-adenosylmethionine; SLC, solute carrier; TCA, tricarboxylic acid; TCR, T cell receptor.

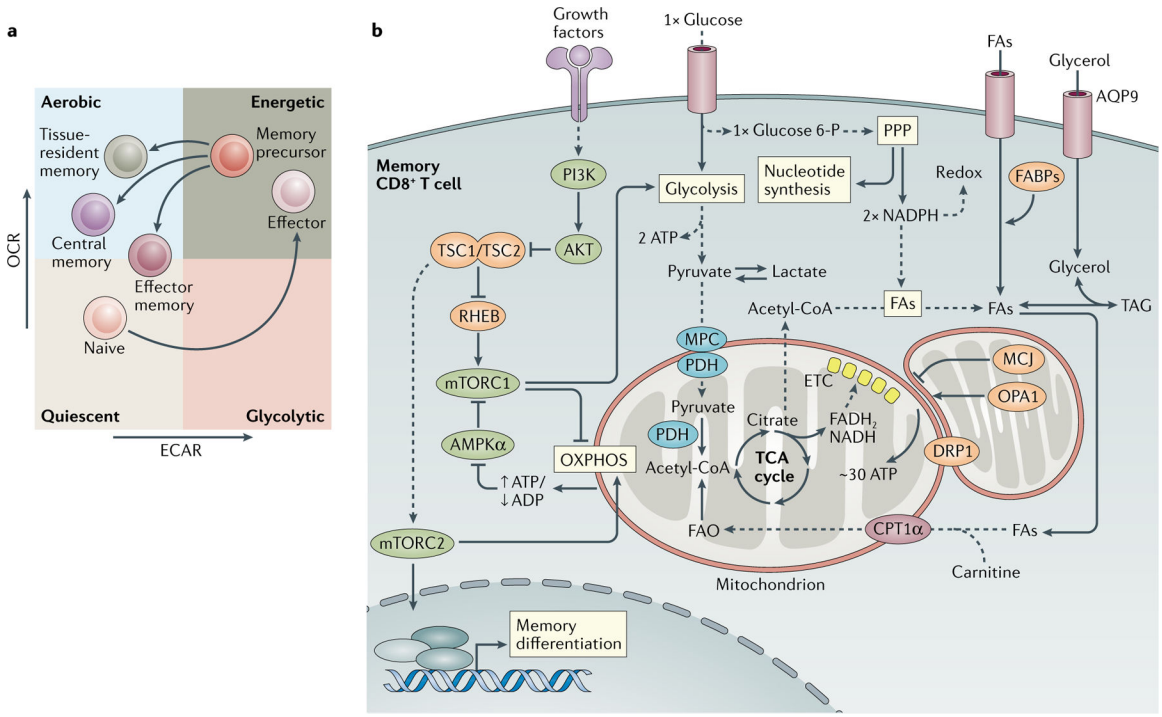

Fig. 3 |. Metabolism of memory CD8$^+$ T cell differentiation.

a | CD8$^+$ T cell subsets along their differentiation trajectory can be segregated on the basis of their extracellular acidification rate (ECAR) and oxygen consumption rate (OCR), which are a proxy for glycolytic and oxidative phosphorylation (OXPHOS) metabolism, respectively, as measured by Agilent Seahorse XF analysers (TABLE 1). **b** | Intrinsic metabolism of memory CD8$^+$ T cells. Glucose-derived carbons are oxidized mostly through OXPHOS in the mitochondria. Fatty acid (FA)-binding proteins (FABPs) coordinate the uptake and intracellular management of lipids, either from extracellular uptake or from synthesis from glucose-derived carbons. Lipids are oxidized in the mitochondria through FA oxidation (FAO) via a mitochondrial transport mechanism involving the carnitine shuttle. The pentose phosphate pathway (PPP) provides the necessary NADPH to maintain adequate redox levels, fuel FA synthesis and provide metabolic intermediates for the synthesis of nucleotides. Growth factors and the overall metabolic and energetic state of the cell control mechanistic target of rapamycin complex 1 (mTORC1)–mTORC2 complexes to coordinate the flux of nutrients into anabolic or catabolic processes. Kinases are coloured green, metabolic enzymes are coloured blue, transporters are coloured pink, transcription factors are coloured light pink and other proteins are coloured orange. AMPKα, 5’-AMP-activated protein kinase catalytic subunit-α; AQP9, aquaporin 9; CPT1α, carnitine palmitoyltransferase 1α; DRP1, dynamin-related protein 1; ETC, electron transport chain; MCJ, methylation-controlled J protein; 6-P, 6-phosphate; PDH, pyruvate dehydrogenase; PI3K, phosphoinositide 3-kinase; TAG, triacylglycerol; TCA, tricarboxylic acid; TSC, tuberious sclerosis complex.

