



ELSEVIER



The emerging role of one-carbon metabolism in T cells

Henry Kurniawan^{1,2,4}, Takumi Kobayashi^{1,2,4} and Dirk Brenner^{1,2,3}

One-carbon metabolism (1CM) supports multiple biological functions, providing 1C units for nucleotide synthesis, epigenetic maintenance, and redox regulation. Although much has been deciphered about the relationship between disruption of 1CM and various diseases, our understanding of 1CM's involvement in the regulation of the immune system is only now evolving. In this review, we summarize key checkpoints of 1CM pathways that govern cellular activities. We also report on recent findings regarding the role of 1CM in T cells and discuss several promising avenues requiring future investigation.

Addresses

¹ Experimental & Molecular Immunology, Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg

² Immunology and Genetics, Luxembourg Centre for System Biomedicine (LCSB), University of Luxembourg, Belval, Luxembourg

³ Odense Research Center for Anaphylaxis, Department of Dermatology and Allergy Center, Odense University Hospital, University of Southern Denmark, Odense, Denmark

Corresponding author: Brenner, Dirk (dirk.brenner@lih.lu)

⁴ Both authors contributed equally.

Current Opinion in Biotechnology 2021, **68**:193–201

This review comes from a themed issue on **Systems biology**

Edited by **Karsten Hiller** and **Dirk Brenner**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 7th January 2021

<https://doi.org/10.1016/j.copbio.2020.12.001>

0958-1669/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Introduction

One-carbon metabolism (1CM) is the term used to describe a series of biochemical reactions resulting in the transfer of one-carbon moieties to various substrates involved in multiple processes critical for cell survival and function [1]. These processes are important not only for energy production but also for the biosynthesis of key molecules such as creatine, polyamines, proteins, phospholipids, and nucleotides. The epigenetic control of DNA and RNA methylation, histone modification, and redox homeostasis also depend on 1CM [1,2]. Physically, 1CM reactions occur in the cytoplasm, nucleus, and mitochondria but are subjected to differential regulation within these compartments [3]. 1CM is particularly vital in cell types that are highly metabolically active, most notably cancer cells, neural cells and immune cells [4].

At the core of the network of reactions constituting 1CM is the naturally derived nutrient folate or its synthetic form called folic acid (Vitamin B9), which are available solely from the diet [5]. The importance of folate is highlighted by the consequences of folate deficiency during embryonic development, which include neural tube malformations constituting birth defects [6]. In adults, a lack of folate has been implicated in anemia, premature ageing, cardiovascular diseases, and cancer [5,7]. Methotrexate, an anti-folate drug that inhibits the conversion of folate into tetrahydrofolate prior entering the 1CM network, is now a standard agent in cancer treatment [8]. This success has led to intensive research on 1CM function in cancer cells, but the role of this network in the immune system is just starting to be unraveled. In particular, how 1CM affects T cell functions has only recently come under study.

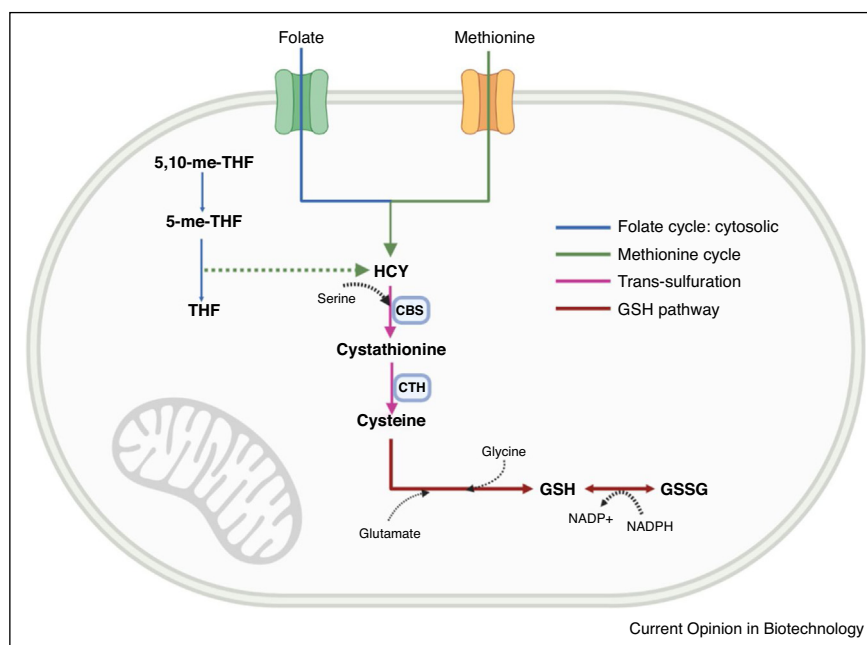
In this review, we highlight key components and summarize critical steps of 1CM and discuss how they contribute to normal and pathological T cell functions. In addition, we will point out various avenues in the cross-talk between 1CM and the immune system that merit future investigation.

