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Peeters, Marlies J.W.; Desler, Claus; Thor Straten, Per

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Mitochondrial-linked de novo pyrimidine synthesis as a regulator of T cell responses

Marlies J. W. Peeters1,*, Claus Desler2, Per thor Straten1,3

Abstract
It has been well established that the metabolism of T cells is integral to their functionality. If a T cell cannot generate enough energy or building blocks, it will not be able to exert its cytotoxic properties to eliminate pathogens and cancer cells. Impairment of mitochondrial oxidative phosphorylation is a well-known disruptor of T cell activation. Dihydroorotate dehydrogenase (DHODH) is a rate-limiting component of the de novo synthesis of pyrimidines and its activity is dependent on functional mitochondrial oxidative phosphorylation. In this regard, DHODH inhibitors have long been used in clinical settings for the treatment of autoimmune diseases, as they potently inhibit lymphocyte proliferation. The exact mode-of-action of these inhibitors in T lymphocytes is not yet exactly understood. In this review, we briefly discuss the critical role of mitochondria in T cell functionality. We also describe how de novo pyrimidine biosynthesis is linked to mitochondrial activity. Finally, we summarize our current knowledge of how mitochondrial-linked de novo pyrimidine biosynthesis modulates T cell responses.

Keywords: T cell metabolism, T cell activation, de novo pyrimidine synthesis, mitochondrial respiration and oxidative respiration

Abbreviations: DHODH: dihydroorotate dehydrogenase, ETC: electron transport chain, OXPHOS: oxidative phosphorylation, AKT: Protein kinase B, IL: Interleukin, MHC: major histocompatibility complex, mTOR: mammalian Target Of Rapamycin, PI3K: Phosphoinositide 3-kinase, SRC: spare respiratory capacity, TCR: T cell receptor

1. Introduction
T cells play a vital role in the establishment of the cellular immune response. This CD4+ and CD8+ T cell-mediated immunity protects a living organism from current and subsequent attacks from various intracellular and extracellular pathogens. On T cell receptor (TCR) triggering by peptide-major histocompatibility complex (MHC) complexes, activated T cells will rapidly expand and differentiate during the clonal expansion phase. Following pathogen clearance, the majority of effector T cells will die via apoptosis during the contraction phase. Finally, a small amount of memory T cells persists for protection against subsequent attacks of the same pathogen (Figure 1A). During these phases, the metabolic activity in T cells adapts rapidly to fulfill their bioenergetic and biosynthetic demands.

In this regard, naïve T cells have a relative dormant metabolism and rely mainly on mitochondrial oxidative phosphorylation (OXPHOS). On TCR activation, the clonal expansion phase results in a massive rewiring in metabolism. This is the Phosphoinositide 3-kinase (PI3K), Protein kinase B (AKT), and mammalian Target Of Rapamycin (mTOR)

pathways drastically increase their activity following TCR and costimulatory signals [1]. Effector T cells will also engage aerobic glycolysis and increase their glucose uptake to fulfill their expanding needs (Figure 1A). Following the contraction phase, memory T cells remain. The persistence of memory T cells relies mainly on mitochondrial OXPHOS and fatty acid oxidation.

In this review, we will briefly discuss the critical role of mitochondria in T cell activation, proliferation, differentiation, and memory responses. This dependence on mitochondrial respiration occurs through direct mitochondrial functions, as well as indirect functions as mitochondria provide the building blocks for other metabolic pathways. In this respect, we will describe how de novo pyrimidine biosynthesis is linked to mitochondrial activity. We acknowledge that other factors affecting the regulation and biogenesis of the mitochondria, as well as other aspects of bioenergetics also regulate T cell activation, but these are outside the scope of this review. Finally, we summarize our current knowledge of how mitochondrial-linked de novo pyrimidine biosynthesis modulates T cell responses.