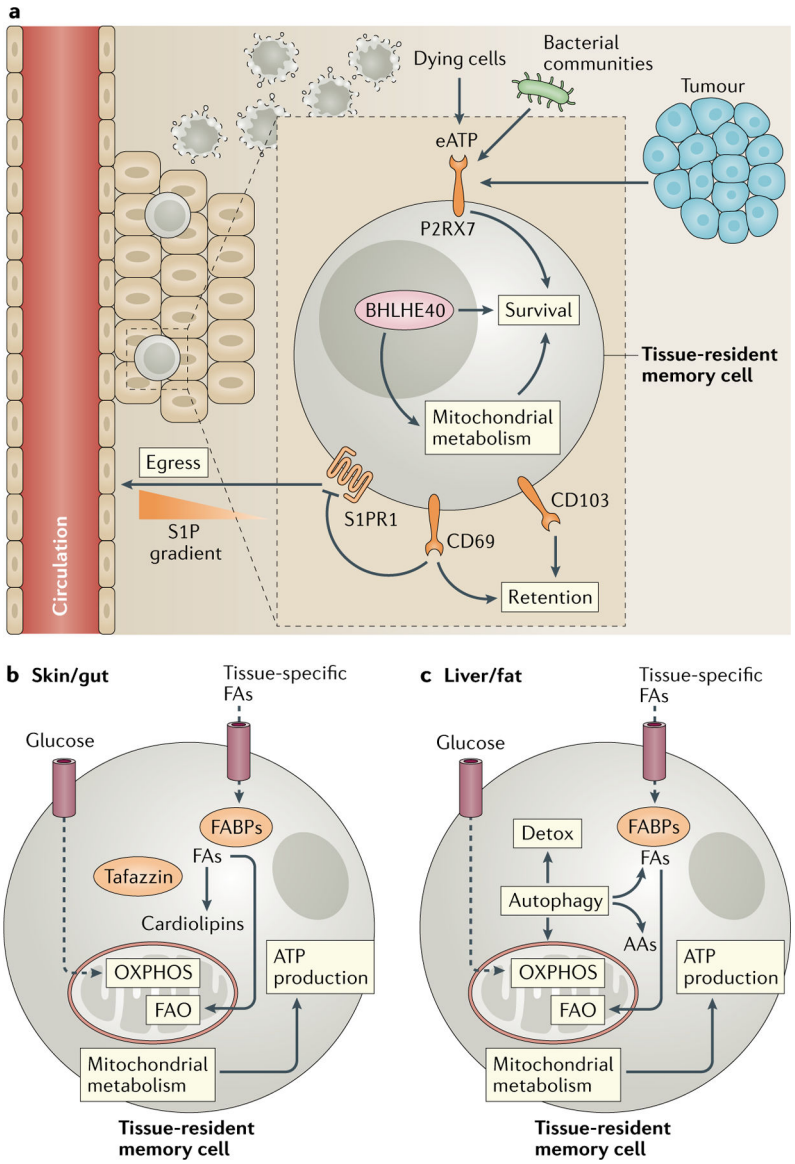

Fig. 4 |. Metabolism of tissue-resident memory CD8$^+$ T cells.

