

Mitochondrial Metabolism in T Cell Activation and Senescence: A Mini-Review

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Key Words

T cells · Aging · Mitochondria · Metabolism

Abstract

The aging immune system is unable to optimally respond to pathogens and generate long-term immunological memory against encountered antigens. Amongst the immune components most affected by aging are T lymphocytes. T lymphocytes are cells of the cell-mediated immune system, which can recognize microbial antigens and either directly kill infected cells or support the maturation and activation of other immune cells. When activated, T cells undergo a metabolic switch to accommodate their changing needs at every stage of the immune response. Here we review the different aspects of metabolic regulation of T cell activation, focusing on the emerging role of mitochondrial metabolism, and discuss changes that may contribute to age-related decline in T cell potency. Better understanding of the role of mitochondrial metabolism in immune cell function could provide insights into mechanisms of immune senescence with the potential for developing novel therapeutic approaches to improve immune responses in aged individuals.

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Introduction

Aging of the immune system is a major risk factor for increased morbidity and mortality of the elderly. This gradual deterioration occurs with natural age advancement and thus affects the entire population. The aging immune system is unable to optimally respond to pathogens and generate long-term immunological memory against encountered antigens. Thus, aged people are less likely to benefit from an active vaccination than younger people, and will be at greater risk upon pathogen exposure [1]. Amongst the immune system components most affected by aging are T lymphocytes, cells of the cell-mediated immune system which can recognize microbial antigens and either directly kill infected cells or help phagocytes kill microbes. Age-related T cell dysfunction is attributed, in part, to the reduced generation of new naïve T cells due to thymic involution, and to the inability of the existing naïve T cells to properly activate. The defect in T cell activation results from an age-dependent accumulation of immunosuppressive cells that repress T cell ability to activate, as well as T cell intrinsic dysfunction [2, 3]. One potential mechanism connecting aging and immune dysfunction in aging is highlighted by the emerging linkage between metabolism and immune cell responses.

Recent work has identified intracellular metabolic pathways as central regulators of T cell function [4, 5] (fig. 1).