2. Mitochondrial activity in T cells
2.1 T cell activation and proliferation
Within the first hours of TCR triggering and thus T cell activation, there is a strong mTORC1-mediated increase of mitochondrial mass and mitochondrial DNA levels, both indispensable for T cell activation [2–3]. Correspondingly, this metabolic rewiring results in higher rates of mitochondrial OXPHOS and metabolic activity in activated T cells. Another component is mitochondrial reactive oxygen species, which acts as an essential signaling molecule during T cell activation [4]. Moreover, inhibition of the mitochondrial electron transport chain (ETC) abrogates proliferation and cytokine production upon TCR-mediated activation [4–7]. Next to directly modulating T cell activation, mitochondria also serve to provide building blocks to various cellular processes that aid proliferation and effecter functions. Although not yet fully understood, this increases the lysosomal
function, production of substrates for one-carbon metabolism, and providing NAD+ for de novo aspartate synthesis \[^{[3-5,8]}\]. Collectively, mitochondria exert both direct and indirect effects, both essential for T cell activation and subsequent proliferation.

### 2.2 T cell differentiation and memory

Not only do mitochondria influence initial activation, they also influence the differentiation of T cells (Figure 1B). For instance, glycolysis promotes differentiation into more inflammatory T cell subsets, like Th1 and Th17 \[^{[8]}\]. In contrast, high OXPHOS promotes differentiation into regulatory T cells \[^{[9,10]}\]. On the CD8+ T cell side, terminally differentiated effector cells destined for apoptosis are more glycolytic \[^{[7]}\]. Memory T cell survival is accompanied by mitochondrial remodeling \[^{[11]}\]. As these memory cells need to be able to respond swiftly to secondary challenges, they possess a high spare respiratory capacity (SRC) \[^{[12]}\]. This allows memory T cells to proliferate and produce cytokines quickly upon re-exposure.

### 3. Mitochondrial-linked de novo pyrimidine biosynthesis in T cells

Resting, naïve T cells are not proliferating outside of regular maintenance. In this state, T cells rely on nucleotide salvage pathways for their nucleotide demands. After T cell activation, cells need...
to proliferate rapidly and as a result, their nucleotide demand increases. Due to an upregulation of the de novo pathway, pyrimidine pools increase eight-fold, while purine pools increase two-fold upon T cell activation \[13\]. To satisfy this increased nucleotide demand, activated T cells need to generate purines and especially pyrimidines via de novo synthesis pathways.

**3.1 De novo pyrimidine biosynthesis pathway**

The flavoenzyme dihydroorotate dehydrogenase (DHODH) is located on the outer leaflet of the inner mitochondrial membrane, connected to the inner membrane space by a single transmembrane domain. De novo pyrimidine synthesis ultimately leads to production of uridine monophosphate nucleotides (UMP) which is further converted to metabolic intermediates that fuel synthesis of RNA, DNA, but also serve as essential precursors in the synthesis of phospholipids, glycolipids, and glycoproteins of the plasma membrane \[14\]. The enzyme catalyzes the conversion of dihydroorotate to orotate by oxidation \[15\]. This makes DHODH an essential step in the de novo pyrimidine synthesis. Following the conversion of orotate into UMP, UMP can be further converted into UTP and CTP, and finally, the nucleotide triphosphates dTTP and dCTP (Figure 2). DHODH is the only enzyme in this pathway located in the mitochondria. DHODH likely associates with complex III of the ETC and its activity is coupled to ubiquinol reduction to ubiquinone, which is an active component of the ETC \[15,16\]. Absence or dysfunction of mitochondrial ETC activity causes a lack of ubiquinone and thus dysfunctional DHODH catalysis. Taken together, DHODH activity is fully dependent on a functional and active mitochondrial ETC.

Two of the most well-known DHODH inhibitors, leflunomide and brequinar, inhibit DHODH activity by blocking the interaction between ubiquinone and DHODH \[17\]. DHODH inhibition subsequently results in a dysfunctional de novo synthesis of pyrimidines. As a consequence, RNA and DNA synthesis are inhibited leading to a cell cycle arrest \[13\]. Treatment of cells with uridines usually completely restores proliferation, underscoring the importance of functional de novo pyrimidine biosynthesis.

**3.2 De novo pyrimidine biosynthesis in T cells**

DHODH inhibitors have long been used in patients, where they reduce the severity of multiple sclerosis and rheumatoid arthritis due to their immunosuppressive properties. As T cells proliferate rapidly upon antigen recognition, both in regular immune responses as in autoimmunity, their demand for de novo pyrimidine biosynthesis is high. This would make DHODH inhibition an effective inhibitor of T cell proliferation. Indeed, many studies find that T cell proliferation is compromised following inhibition of de novo pyrimidine biosynthesis (overview in Table 1).