Components and functions of 1CM

Folate cycle

The folate cycle lies at the heart of 1CM. As illustrated in [Figure 1](#), dietary folate is first reduced to dihydrofolate (DHF) in the cytosol by the 1CM enzyme dihydrofolate reductase (DHFR). DHF is further converted by DHFR into its active form tetrahydrofolate (THF) and drives the folate cycle in both cytoplasm and mitochondria [9]. In both compartments, THF receives a 1C unit upon the conversion of serine into glycine in a reversible reaction catalyzed by either serine hydroxymethyltransferase-1 (SHMT1) or SHMT2, leading to the production of 5,10-methylene-THF (5,10-me-THF) [5]. THF and 5,10-me-THF are central intermediates in 1CM, being the launch points for several interconnected metabolic pathways such as the methionine cycle, the trans-sulfuration pathway, DNA/histone methylation, and nucleotide biosynthesis. The importance of serine and glycine as 1C donors in the 1CM network should not be underestimated, particularly in the context of tumorigenesis. In multiple mouse models of cancer development, serine and glycine starvation *in vivo* attenuates tumor growth and prolongs mouse survival [10,11].

Figure 2



1C metabolism in redox regulation. Sufficient levels of extracellular methionine and folate entering a cell drive cytosolic 1CM involving folate as well as the methionine cycle, which can branch into the trans-sulfuration pathway. Homocysteine (HCY) can be shuttled back into methionine via THF or can participate in the trans-sulfuration pathway to produce cysteine. Cysteine is a precursor of glutathione (GSH), a major regulator of cellular redox status. GSH donates electrons to NADP^+ and becomes oxidized to form GSSG. GSSG can be converted back into GSH in a NADPH-dependent reaction.

Nucleotide biosynthesis

5,10-me-THF is a critical component in nucleotide biosynthesis. In the cytoplasm, 5,10-me-THF is converted by methylenetetrahydrofolate dehydrogenase-1 (MTHFD1) into 10-formyl-THF, from which purines are derived (Figure 1). Indeed, purine synthesis accounts for the largest demand of folate 1C units in mammalian cells, and these units are exclusively provided by 1CM [5]. On the pyrimidine side, mitochondrial 5,10-me-THF is used as methyl donor for the methylation of deoxyuridine monophosphate (dUMP) by thymidylate synthase (TYMS), generating thymidine monophosphate (dTMP) moieties used for DNA synthesis.

Formate synthesis and 1CM recycling

In the mitochondria, a small portion of 10-formyl-THF is used for the formylation of mitochondrial RNA [16]. Nevertheless, the majority of mitochondrial 1C units are destined to fuel cytosolic and nuclear reactions [17]. Therefore mitochondrial 10-formyl-THF must be cleaved by MTHFD1L to generate formate, which can be exported to the cytosol by an unknown mechanism [18]. In the cytosol, MTHFD1 regenerates formate into 10-formyl-THF for *de novo* purine synthesis, and 5,10-methylene-THF for methionine cycle. The folate cycle is

completed in the cytosol by SHMT1 which converts glycine into serine while regenerates THF [19].

An important feature of 1CM is its compartmentalization, mediated by the enzymatic reactions linking serine to formate in the cytosol and mitochondria (Figure 1). This compartmentalization enables not only parallel metabolic processes, but also a full oxidative/reductive cycle in which serine is synthesized in the cytosol but catabolized in the mitochondria [4]. This cycle is driven by the differences in the electrochemical potential between mitochondrial NADH (and also NADPH) and cytosolic NADPH. For instance under physiological condition, MTHFD1 consumes NADPH while MTHFD2L can use both NADH and NADPH. Thus, high cytosolic NADPH/ NADP^+ favors flux from formate toward serine in the cytosol. The importance of these cofactors in 1CM is discussed in the next section.

Roles of NADH and NADPH in 1CM

Driving forces

The enzymatic reactions of 1CM largely depend on balanced pools of the cofactors NAD^+/NADH and $\text{NADP}^+/\text{NADPH}$ [19], which act as key electron donors

[8]. 1CM is thus closely linked to the pathways of cellular respiration that generate these cofactors as they maintain ATP production.

NADPH is considered to be the main regulator of 1CM [20,21]. NADPH is also crucial for regulating cellular ROS generated during mitochondrial OXPHOS [22,23]. NADPH is generated when glycolysis branches into the pentose phosphate pathway (PPP). *De novo* serine synthesis stemming from glycolysis pathways also regenerates NADPH [24]. Inhibiting serine synthesis by PHGDH inhibition dampens 1CM and reduces intracellular levels of NADPH [24,25*].

Within the 1CM network, the conversion of folate to DHF, and that of DHF to THF, occur via NADPH-dependent reactions (Figure 1). Thus, NADPH consumption is a prominent driver of 1CM. However, 1CM also regenerates NADPH. During catabolic metabolism, a high demand for purine synthesis drives the conversion of 5,10-me-THF into 10-formyl-THF via MTHFD1, a reaction that enables the cell to form NADPH from NADP⁺ [26,27]. The importance of MTHFD1 in regenerating NADPH in 1CM has been demonstrated in a study where knockdown of *Mthfd1* significantly reduced NADPH-derived serine [28]. The NADPH needed to support 1CM can also be generated from the processing of 10-formyl-THF into purines, and the oxidation of 10-formyl-THF to CO₂ [17,29] (Figure 1).