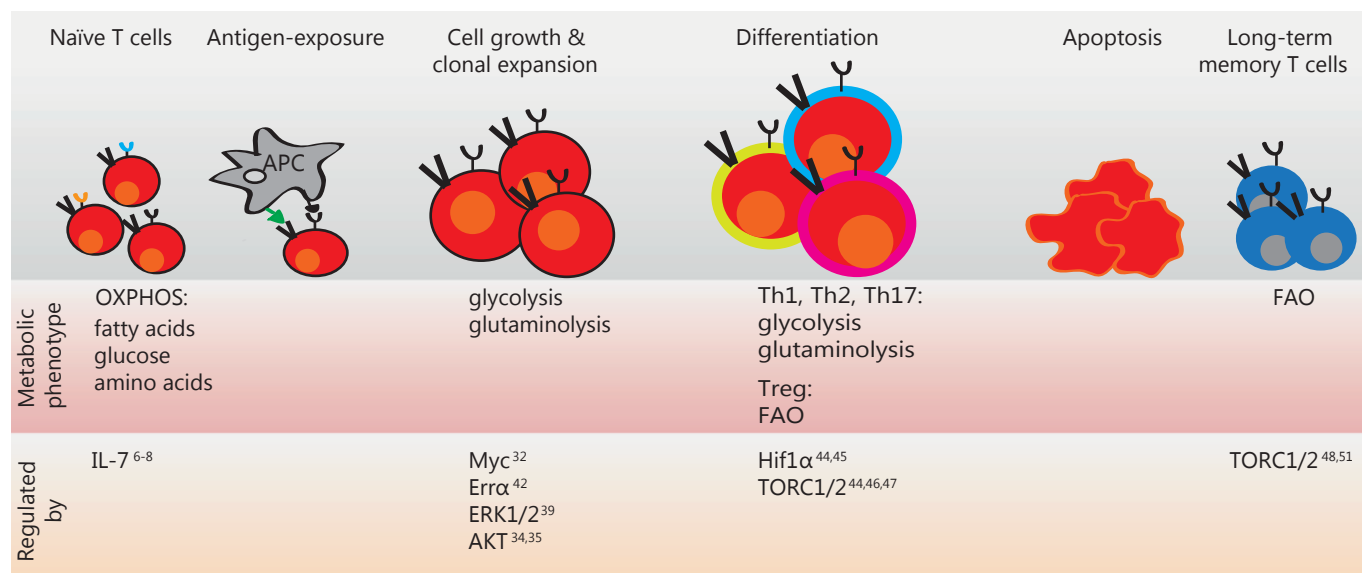


Fig. 1. In the course of the immune response, T cells undergo metabolic shifts to accommodate their changing metabolic and bioenergetics requirements at every stage. The schematic specifies the different stages of T cell-mediated immune response, with the metabolic phenotype at each stage. The bottom panel lists the dif-

ferent transcription factors/signaling molecules shown to regulate those metabolic switches, with the relevant reference. OXPHOS = Oxidative phosphorylation; FAO = fatty acid oxidation; APC = antigen presenting cell.

Upon activation, T cells undergo rapid cell growth, followed by massive proliferation and differentiation to effector cell lineages, as dictated by the specificities of the imposed threat. Most of these effector T cells die shortly after pathogen clearance, whereas a small population of T cells become memory T cells that respond faster and better upon subsequent exposure to the inciting pathogen. Transition between the different phases of the T cell response is accompanied by metabolic reprogramming to comply with the cell's specific needs at each phase [4, 5] (fig. 1). In this review we discuss the major metabolic pathways regulating T cell function during the different stages of an immune response, from the early events during the initial stimulation of naïve T cells to long-term memory formation. Finally, we speculate how impaired mitochondrial metabolism could contribute to age-related deterioration of T cell immunity.

Metabolic Regulation of the Naïve T Cell Pool

T lymphocytes can be divided to different subpopulations with distinct functions. CD4⁺ T helper cells (Th), as suggested by their name, support the functions

of other immune cells including B cells (which can become antibody-producing cells), macrophages, and T cells. CD8⁺ cytotoxic T cells (Tc) kill cells that express foreign antigens, such as cells infected by viruses or other microbes that replicate intracellularly. In the young, naïve T cells can metabolize glucose, amino acids, and lipids to catabolically fuel ATP generation in the mitochondria [6] (fig. 2). T cells are constantly exposed to high nutrient availability in the circulation; thus, the metabolic state of naïve T cells is regulated by the availability of trophic signals rather than by the availability of nutrients. These trophic signals include signals downstream of the T cell receptor (TCR) [7] and homeostatic cytokines, such as IL-7 [8]. Weak TCR signaling is needed to maintain expression of the glucose transporter Glut-1 [7]. Similarly, IL-7 regulates glucose uptake and glycolysis through activation of various glycolytic enzymes mediated by the Jak/stat5 pathway, and by inducing a low and sustained activation of Akt [8]. In the absence of these necessary cell extrinsic signals, T cells are unable to maintain sufficient glucose uptake and metabolism, causing a reduction in mitochondrial membrane potential and cellular ATP, and leading to cell death [7].