a | Environmental sensing and survival in peripheral tissues. Tissue-resident memory CD8$^+$ T cells sense concentrations of extracellular ATP (eATP) through P2RX7 to engage signalling that impacts survival. Expression of BHLHE40, which is restricted to tissue-resident memory CD8$^+$ T cells, increases survival and mitochondrial metabolism to favour tissue residency. Expression of surface proteins, such as CD103 and CD69, favours tissue retention by blocking tissue egress signals, such as the sphingosine 1-phosphate (S1P) gradient established between the circulation and peripheral tissues. Transcription factors are coloured light pink and other proteins are coloured orange. **b** | Tissue-resident memory CD8$^+$ T cells at barrier surfaces, such as skin and gut, use glucose and lipids to fuel the production of mitochondrial ATP. Fatty acid (FA)-binding protein (FABP) isoform expression is tissue specific and adapted to environmental lipid species. The cardiolipin composition of the mitochondria, controlled by the protein tafazzin, is a key aspect of

their functionality. Transporters are coloured pink **c** | Tissue-resident memory $CD8^+$ T cells at non-barrier surfaces, such as liver and fat, use glucose and lipids to fuel the production of mitochondrial ATP. FABP isoform expression is tissue specific and adapted to environmental lipid species. Autophagy promotes intracellular detoxification of damaged organelles, recycles nutrients and ensures mitochondrial homeostasis. Metabolic enzymes are coloured blue, transporters are coloured pink, transcription factors are coloured light pink and other proteins are coloured orange. AA, amino acid; FAO, fatty acid oxidation; OXPHOS, oxidative phosphorylation; S1PR1, sphingosine-1-phosphate receptor 1.