NADH is another vital driver of 1CM. The majority of intracellular NADH is generated in the cytoplasm during glycolysis and in the mitochondria during Krebs cycle. *De novo* serine synthesis downstream of glycolysis involves the regeneration of NADH from NAD⁺ in a reaction catalyzed by phosphoglycerate dehydrogenase (PHGDH) and phosphoserine aminotransferase 1 (PSAT1) [30]. In addition, NADH is produced by the Krebs cycle in the mitochondria, providing electrons for electron transport chain (ETC)-mediated ATP production. Mitochondrial NADH is also generated when 5,10-me-THF is converted to 10-formyl-THF, further linking 1CM to the respiratory state of the cell [5]. CRISPR-Cas9 genomic deletion of *Shmt2*, whose product converts THF to 5,10-me-THF in the mitochondria, abrogated the expression and activity of complex I of the ETC, leading to a significant reduction in intracellular ATP and decreased cellular proliferation [31*]. These observations emphasize the close ties between 1CM, cellular respiration, and the preservation of reducing power.

Several lines of evidence reinforce this concept of crosstalk between 1CM and cellular respiration. An early study by Meiser et al. showed that inhibition of complex I of the ETC led to drastic reductions in serine catabolism and formate release [32]. A more recent study by Yang *et al.*

demonstrated that folate-dependent serine catabolism generates substantial amounts of NADH, and that a reciprocal relationship exists between 1CM and cellular respiration [33]. Yang *et al.* showed that, in mice that were deficient for mitochondrial complex I and thus suffered from defective respiration, serine catabolism through MTHFD2L became a major source of NADH to facilitate cell growth [34*]. Similarly, mitochondrial DNA depletion that was induced via doxycycline-triggered expression of DNA polymerase gamma, led to abrogated cellular respiration and resulted in increased expression of ATF4-mediated serine synthesis genes (PHGDH, PSAT1, PSPH), thus generating serine to feed into 1CM [35]. Another study by Nikannen *et al.* further showed that mitochondrial DNA depletion *in vivo*, achieved using mutation of the mitochondrial DNA helicase TWINKLE, promoted the expression of two key enzymes of mitochondrial 1C cycle MTHFD2 and MTHFD1L [36]. Taken together, these studies establish that impaired mitochondrial respiration induces a compensatory response to increase 1CM enzymes, although these changes are insufficient to regain full functionality.

Redox regulation

Although NAD⁺/NADH and NADP⁺/NADPH are crucial players in 1CM, the intracellular pools of these electron donors need to be properly balanced because their disbalance can lead to apoptosis [37]. To this end, the utilization of NADH and NADPH in 1CM is offset by their use in OXPHOS and glycolysis. This balancing act adds another layer of regulatory complexity to the 1CM story: on one hand the transfer of electron from NADH to the electron transport chain during OXPHOS is a major producer of harmful metabolic reactive oxygen species (ROS), whereas 1CM and NADPH are involved in ROS detoxification.

1CM also helps to maintain proper cellular redox status in two ways related to glutathione (GSH), the cell's most abundant antioxidant. First, as noted above, 1CM is involved in the trans-sulfuration cascade that generates cysteine. In a reaction requiring the activity of glutamate cysteine ligase (GCL), cysteine combines with glycine and glutamate to form GSH (Figure 2) [38,39]. Secondly, 1CM maintains the correct NADP⁺/NADPH ratio necessary for consistent GSH regeneration (Figure 2). NADPH is consumed during the reduction of oxidized GSH and thioredoxin, and 1CM generates NADPH needed [40]. Thus, 1C metabolism is critical in the maintenance of cellular redox by fostering the production of cysteine as a precursor for GSH synthesis and by maintaining the NADPH pool for GSH regeneration.

The links between 1CM and T cell function

Cellular metabolism is known to be a critical control mechanism in many cell types that are highly

metabolically active, including immune cells [11]. T cells mediate the cellular adaptive immune response and are defined by surface expression of CD3 molecules or conventional T cell receptors (TCR). T cells that express CD8 are cytotoxic T cells that are critical to defend against intracellular pathogens and tumors. Another important subset is CD4 T cells that are either pro-inflammatory helper T cells (Th cells) that secrete cytokines to instruct specific immune response or suppressive regulatory T helper cells (Tregs) that dampen the immune response. Th cells are further categorized by the expression of lineage specific transcription factors, such as TBET for Th1 cells that produce IFN- γ and TNF, GATA3 for Th2 cells that produce IL-4, and ROR γ T for Th17 cells that produce IL-17 [41]. In contrast, Tregs are defined by the high expression of the IL-2 receptor CD25 and the master transcriptional regulator FOXP3 [42].

Although the importance of cellular metabolism in regulating T cell function is increasingly appreciated, it is only recent that 1CM has been examined in T cells [43]. In this section, we discuss recent studies delineating the important roles of three critical elements of 1CM, namely folate, serine and methionine, in T cell biology.

Folate

As noted in Section (Introduction) and illustrated in Figure 1, 1CM depends to a large extent on the cyclic reduction and oxidation of THF, which receives 1C units donated by serine and glycine [4]. Demand for folate is most acute in cell types with high proliferative potential, such as neural cells, cancer cells and activated T cells [4,44^{**},45]. All of these cell types, including various T cell subsets face a major requirement for nucleotide biosynthesis to support their rapidly expanding biomass (Figure 3a). For example, the mitogen-activated proliferation of human CD8⁺ T cells *in vitro* is impaired by folate deprivation [45]. Among murine CD4⁺ T cells restimulated *in vitro*, folate receptor 4 (FR4) is expressed on CD25⁻ Foxp3⁻ T helper (Th) cells that are more proliferative and produce more IL-2 than on less proliferative IFN γ -producing Th1-like cells. This elevated FR4 expression was found to correlate with CD44⁺CD62L⁺ expression, that is, on CD4⁺ T cells with central memory characteristics [46]. FR4 was expressed on CD44⁺CD62L⁻ CD4⁺ T cells with effector and/or effector memory functions. This same study revealed that FR4 expression was also strongly associated with natural regulatory T cell (nTregs) markers, including Foxp3, CD25, CTLA-4 and GITR, as well as with the T cell activation markers CD44 and CD69. In addition, FR4⁺CD25⁺ T cells expanded much more after antigenic stimulation than did FR4⁻CD25⁺ T cells, suggesting that antigen-specific nTreg survival and expansion rely on FR4. Accordingly, blockade of FR4 by anti-FR4 Fab fragment or complete anti-FR4 antibody enhanced anti-tumor immunity and exacerbated autoimmune disease in young mice [46]. Whether folate