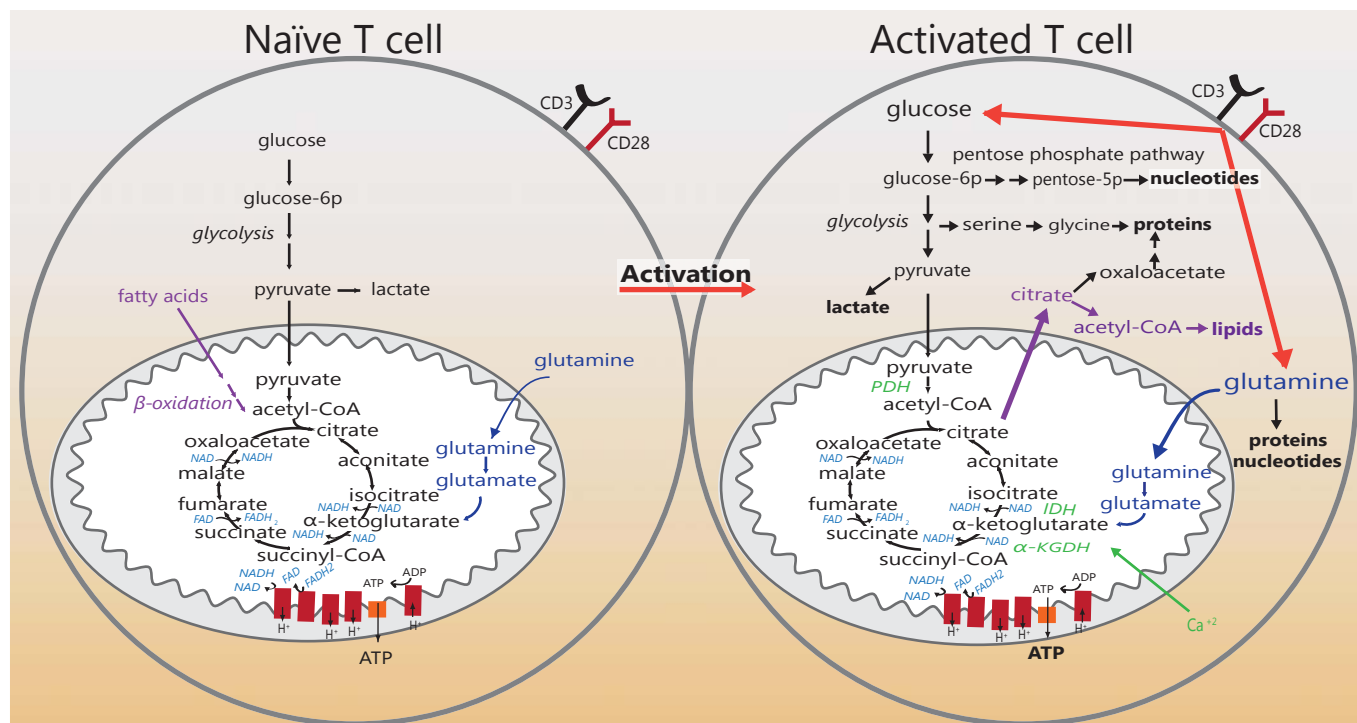


Fig. 2. A schematic describing the metabolic shift that accompanies the transition from a naïve T cell to a fully activated, highly proliferative, T cell. In brief: the main fuel of naïve cells (left panel) is derived from oxidative phosphorylation of low amounts of glucose, amino acids, and fatty acids. Most of the carbon is fully oxidized in the mitochondria. The activated cells (right panel) dra-

matically increase their glucose and glutamine metabolism. A large percentage of the carbon derived from these metabolites is being used for synthesis of proteins, lipids, and nucleic acids. IDH = Isocitrate dehydrogenase; α -KGDH = α -ketoglutarate dehydrogenase.

Metabolism during Early T Cell Activation: The Role of Mitochondria in Ca^{+2} Buffering, Energy Production, and Reactive Oxygen Species Signaling

Proper activation of a naïve T cell requires two signals received from the antigen-presenting cell. The first signal is delivered by binding of cognate antigen presented by major histocompatibility complex molecules on the antigen-presenting cell to the TCR/CD3 complex. The second signal, which is also referred to as the 'costimulatory signal', involves various receptors whose activation will determine whether the T cell will become fully activated or anergic [9, 10]. Activation of naïve T cells initiates an immediate burst of cytosolic Ca^{+2} that is released from the endoplasmic reticulum in response to binding of inositol triphosphate (IP3) to its receptor, IP3R, on the endoplasmic reticulum (reviewed in [11]). Elevated Ca^{+2} levels in the cytoplasm trigger the activation of key transcriptional factors that regulate T cell responses, including the nu-

clear factor of activated T cells (NFAT1), and NF- κ B [12, 13]. Ca^{+2} activates these pathways in a highly specific fashion, determined by the frequency of the Ca^{+2} spikes [12–14]. The effective long-lasting activation of this Ca^{+2} -triggered signaling requires Ca^{+2} influx from the extracellular space, mainly through calcium release-activated channels (CRACs). In contrast to IP3 signaling that declines within minutes, CRACs can be open for hours by sustained TCR engagement [11].