In 1994, it was discovered that brequinar markedly inhibited expression of Interleukin (IL)-2 upon human T cell activation, due to a lack of cell cycle progression into S phase \[27\]. One year later, leflunomide was discovered to have similar effects \[30\]. Not long thereafter, it was suggested that these inhibitors affected pyrimidine synthesis in proliferating T cells \[26,30\]. Indeed, addition of the pyrimidine uridine could restore T cell functionality \[19,28–30\]. In contrast, other studies have suggested that addition of uridine could not completely restore T cell functionality, suggesting that there could be pyrimidine-independent effects \[24,25,28\]. Leflunomide and teriflunomide has been demonstrated to preferentially target ATP synthase and thereby directly inhibit the ETC \[31\].

Functionally, de novo pyrimidine biosynthesis plays various reported roles in T cells. Overall, inhibition of this pathway strongly inhibits T cell proliferation \[11,19,21,29\]. In contrast, expression of effector molecules like interferon-gamma and granzyme

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**Figure 2. Overview of the de novo pyrimidine biosynthesis pathway.** (I–IV) Electron transport chain complexes I to IV. (V) ATP synthase. DHODH converses dihydroorotate into orotate. Following the conversion of orotate into uridine monophosphate nucleotides, UMP can be further converted into UTP and CTP, and finally, the nucleotide triphosphates dTTP and dCTP. dCTP: Deoxycytidine triphosphate, dTTP: Deoxythimidine triphosphate, DHODH: Dihydroorotate dehydrogenase, UMP: Uridine monophosphate, UTP: Uridine 5’-triphosphate.
B by activated but nonproliferating T cells is not affected [18,23,29]. Klotz et al [18] moreover reported decreased TH1 differentiation, in line with earlier results where TH2 differentiation was favored during DHODH inhibition [18,22]. CD8+ T cells failed to differentiate into memory cells upon inhibition of pyrimidine biosynthesis [23]. Interestingly, in a human family lacking CTP synthase 1, T cells fail to proliferate resulting in severe immunodeficiency, while TCR signaling remains unaffected [20]. Taken together, pyrimidine synthesis modulates T cell proliferation and thus differentiation, but does not affect effector functions on a per cell basis.

Next to affecting proliferation, various studies found that DHODH inhibition interfered with mitochondrial OXPHOS, although only one of these studies was focused on T cells [13,31,32]. In the study from Klotz et al [18] DHODH inhibition resulted in decreased mitochondrial respiration, both in basal and maximal respiration. These results could be due to toxicity of the DHODH inhibitor used, as concentrations used in this study halved the percentage of viable cells. Various other studies reported only a cytostatic (ie, proliferation arrest), but not a cytotoxic effect of DHODH inhibition [19,22,29]. As the mitochondrial ETC does not functionally depend on the DHODH enzyme, OXPHOS is unlikely to be affected by DHODH inhibition [15]. Indeed, we showed that mitochondrial respiration was unaffected when using nontoxic amounts of brequinar [29]. In addition, although pyrimidine supplementation could rescue T cell proliferation, we did not see a full depletion of pyrimidine or purine levels. Collectively, this leads us to believe that other pathway-related metabolites regulate T cell proliferation, instead of the direct availability of pyrimidines. This underscores the need for more studies into the mechanisms of the OXPHOS-related De novo pyrimidine pathway in T cells.

4. Conclusions and perspectives

The general importance of various metabolic pathways in T cells are now firmly proven. Clearly, mitochondria are more than just the powerhouse of the cell. They control T cell activation, proliferation, differentiation, and memory formation. Disturbances in mitochondrial activity can compromise both short-term and long-term immune responses in many ways. To be able to control mitochondrial metabolism to reinvigorate T cells, more knowledge on why mitochondria are important for T cells is needed. This increased knowledge could correct autoimmune diseases and chronic inflammation, as well as counteract T cell exhaustion in cancer.

De novo pyrimidine synthesis is strongly intertwined with OXPHOS through the mitochondrial location of DHODH. Pinpointing the mechanism behind DHODH's strong effect on T cell functionality, will potentially open new avenues for the modulation of their metabolic fitness. We excitingly await future studies that shed light on the integral functions of mitochondria in T cells.

Conflicts of interest

The authors declare no conflicts of interest.
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References