sequestration by Tregs leads to folate depletion in the surrounding environment and thus contributes to regulatory mechanisms remains unclear. Overall, this observation clearly indicates that FR4 is crucial for Treg expansion *in vivo*.

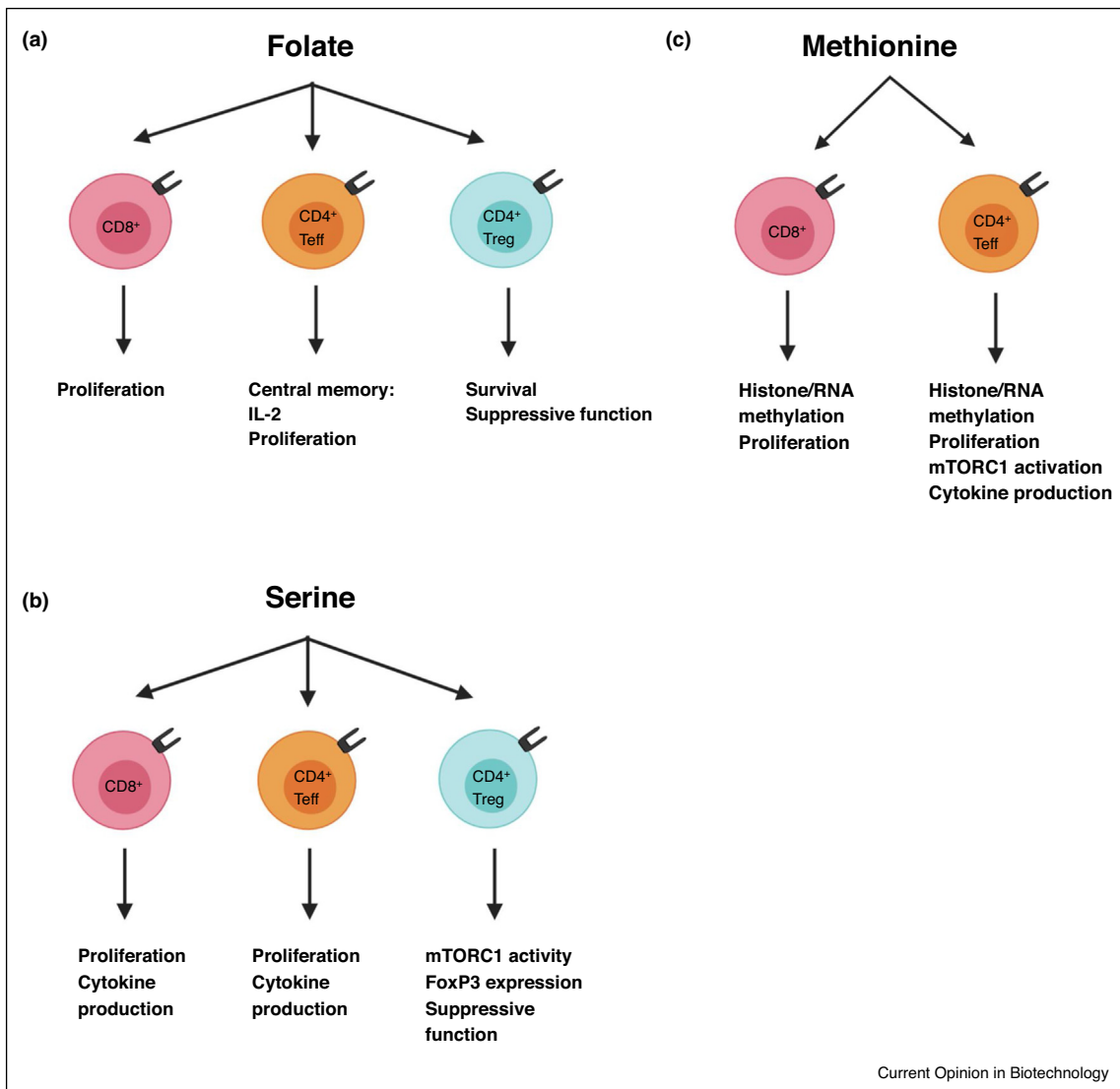
Furthermore, folic acid absorption from dietary sources depends on gut microflora that interact with the immune system [47,48]. Indeed, mice fed on a folate-deficient diet showed selective depletion of nTregs normally resident in the colon or small intestine that was caused by effects on the anti-apoptotic molecules BCL-2 and BCL-XL [49,50]. Thus, folate and 1CM are critical for the functions of rapidly proliferating T cells, especially effector T cells and nTregs.