Recent studies have demonstrated that CRACs tend to accumulate at the immune synapse [15], in the vicinity of mitochondria. It has been suggested that Ca^{+2} entry through CRACs may drive mitochondrial translocation to the immune synapse. A small increase in cytosolic Ca^{+2} concentration initiates mitochondrial motility, whereas the increased Ca^{+2} concentration in the vicinity of the plasma membrane and the immune synapse inhibits mitochondrial motility and causes their on-site accumulation [16, 17]. At the immune synapse, mitochondrial Ca^{+2}

uptake prevents Ca^{+2} -dependent inactivation of the channels, and thereby supports sustained Ca^{+2} -dependent signaling [16–19]. In addition, Ca^{+2} uptake has a direct effect on mitochondrial function [20, 21]; Ca^{+2} directly activates key mitochondrial enzymes, causing an increase in tricarboxylic acid (TCA) cycle flux (reviewed in [22]).

Among the mitochondrial enzymes that are activated by Ca^{+2} , although not specifically shown in T cells, are pyruvate dehydrogenase (PDH) [23, 24], isocitrate dehydrogenase [25], and α -ketoglutarate dehydrogenase [26]. PDH catalyzes the entrance of pyruvate-derived carbon into the mitochondrial TCA cycle, through conversion of pyruvate and CoA to acetyl-CoA and CO_2 , in conjunction with reduction of NAD^+ to NADH. Acetyl-CoA is then further metabolized in the TCA cycle. PDH activity is rate limiting for glucose oxidation by the mitochondria, and therefore directly affects the rate of mitochondrial respiration and ATP production [27]. Isocitrate dehydrogenase is a TCA cycle enzyme that catalyzes the oxidative decarboxylation of isocitrate to produce α -ketoglutarate and CO_2 , while reducing NAD^+ to NADH [22]. α -Ketoglutarate dehydrogenase, another TCA cycle enzyme, converts α -ketoglutarate and CoA to succinyl-CoA and CO_2 . Similarly to the two other enzymes, this reaction also generates NADH from NAD^+ [22]. The increased flux through the mitochondrial TCA cycle generates NADH and FADH_2 , which are used as electron donors in the electron transport chain, thus coupling increased metabolite oxidation in the TCA cycle to increased mitochondrial respiration and ATP production (fig. 2).

Although mitochondrial function in early naïve T cell activation has not been studied in detail, it has been shown that mitochondrial reactive oxygen species (ROS) produced by the electron transport chain is required for proper T cell activation, as evidenced by studies using the mitochondrial complex I inhibitor, rotenone, which reduced IL-2 production by activated Th cells [28]. It was suggested that complex I-derived mitochondrial ROS may facilitate cytokine production through activation of AP-1 and NF- κ B [28]. A more recent study, however, argued that these effects could be explained by the inhibition of complex III that is downstream of complex I in the electron transport chain by using mice carrying a targeted mutation in Rieske iron-sulfur polypeptide 1 (Uqcrrs1), a subunit of the ubiquinol-cytochrome c reductase complex (complex III). Mitochondrial ROS generated in complex III in T cells was significantly reduced in this strain, without affecting T cell viability [29]. This Uqcrrs1 mutant mouse showed that complex III-derived ROS is needed for antigen-specific T cell expansion and IL-2

production [29]. In addition, these mitochondrial ROS-deficient T cells failed to induce NFAT1 translocation to the nucleus, resulting in reduced IL-2 production and impaired activation [29]. Taken together, these studies demonstrate that mitochondria play a key role in the early signaling events that control T cell activation. Increased Ca^{+2} influx that is maintained by the mitochondria at the immune synapse as well as mitochondrial ROS production both induce the activation of key transcription factors that regulate T cell function.