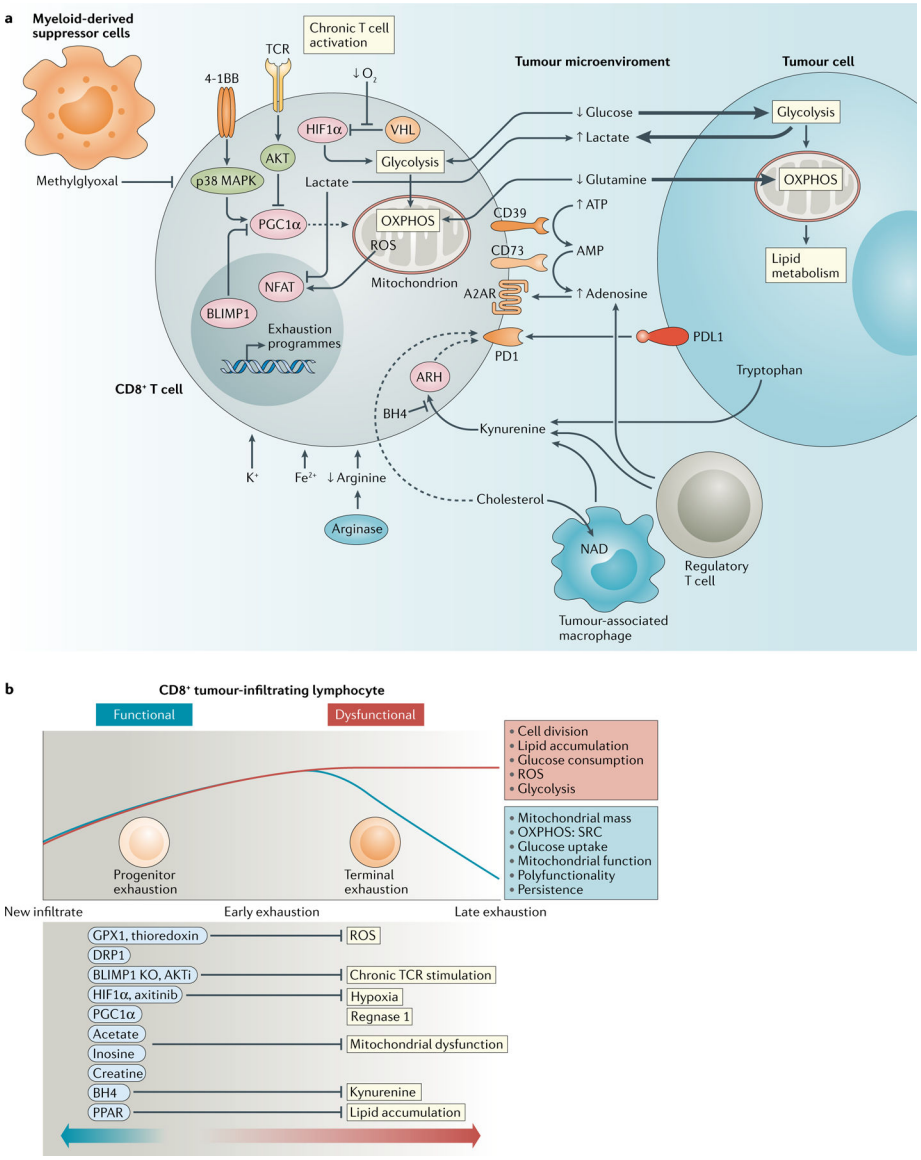

Fig. 5 |. Metabolic insufficiency in chronic infection and cancer.

a | Metabolic insufficiency by nutrient competition and intercellular exchanges in the tumour microenvironment (TME). $CD8^+$ T cells compete for nutrients with other cells in the TME, predominantly with cancer cells, which can deplete glucose and glutamine pools, and release immunosuppressive metabolites and ions, such as lactate, kynurenine and potassium ions. Additional immune cells, such as myeloid-derived suppressor cells, tumour-associated macrophages and regulatory T cells also contribute to the generation and secretion of metabolites that suppress $CD8^+$ T cells. Chronic antigen presentation and low oxygen concentrations promote $CD8^+$ T cell dysfunction by driving AKT-mediated and BLIMP1-mediated suppression of PGC1α, causing mitochondrial production of reactive oxygen species (ROS). Mitochondrial-derived ROS promote NFAT-driven exhaustion programmes. In addition to signalling through P2RX7, extracellular ATP can be subsequently converted to AMP and adenosine by the actions of CD39 and CD73, respectively. Adenosine

signalling through adenosine receptor A_{2A} (A2AR) drives expression of PD1 on $CD8^+$ T cells. PD1 signalling triggered by its ligand PDL1 on the surface of surrounding cells inhibits further $CD8^+$ T cell activation, acting in part through its suppressive effect on mitochondrial metabolism. Secretion of arginase by tumour-associated macrophages and other cells depletes arginine pools from the TME and limits the antitumour effect of $CD8^+$ T cells. Kinases are coloured green, metabolic enzymes are coloured blue, transporters are coloured pink, transcription factors are coloured light pink and other proteins are coloured orange. **b** | Metabolic insufficiency and exhaustion programmes. Upon infiltration into the tumour microenvironment, $CD8^+$ T cells increase their metabolism to increase proliferation and cytotoxicity. However, prolonged exposure to persistent antigen and an immunosuppressive microenvironment drives them to dysfunction, a spectrum of differentiation states also known as exhaustion. A combination of metabolic imbalances and opposing cues (that is, chronic stimulation and pro-proliferation cues combined with lack of functional mitochondria and nutrients and diminished glucose uptake or lipid accumulation) pushes them further into a late non-reversible exhaustion state that is associated with failed antitumour immune responses. Manipulation of $CD8^+$ T cell metabolism through genetic, pharmacological and metabolic interventions can oppose immunosuppressive factors and prevent differentiation to a dysfunctional state. AHR, aryl hydrocarbon receptor; AKTi, AKT inhibitor; BH4, tetrahydrobiopterin; DRP1, dynamin-related protein 1; GPX1, glutathione peroxidase 1; HIF1α, hypoxia inducible factor 1α; KO, knockout; NFAT, nuclear factor of activated T cells; OXPHOS, oxidative phosphorylation; PGC1α, peroxisome proliferator activated receptor gamma coactivator 1α; PPAR, peroxisome proliferator-activated receptor; SRC, spare respiratory capacity; TCR, T cell receptor; VHL, von Hippel–Lindau disease tumour suppressor.