Serine

As noted above, serine is a non-essential amino acid that fuels 1CM by donating 1C units. Like folic acid, serine also contributes to T cell proliferation. Several studies have demonstrated that the early stages of TCR-dependent T cell activation are accompanied by elevated expression of genes that participate in 1CM [51,52,53^{**}]. Drastic proteome remodeling occurs in the mitochondria of activated T cells that leads to increased expression of SHMT2 [4,52]. Inhibition of SHMT2 in mouse CD4⁺ and CD8⁺ T effector T cells, or culture under serine/glycine-deficient conditions, does not alter their expression of activation markers or cytokine production upon TCR or antigenic stimulation, but these cells exhibit defects in survival and proliferation (Figure 3b) [51,52]. Provision of glycine plus the antioxidant N-acetyl cysteine (NAC), or supplementation with formate, can restore the functions of SHMT2-inhibited T effector cells [51,52]. These observations reinforce the conclusion that serine's participation in 1CM is most pertinent to controlling redox balance and purine synthesis, both of which are critical for effector T cell proliferation.

Although both cancer cells and activated effector T cells are highly proliferative, they differ in their use of 1CM. In cancer cells, Reid *et al.* showed that *de novo* serine synthesis via PHGDH was required even in the presence of abundant extracellular serine, and that inhibition of PHGDH induced alterations in multiple pathways, including decreases in PPP, glycolysis and the TCA cycle [54^{*}]. In contrast, in effector CD8⁺ T cells, neither silencing PHGDH nor removing extracellular serine affected the expression of T cell activation markers such as CD44 and CD25 [55^{**}]. However, either PHGDH inhibition or serine starvation reduced T cell proliferation, and that the blockade of both serine synthesis and uptake completely abrogated proliferation [55^{**}]. These results indicate that, in T cells, while *de novo* serine synthesis or serine uptake may be able to partially compensate for the loss of a single pathway of serine

Figure 3



Support of T cell subset functions by key 1CM intermediates. Folate, serine and methionine are 1CM elements that are critical for T cell proliferation and functions. **(a)** Folate supports CD8⁺ and CD4⁺ effector T cell proliferation and cytokine production, while also promoting the survival and suppressive function of CD4⁺ regulatory T cells. **(b)** Serine supports CD8⁺ and CD4⁺ effector T cell proliferation and cytokine production, and also preserves the metabolism and suppressive function of CD4⁺ regulatory T cells. **(c)** Methionine is mostly important for the histone/RNA methylation that imposes epigenetic regulation in CD8⁺ and CD4⁺ effector T cells.

acquisition, both must operate to maintain optimal T cell expansion. Interestingly, Ma *et al.* showed that effector CD4⁺ T cells cultured without serine/glycine showed a significant reduction in proliferation but normal glycolysis-dependent lactate fermentation and mitochondrial respiration producing ATP [51]. CD4⁺ T cells derived from aged mice showed suboptimal upregulation of mitochondrial protein synthesis upon stimulation, which was attributed to defective upregulation of 1CM enzymes [56]. Accordingly, *in vitro* glycine and formate

supplementation to culture was able to rescue the survival and proliferation of their T cells [56].

GSH is an important regulator of metabolism and cellular redox balance in both effector T cells and Tregs [53^{••},57]. GSH depletion leads to increased ROS, which interferes with T cell signaling at multiple points [58]. Kurniawan *et al.* observed that Tregs genetically deficient for the catalytic subunit of GCL (*Gclc*), which catalyzes GSH synthesis increased both their synthesis and uptake of serine [53^{••}]. As a result, 1CM was stimulated and drove increased Treg

proliferation. Further analysis revealed that this heightened need for serine by these mutant cells was fueled by increases in glycolysis and OXPHOS. This observation indicates that normal Treg expansion depends on serine-driven 1CM, a result in line with findings in CD8⁺ effector T cells [51]. Strikingly, the increased serine levels and 1CM activity in redox-imbalanced Tregs not only increased Treg proliferation but also activated mTORC1 and reduced Foxp3 expression, leading to impaired Treg suppressive function and spontaneous autoimmunity *in vivo* [53**] (Figure 3b). Pharmacologic or genetic inhibition of PHGDH restored normal glycolysis and suppressive function to the mutant Tregs [53**]. Moreover, when mutant mice with a Treg-specific *Gclc* deficiency were fed on a serine-deficient diet, they were protected against developing lethal autoimmunity [53**]. These findings highlight the intertwined nature of serine acquisition, 1CM and ROS regulation *in vivo*. They also affirm the importance of serine in promoting 1CM to support the expansion and functions of effector T cells and Tregs, all vital for normal T cell homeostasis.

Methionine

Methionine is another amino acid that can be synthesized by the 1CM network [17]. As noted above, methionine is an essential methyl donor for DNA, RNA and histone methylation [1]. Ma *et al.* showed that activated T cells increase their expression of genes involved in the methionine cycle, but that these cells do not carry out *de novo* synthesis of methionine from serine or glucose [51]. Thus, the methionine cycle has a non-redundant role in activated T cells.