Mitochondrial Metabolism in T Cell Growth and Clonal Expansion

Successful initial activation of naïve T cells leads to a phase of rapid cell growth, followed by massive clonal expansion, with increased energy demand. Compared to naïve T cells, stimulated T cells dramatically increase the activity of the nonoxidative arm of glucose catabolism, with the bulk of glucose-derived carbon being released as lactate [4, 5] (fig. 2). A similar switch to glycolytic metabolism in highly proliferative cells despite the availability of sufficient oxygen was described in tumor cells and is known as the ‘Warburg effect’ [30]. In activated T cells, similar to proliferating tumor cells, induced glycolysis promotes biosynthesis. For example, many of the glycolysis intermediates are further processed by enzymes of the pentose phosphate pathway for synthesis of nucleic acids and NADPH, which is an important reducing agent in many anabolic reactions, and used for reduction of glutathione [31, 32].

The transition from catabolic to anabolic metabolism in the activated T lymphocytes is also characterized by a significant repression of mitochondrial fatty acid oxidation [31], making fatty acids available for lipid synthesis (fig. 2). In addition, the mitochondria supply precursors that can be utilized by biosynthetic pathways. For example, the TCA cycle intermediate citrate is exported from the mitochondria to the cytosol where it is used for lipid synthesis (fig. 2). One consequence of the use of TCA cycle intermediates for biosynthesis is the need to constantly replenish the TCA cycle (anaplerosis). In activated T cells (and tumor cells), anaplerosis is accomplished, in part, by glutamine. Glutamine is deaminated in the mitochondria to generate α -ketoglutarate, which enters the TCA cycle through the activity of α -ketoglutarate dehydrogenase (fig. 2). In addition to its anaplerotic role in activated T cells, glutamine contributes to the biosynthesis of polyamines, and glutamine deprivation inhibits T cell growth and proliferation [32, 33].

Thus, the metabolic reprogramming in the activated T cells provides the building blocks and reducing power needed for synthesis of biomass.

Signaling Pathways Regulating Metabolism in Activated T Cells

The same mechanisms that drive the Warburg effect in cancer cells also appear to regulate T cell activation. Stimulation of the TCR together with CD28-dependent costimulation in naïve T cells triggers the activity of phosphatidylinositol 3'-kinase, which, through conversion of the membrane lipid PIP2 to PIP3, leads to the activation of the serine-threonine kinase, Akt [34, 35]. Importantly, Akt activity in stimulated T cells is much higher than its activity in the naïve, 'resting' T cells. Akt stimulates glycolysis by promoting surface expression of the glucose transporter Glut-1 [34, 36], and enhancing the activity of the glycolytic enzymes phosphofructokinase and hexokinase [37, 38]. Hexokinase is further induced by Erk, in response to TCR activation [39]. Akt also activates mammalian target of rapamycin (mTOR) signaling to promote protein synthesis [40], and ATP citrate lyase to promote lipid synthesis [41].

The robust metabolic changes after T cell activation are orchestrated by a global change in the cell metabolic transcriptome, of which Myc is a known master regulator [32]. Studies using a T cell-specific conditional KO of Myc showed that activation-dependent glycolysis, glutamine oxidation, and biosynthesis were all defective in T cells in the absence of Myc [32]. Interestingly, mitochondrial pathways were not strongly affected by Myc deficiency: activation of Myc-deficient T cells resulted in reduced lipid oxidation and increased oxygen consumption, similar to WT cells [32].

The orphan nuclear receptor, estrogen-related receptor- α (Err α), is another transcription regulator that plays a role in the metabolic transition upon T cell activation. Peripheral T cells in Err α KO mice failed to accumulate effector/memory phenotypes and remained phenotypically naïve [42]. Metabolically, Err α inhibitors repressed electron transport genes and altered genes involved in glucose metabolism. Interestingly, Err α inhibition reduced expression of dihydrolipoamide S-acetyl-transferase (DLAT), a component of the PDH complex, and induced expression of the PDH inhibitor, pyruvate dehydrogenase kinase-I (PDK-1). Together, those changes could reduce pyruvate flux into the mitochondrial TCA cycle [42]. Further work is needed to determine the role

of mitochondria in the malfunction of T cells in Err α -deficient mice, yet those studies suggest that mitochondrial activation and fuel utilization are important for proper T cell activation.