Table 1 |

Techniques for probing $CD8^+$ T cell metabolism

	Process or technique to probe pathway	Pathway perturbations	Considerations	Refs
Not pathway specific				
Targeted and untargeted metabolomics	Chemical fractionation of biological samples coupled with chromatography-based separation; MS-based detection methods in combination with titration of known metabolites (targeted); database-mediated metabolite chemical composition identification strategies (untargeted)	NA	Lack of metabolite annotation data limits metabolite identification in untargeted metabolomics	–
Metabolic tracing and flux analysis	Labelled metabolites can be traced in vitro and in vivo by following the incorporation of their labelled groups into their metabolic derivatives; metabolic flux allows quantification of molecule turnover	NA	–	37
Pathway specific				
Glycolysis	Glucose uptake: injection of [^{18}F]-fluorodeoxyglucose, incubation with the fluorescent glucose analogue 2-NBDG, radioactive incorporation with [3-3H] glucose, surface expression of glucose transporters Glycolytic capacity: maximum medium acidification upon inhibition of ATP synthase Glycolysis: medium acidification associated with oxidizing glucose Glycolytic reserve: difference between glycolytic capacity and glycolysis; it measures the capacity of the glycolysis to compensate for the loss of mitochondrial ATP synthesis	Genetic: *VHL* KO, *HIF1A* KO Pharmacologic: 2-deoxyglucose (blocks phosphoglucose isomerase and hexokinase reactions), 3-bromopyruvate (hexokinase II inhibitor), ritonavir (HIV protease inhibitor), dichloroacetate (pyruvate dehydrogenase kinase inhibitor), FX11 (LDHA inhibitor), 4-CIN (inhibition of mitochondrial pyruvate transport)	Supplemental glucose or pyruvate in Seahorse media will change glycolysis and the interpretation of data RPMI and DMEM have different levels of glucose, which are not physiological. Culturing T cells in these media may not reflect glucose consumption ex vivo	26,52,59,73,81,89,216–220
Oxidative phosphorylation	OCR (pmol O_2/min) (Seahorse): Reserve respiratory capacity/spare respiratory capacity: difference between the basal and maximal OCR. This indicates the ability of a cell to meet an increased energy demand Basal respiration: required to maintain homeostatic ATP demand ATP-linked respiration: oligomycin-dependent OCR; it estimates the OCR associated with mitochondrial ATP synthesis Maximal respiration: OCR levels when mitochondria are forced to work at maximum capacity to maintain mitochondrial membrane potential in the presence of a protonophore Proton leak: OCR not coupled to ATP synthase activity OCR-to-ECAR ratios ATP levels with ATP probes	Genetic: *VHL* KO, *HIF1A* KO Pharmacologic: rotenone (ETC complex I inhibitor), FCCP (protonophore), oligomycin (ATP synthase inhibitor), antimycin A (ETC complex III inhibitor), UK5099 (mitochondrial pyruvate transporter inhibitor), methylpyruvate (cell-permeant metabolite)	Supplemental glucose or pyruvate in Seahorse media will change glycolysis and the interpretation of data RPMI and DMEM have different levels of glucose, which are not physiological. Culturing T cells in these media may not reflect glucose consumption ex vivo	69,219,220
Mitochondrial dynamics	Mitochondrial mass and shape: mitochondrial dyes (MitoTracker Green and MitoTracker Red), membrane polarization dyes (TMRE), electron microscopy Mitochondrial biogenesis: mtDNA/nDNA ratio, PGC1α expression, TFAM expression, mRNA expression of	Genetic: *DRP1* KO, *OPA1* KO, *TAZ* KO	Sodium azide (commonly used in flow cytometry staining buffers) is an ATP synthase inhibitor and can jeopardize interpretation of mitochondrial read-outs	11,55,66,81,94,104,163,221