Upon activation, T cells rapidly take up methionine, which is transferred to SAM, the methyl group donor for methylation processes [59*]. In CD4⁺ T cells, TCR engagement also upregulates methyltransferases associated with H3K4me3 (transactivating) or H3K27me3 (repressive) histone methylation [60] (Figure 3c). Accordingly, T cells deprived of extracellular methionine exhibit decreased RNA methylation, as indicated by decreases in N6 adenosine (m6A) and methyl-cytosine (5mc). Methionine deprivation also leads to reduced histone methylation and impaired cell cycling, proliferation, and/or cytokine production by both CD4⁺ and CD8⁺ effector T cells, but has no impact on expression of Th lineage transcription factors or effector T cell differentiation [59*,60]. H3K4me3 methylation is particularly sensitive to varying concentrations of extracellular methionine, whilst other types of methylation are unaffected by methionine levels [59*]. In mice induced to develop experimental autoimmune encephalomyelitis (EAE), a murine model of human multiple sclerosis, the feeding of a methionine-limited diet leads to milder disease associated with reduced levels of inflammatory cytokines and pathological T cells in the brain [59*]. Furthermore, silencing of *Mat2a*, a gene required for S-adenyl methionine (SAM) required for methylation, reduced abundance of myelin

oligodendrocyte glycoprotein peptide (MOG)-peptide specific pathogenic T cells in spleen and brain upon adoptive transfer in EAE model [59*]. These findings imply that 1CM and methionine are involved in pathological T cell function. Taken together, the data indicate that activated T cells increase their extracellular methionine uptake to enhance the methylation processes required for T cell function without affecting lineage differentiation.

Unanswered questions

Although 1CM in the immune system has been keenly explored over the last decade, the complex mechanisms regulating immune responses and the metabolism of immune cells present significant challenges to pinpointing the role of a specific metabolic checkpoint. Since either understimulation or overstimulation of T cells has deleterious effects on the host, understanding the mechanisms by which 1CM influences immune homeostasis is critical. The fact that there are already intricate links between 1CM and various cellular functions exacerbates these difficulties. Moreover, unlike in other cell types, glucose-fueled serine synthesis and/or uptake does not foster methionine synthesis, indicating that the regulation of 1CM can be cell type-specific. Detailed understandings of the functional links between 1CM and T cell function will thus provide novel mechanistic insights for T cell biology.

Another important issue to be further investigated is how the contributions of 1CM in different compartments (cytosolic versus mitochondrial) affect the regulation of T cell functions. Future studies should also aim to investigate the subset-specific regulation of various 1CM substrates (particularly methionine) in Tregs. In addition, dissecting the roles of the post-translational modifications promoted by 1CM (e.g. histone methylation, acetylation) may reveal an underlying mechanism that regulates functional specification of different T cell subsets.

It has only recently become clear that 1CM is vital in T cells. While defects in 1CM impair effector T cell functions [51,52,56,60], over-activation of 1CM due to impaired redox homeostasis abrogates Treg function and increases pathologic T cell responses [53**]. However, diets depleted of specific amino acids implicated in certain disease settings have been shown to ameliorate symptoms of these conditions. Furthermore, there seem to be subset-specific effects of 1CM on different T cell populations. It is therefore possible that targeted dietary therapy to manipulate 1CM can be exploited to modulate T cell responses in particular disorders.

Conclusion

1CM is instrumental in a wide range of cellular functions, including nucleotide synthesis, cell proliferation,

epigenetic regulation, and redox homeostasis. The 1CM network is reciprocally linked to cellular metabolic reactions such as glycolysis and OXPHOS by various enzymatic products and cofactors, including NADH and NADPH. Dysregulated 1CM has long been associated with tumorigenesis, and more recent evidence highlights a link between 1CM and immune cell functions. In particular, 1CM-related nutrients such as folate, serine and methionine have been implicated in supporting T cell functions. Alterations to 1CM in T cells are associated with various pathologies *in vivo*, including autoimmune diseases and cancer. Thus, a better understanding of 1CM's role in T cells may offer novel opportunities to improve immunotherapies for a broad spectrum of human disorders.

Conflict of interest statement

Nothing declared.