Metabolic Regulation of T Cell Differentiation and Cytokine Production

Naïve CD4⁺ T cells can differentiate into a variety of subsets including Th1, Th2, Th17, and induced regulatory T cells (iTreg). These different subsets differ in the cytokines they produce and in their functions in host defense. Th1 cells secrete high levels of IFN γ and mediate defense against intracellular microbes. Th2 cells secrete high levels of IL-4, IL-5, and IL-13, and mediate defenses against helminthes. Th17 cells produce IL-17 and IL-22 and defend against extracellular bacteria and fungi. iTreg cells suppress immune responses. These specific T cell subsets possess different metabolic characteristics, and metabolic pathways play a central role in regulation of T cell differentiation.

Effector cells (Th1, Th2, and Th17) rely mainly on oxidative glycolysis and glutaminolysis, while iTreg cells depend on lipid oxidation [43] (fig. 1). Addition of fatty acids to culture media of CD4⁺ T cells during initial activation selectively repressed Th1, Th2, and Th17 related cytokines and induced differentiation into iTreg [43]. Similarly, administration of 2-DG, a common inhibitor of glycolysis, inhibited Th17 differentiation and promoted iTreg generation [44]. The transcription factor hypoxia-inducible factor 1 α (Hif1 α) was shown to play a central role in promoting glycolysis in Th17 cells [44]. In agreement, lack of Hif1 α diminished Th17 differentiation and enhanced iTreg generation. Hif1 α further promoted Th17 differentiation by directly interacting with the IL-17 promoter, thereby regulating Th17 signature gene expression [45].

Differentiation of naïve CD4⁺ T cells into Th1, Th2, and Th17 cells also involves the kinase mTOR. mTOR is a central regulator of metabolism that translates environmental cues such as amino acids, insulin, and growth factor concentrations into the metabolic outcome. mTOR forms two distinct signaling complexes known as mTORC1 and mTORC2. Interestingly, the mTORC1-signaling complex specifically regulates differentiation into Th1 and induced-Th17 subtypes, whereas differentiation to Th2 as well as natural Th17 cells requires signaling through the mTORC2 complex [46, 47]. Activation of the mTOR pathway is further required for Hif1 α induction during TH17 differentiation [44].

Metabolic Regulation of Memory Cell Formation

Generation of long-lasting memory T cells is a key feature of adaptive immunity. Existence of immunological memory ensures that subsequent exposure to a specific microbial threat will evoke a more rapid and effective immune response. Studies that characterize the metabolic phenotype of memory cells have focused on CD8⁺ memory T cells. Memory CD8⁺ T cells, similar to naïve T cells and induced regulatory T cells, rely on fatty acid oxidation as their source of energy. This makes sense as memory T cells, similar to naïve T cells, are in a 'resting' state in contrast to the highly proliferative state of effector T cells. Pearce et al. [48] connected reduced fatty acid metabolism in TNF receptor-associated factor 6-deficient mice to impaired memory cell formation. Administration of the antidiabetic drug metformin restored fatty acid oxidation and CD8 memory T cell formation in TNF receptor-associated factor 6-deficient mice [48]. A later study demonstrated that memory cells rely on fatty acid oxidation to maintain high mitochondrial spare respiratory capacity levels [49]. In addition, memory CD8⁺ T cells were shown to have increased mitochondrial mass compared to naïve T cells, which supports their ability to rapidly reactivate upon reexposure to their specific antigen [50]. The mTOR pathway was shown to regulate memory T cell differentiation. Administration of rapamycin (an inhibitor of the mTORC1 complex) during the proliferative phase of the T cell response increases the number of memory T cells generated, due to an enhanced commitment of effector cells to become memory cells [51]. mTOR inhibition in effector T cells may inhibit anabolic metabolism and drive the cells back into the catabolic state and fatty acid oxidation that are characteristic of memory cells [52].