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	Process or technique to probe pathway	Pathway perturbations	Considerations	Refs
	mitochondrial genes Mitochondrial function and dysfunction: mitochondrial ROS, cellular ROS			
Serine, glycine and one-carbon pathway	Methionine and SAM concentrations Serine and glycine uptake Expression of PHGDH, PSAT1, SHMT1 and SHMT2	–	–	36,129
Pentose phosphate pathway	NADPH production in the presence/absence of G6PDH activity	Pharmacologic: 6-aminonicotinamide (G6PDH inhibitor), G6PDi-1 (G6PDH inhibitor)	–	61,62
Glutaminolysis	Incorporation of glutamine in TCA cycle intermediates Radioactive incorporation with [U-^{14}C]glutamine	–	–	26,128
mTORC1 activity	Downstream signalling: anti-phospho-p70 S6 kinase (T421/424), anti-S6, anti-pS6 (S235/236), anti-pS6 (S240/244)	Genetic: *TSC1* KO, *TSC2* KO, *RPTOR* KO Pharmacologic: rapamycin (mTORC1 inhibitor)	–	57,71,72
mTORC2 activity	Downstream signalling: anti-pAKT (S473)	Genetic: *TSC1* KO, *RICTOR* KO	–	57
Protein synthesis	Downstream mTORC1 signalling: anti-p4EBP1 (Thr37/46) Total protein Polysome-associated RNA	Pharmacologic: cycloheximide (ribosomal subunits)	–	222,223
AMPK activity	Downstream signalling: anti-pAMPK (T172), anti-phospho-Raptor (S792), anti-pACC (S79), anti-pATGL	Pharmacologic: metformin (AMPK activator)	–	161,222
Redox state	GSSH/GSH ratios NADPH/NADP$^+$ ratios	Pharmacologic: buthionine sulfoximine (glutathione synthesis inhibitor), γ-glutamylcysteine synthetase inhibitor	–	61
Glycogenesis	Glycogen assays Electron microscopy of glycogen deposits	Pharmacologic: 3-MPA (PCK1 inhibitor)	–	61
Autophagy	Endosome-bound LC3 by flow Lipidated LC3 ratios	Genetic: *ATG5* KO Pharmacologic: bafilomycin (V-ATPase inhibitor)	–	114,115,128
Fatty acid oxidation	OCR in the presence of oleate or palmitate Radioactive incorporation with [$^{13}C_{16}$]palmitate and [9,10-^{3}H]palmitic acid	Genetic: *CPT1A* KO	–	11,26,52,54,58,177
Fatty acid synthesis	Expression of SREBP target genes DGAT1 and DGAT2 activity	Pharmacologic: statins (HMG-CoA reductase inhibitor), C75 (FASN inhibitor), PF-04620110 (DGAT inhibitor), amidepsine A (DGAT inhibitor)	–	52,104,107,224
Fatty acid uptake, storage and transport	In vivo systemic injection or in vitro incubation with BODIPY-conjugated lipid probes TAG, DAG, glycerol and neutral lipid by LC–MS and BODIPY 493/503 staining	Genetic: *CD36* KO, *LDLR* KO, *FABP* KO Pharmacologic: phloretin (AQP inhibitor)	In vitro and ex vivo uptake assays lack the nutrient competition effect from other cell types of their tissues of origin and can substantially differ from in vivo read-outs	52,54,110,210,225

4-CIN, α-cyano-4-hydroxycinnamic acid; CPT1α, carnitine palmitoyltransferase 1α; DAG, diacylglycerol; DRP1, dynamin-related protein 1; ECAR, extracellular acidification rate; ETC, electron transport chain; FABP, fatty acid-binding protein; FCCP, trifluoromethoxy carbonylcyanide phenylhydrazone; KO, knockout; LC, liquid chromatography; 3-MPA, 3-mercaptopicolinic acid; MS, mass spectrometry; mtDNA, mitochondrial DNA; mTORC: mechanistic target of rapamycin complex; NA, not applicable; nDNA, nuclear DNA; 2-NBDG, (2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose); OCR, oxygen consumption rate; ROS, reactive oxygen species; SAM, *S*-adenosylmethionine; SREBP, sterol regulatory element-binding protein; TAG, triacylglycerol; TCA, tricarboxylic acid; TMRE, tetramethylrhodamine, ethyl ester.