Acknowledgements

We acknowledge the valuable work of all investigators that we were unable to cite due to space limitations. D.B. and H.K. are supported by the FNR-ATTRACT program (A14/BM/7632103), and D.B. by the FNR-CORE (C18/BM/12691266) and the FNR-PRIDE (PRIDE/11012546/NEXTIMMUNE) scheme. The figures are created using Biorender.com.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Clare CE *et al.*: **One-carbon metabolism: linking nutritional biochemistry to epigenetic programming of long-term development.** *Annu Rev Anim Biosci* 2019, **7**:263-287.
 2. Suh E, Choi S-W, Friso S: **One-carbon metabolism.** *Molecular Basis of Nutrition and Aging.* 2016:513-522.
 3. Stover PJ, Field MS: **Trafficking of intracellular folates.** *Adv Nutr* 2011, **2**:325-331.
 4. Ducker GS, Rabinowitz JD: **One-carbon metabolism in health and disease.** *Cell Metab* 2017, **25**:27-42.
 5. Ducker GS, Rabinowitz JD: **One-carbon metabolism in health and disease.** *Cell Metab* 2017, **25**:27-42.
 6. van Gool JD *et al.*: **Folic acid and primary prevention of neural tube defects: a review.** *Reprod Toxicol* 2018, **80**:73-84.
 7. Rosenzweig A, Blenis J, Gomes AP: **Beyond the warburg effect: how do cancer cells regulate one-carbon metabolism?** *Front Cell Dev Biol* 2018, **6**:90.
 8. Newman AC, Maddocks ODK: **One-carbon metabolism in cancer.** *Br J Cancer* 2017, **116**:1499-1504.
 9. Zheng Y, Cantley LC: **Toward a better understanding of folate metabolism in health and disease.** *J Exp Med* 2019, **216**:253-266.
 10. Maddocks ODK *et al.*: **Modulating the therapeutic response of tumours to dietary serine and glycine starvation.** *Nature* 2017, **544**:372-376.
 11. Amelio I, Melino G, Frezza C: **Exploiting tumour addiction with a serine and glycine-free diet.** *Cell Death Differ* 2017, **24**:1311-1313.
 12. Mentch SJ, Locasale JW: **One-carbon metabolism and epigenetics: understanding the specificity.** *Ann N Y Acad Sci* 2016, **1363**:91-98.
 13. Su X, Wellen KE, Rabinowitz JD: **Metabolic control of methylation and acetylation.** *Curr Opin Chem Biol* 2016, **30**:52-60.
 14. Škovierová H *et al.*: **The molecular and cellular effect of homocysteine metabolism imbalance on human health.** *Int J Mol Sci* 2016, **17**.
 15. Eriksson S *et al.*: **Dietary methionine can sustain cytosolic redox homeostasis in the mouse liver.** *Nat Commun* 2015, **6**:6479.
 16. Nilsson R *et al.*: **Metabolic enzyme expression highlights a key role for MTHFD2 and the mitochondrial folate pathway in cancer.** *Nat Commun* 2014, **5**:3128.
 17. Ducker GS *et al.*: **Reversal of cytosolic one-carbon flux compensates for loss of the mitochondrial folate pathway.** *Cell Metab* 2016, **23**:1140-1153.
 18. Brosnan ME, Brosnan JT: **Formate: the neglected member of one-carbon metabolism.** *Annu Rev Nutr* 2016, **36**:369-388.
 19. Yang M, Vousden KH: **Serine and one-carbon metabolism in cancer.** *Nat Rev Cancer* 2016, **16**:650-662.
 20. Tedeschi PM *et al.*: **Contribution of serine, folate and glycine metabolism to the ATP, NADPH and purine requirements of cancer cells.** *Cell Death Dis* 2013, **4**:e877.
 21. Maddocks OD *et al.*: **Serine metabolism supports the methionine cycle and DNA/RNA methylation through de novo ATP synthesis in cancer cells.** *Mol Cell* 2016, **61**:210-221.
 22. Liu CL *et al.*: **Targeting the pentose phosphate pathway increases reactive oxygen species and induces apoptosis in thyroid cancer cells.** *Mol Cell Endocrinol* 2020, **499**:110595.
 23. Koo SJ *et al.*: **Pentose phosphate shunt modulates reactive oxygen species and nitric oxide production controlling trypanosoma cruzi in macrophages.** *Front Immunol* 2018, **9**:202.
 24. De Santis MC *et al.*: **Signaling pathways regulating redox balance in cancer metabolism.** *Front Oncol* 2018, **8**:126.
 25. Bradshaw PC: **Cytoplasmic and mitochondrial NADPH-coupled redox systems in the regulation of aging.** *Nutrients* 2019, **11**.
- This review highlights the importance of NADPH, one of the driving force in one carbon metabolism in cellular aging. NADPH provides the reducing equivalents to antioxidants such as glutathione to protect the cell from ROS toxicity. NADPH depletion is shown to be linked to aging processes.
26. Newman AC, Maddocks ODK: **Serine and functional metabolites in cancer.** *Trends Cell Biol* 2017, **27**:645-657.
 27. Lewis CA *et al.*: **Tracing compartmentalized NADPH metabolism in the cytosol and mitochondria of mammalian cells.** *Mol Cell* 2014, **55**:253-263.
 28. Fan J *et al.*: **Quantitative flux analysis reveals folate-dependent NADPH production.** *Nature* 2014, **510**:298-302.
 29. Piskounova E *et al.*: **Oxidative stress inhibits distant metastasis by human melanoma cells.** *Nature* 2015, **527**:186-191.
 30. Meiser J, Vazquez A: **Give it or take it: the flux of one-carbon in cancer cells.** *FEBS J* 2016, **283**:3695-3704.
 31. Lucas S *et al.*: **Serine catabolism is essential to maintain mitochondrial respiration in mammalian cells.** *Life Sci Alliance* 2018, **1** p. e201800036
- The study shows the importance of one-carbon metabolic apparatus in maintaining cellular respiration. It is shown that the absence of SHMT abrogates cellular respiration, whereas the supplementation of formate (a bona fide 1C donor) restores mitochondrial complex I assembly.
32. Meiser J *et al.*: **Serine one-carbon catabolism with formate overflow.** *Sci Adv* 2016, **2**:e1601273.
 33. Maynard AG, Kanarek N: **NADH ties one-carbon metabolism to cellular respiration.** *Cell Metab* 2020, **31**:660-662.
 34. Yang L *et al.*: **Serine catabolism feeds NADH when respiration is impaired.** *Cell Metab* 2020, **31**:809-821 e6
- The study demonstrates that when cellular respiration is impaired, serine catabolism contributes to toxic NADH accumulation. The authors show

that elevated level of NADH-derived serine catabolism inhibits cell growth. Inhibition of mitochondrial serine catabolism normalizes NADH level and promotes cell growth.

35. Bao XR *et al.*: **Mitochondrial dysfunction remodels one-carbon metabolism in human cells.** *eLife* 2016, **5**.
36. Nikkanen J *et al.*: **Mitochondrial DNA replication defects disturb cellular dNTP pools and remodel one-carbon metabolism.** *Cell Metab* 2016, **23**:635-648.
37. Cui Y *et al.*: **NADPH accumulation is responsible for apoptosis in breast cancer cells induced by fatty acid synthase inhibition.** *Oncotarget* 2017, **8**:32576-32585.
38. McCarty MF, O'Keefe JH, DiNicolantonio JJ: **Dietary glycine is rate-limiting for glutathione synthesis and may have broad potential for health protection.** *Ochsner J* 2018, **18**:81-87.
39. Gould RL, Pazardo R: **Impact of supplementary amino acids, micronutrients, and overall diet on glutathione homeostasis.** *Nutrients* 2019, **11**.
40. Martinez-Reyes I, Chandel NS: **Mitochondrial one-carbon metabolism maintains redox balance during hypoxia.** *Cancer Discov* 2014, **4**:1371-1373.
41. Zhu J, Yamane H, Paul WE: **Differentiation of effector CD4 T cell populations.** *Annu Rev Immunol* 2010, **28**:445-489.
42. Fan MY *et al.*: **Differential roles of IL-2 signaling in developing versus mature tregs.** *Cell Rep* 2018, **25**:1204-1213 e4.
43. Geltink RIK, Kyle RL, Pearce EL: **Unraveling the complex interplay between T cell metabolism and function.** *Annu Rev Immunol* 2018, **36**:461-488.
44. Clare CE *et al.*: **One-carbon metabolism: linking nutritional •• biochemistry to epigenetic programming of long-term development.** *Annu Rev Anim Biosci* 2019:263-287
This review extensively highlights the crucial role of one carbon metabolism and its interlinking metabolic pathways that are central to cellular function, DNA, polyamines and amino acid synthesis, and epigenetic regulation. It is also shown that one carbon metabolism serves as key biochemical link between parental environment and epigenetic regulation of early development.
45. Courtemanche C *et al.*: **Folate deficiency inhibits the proliferation of primary human CD8+ T lymphocytes in vitro.** *J Immunol* 2004, **173**:3186-3192.
46. Yamaguchi T *et al.*: **Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor.** *Immunity* 2007, **27**:145-159.
47. Engevik MA *et al.*: **Microbial metabolic capacity for intestinal folate production and modulation of host folate receptors.** *Front Microbiol* 2019, **10**:2305.
48. Neumann C *et al.*: **c-Maf-dependent T(reg) cell control of intestinal T(H)17 cells and IgA establishes host-microbiota homeostasis.** *Nat Immunol* 2019, **20**:471-481.
49. Kinoshita M *et al.*: **Dietary folic acid promotes survival of Foxp3 + regulatory T cells in the colon.** *J Immunol* 2012, **189**:2869-2878.
50. Kunisawa J *et al.*: **A pivotal role of vitamin B9 in the maintenance of regulatory T cells in vitro and in vivo.** *PLoS One* 2012, **7**:e32094.
51. Ma EH *et al.*: **Serine is an essential metabolite for effector T cell expansion.** *Cell Metab* 2017, **25**:345-357.
52. Ron-Harel N *et al.*: **Mitochondrial biogenesis and proteome remodeling promote one-carbon metabolism for T cell activation.** *Cell Metab* 2016, **24**:104-117.
53. Kurniawan H *et al.*: **Glutathione restricts serine metabolism to •• preserve regulatory T cell function.** *Cell Metab* 2020
The study highlights the role of antioxidant glutathione in modulating serine metabolism to maintain regulatory T cell function. The authors show that glutathione serves as a feedback loop in controlling one carbon metabolism in regulatory T cells. This feedback loop is critical to preserve regulatory T cell function and prevent autoimmunity.
54. Reid MA *et al.*: **Serine synthesis through PHGDH coordinates • nucleotide levels by maintaining central carbon metabolism.** *Nat Commun* 2018, **9**:5442
This study highlights the importance of de novo serine synthesis via PHGDH even when serine uptakes are highly accessible. Inhibition of serine synthesis disrupts the mass balance within central one carbon metabolism.
55. Ma EH *et al.*: **Metabolic profiling using stable isotope tracing •• reveals distinct patterns of glucose utilization by physiologically activated CD8(+) T cells.** *Immunity* 2019, **51**:856-870 e5
The study shows that immune aging is linked to one carbon metabolism. The authors compare the proteome of T cells between young and old mice. They show that aged T cell exhibit deficit in the induction of enzymes of one carbon metabolism. Boosting one carbon metabolism enhances the activation of aged T cells.
56. Ron-Harel N *et al.*: **Defective respiration and one-carbon metabolism contribute to impaired naïve T cell activation in aged mice.** *Proc Natl Acad Sci U S A* 2018, **115**:13347-13352.
57. Mak TW *et al.*: **Glutathione primes T cell metabolism for inflammation.** *Immunity* 2017, **46**:675-689.
58. Franchina DG, Dostert C, Brenner D: **Reactive oxygen species: involvement in t cell signaling and metabolism.** *Trends Immunol* 2018, **39**:489-502.
59. Roy DG *et al.*: **Methionine metabolism shapes T helper cell • responses through regulation of epigenetic reprogramming.** *Cell Metab* 2020, **31**:250-266.e9
The study shows that methionine metabolism, which is the interlinking metabolic pathways in one carbon metabolic network, is important for T helper cell response. Methionine shapes epigenetic reprogramming in T helper cell and support the inflammatory T cell function.
60. Sinclair LV *et al.*: **Antigen receptor control of methionine metabolism in T cells.** *eLife* 2019.