Characteristics of Age-Related T Cell Malfunction

The activity of both Th and Tc cells deteriorate with aging, partly due to cell-intrinsic defects. The mechanisms leading to the cell intrinsic age-associated defects in T cells have been recently reviewed elsewhere [2, 3]. In brief, naïve CD4⁺ T cells in aged mice survive longer in the circulation compared to naïve CD4⁺ T cells from young mice, and express reduced levels of Bim, a proapoptotic Bcl family member [53]. This longevity has been suggested to serve as a homeostatic mechanism to maintain the size of the naïve T cell population in aged mice, when the number of recent thymic emigrants (newly generated naïve T cells) is reduced due to thymic involution [54]. When activated,

these long-lived naïve CD4⁺ T cells show lower Ca⁺² influx in response to TCR triggering, and reduced clonal expansion as compared to naïve CD4⁺ T cells from younger mice. As a result, aged naïve CD4⁺ T cells give rise to less efficient effector cells. For example, impaired helper function results in poor T cell-dependent B cell responses and antibody secretion in aged mice [55]. Finally, the memory cells that develop from aged naïve CD4⁺ T cells are dysfunctional, showing a reduction in cytokine production and inability to expand after restimulation [56].

In general, the same is true for aged Tc cells. Aged individuals have a reduced naïve CD8⁺ T cell pool, with a restricted repertoire. Upon activation, these aged effector CD8⁺ T cells show impaired proliferation, differentiation, and cytokine production. Interestingly, adoptive transfer of naïve CD8⁺ T cells from an aged donor to a young recipient does not rescue the impaired phenotype [57, 58], suggesting that the functional deficit of aged naïve CD8⁺ T cells is, at least in part, due to cell autonomous malfunction [3]. Thus, long-lived naïve T cells gradually lose function over time, highlighting the need to understand molecular changes that occur in immune cells during aging. In addition, there is an increase in expression of inhibitory receptors including PD-1, LAG-3, CD160, and CD44 on T cells in aged mice [59]. Here we will focus on studies pointing to a role for mitochondrial malfunction in this age-related gradual deterioration.

Age-Related Mitochondrial Malfunction and T Cell Senescence: Are They Connected?

The importance of mitochondrial function in T cell biology suggests an interesting connection between T cells and mitochondrial decline. It is well established that mitochondrial function is compromised with aging [60]. Considering the multiple ways mitochondria support proper T cell activation, we propose that mitochondrial dysfunction contributes to the reduced potency of the naïve T cell pool in aged individuals.

Impaired Ca⁺² buffering capacity, reduced coupling efficiency for ATP production, and exacerbated generation of mitochondrial ROS are characteristics of mitochondrial malfunction in aged cells, and all of these alterations have the potential to impair the early phase of naïve T cell activation. Aged T cells show reduced Ca-mediated signaling [61], which could be attributed, at least in part, to Ca⁺² buffering deficits found in mitochondria of aged cells [62]. Impaired Ca⁺² uptake by the mitochondria will prevent sustained opening of CRAC channels at the immune synapse

and impair Ca^{+2} -mediated activation of key transcription factors like NFAT1 and NF- κ B. Bioenergetic studies of postmitotic tissues suggest a decline in mitochondrial respiratory function with age. This reduction in oxidative phosphorylation is attributed to many factors, including a decline in activity of respiratory enzyme complexes, a decrease in mitochondrial membrane potential, and an increase in proton leak (reviewed in [63]). Impaired function of the electron transport chain results in reduced ATP production. Failure to provide sufficient ATP during the initial activation of naïve T cells may result in defects in signaling and impaired synthesis of biomass, and thereby impair early cell growth. Sahin et al. [64] recently described one mechanism explaining mitochondrial dysfunction with aging. They showed that p53 could be activated by telomere dysfunction in aged cells and repress peroxisome proliferator-activated receptor- γ , coactivator 1 (Pgc1), the master mitochondrial regulator, resulting in inhibition of mitochondrial biogenesis and function [64].

Various interventions have been shown to delay or rescue age-associated deficit in mitochondrial function. For example, calorie restriction can partially prevent age-related decline in mitochondrial gene expression and increase mitochondrial biogenesis through Pgc1 activation [65]. Similarly, enduring exercise induces mitochondrial

biogenesis in skeletal muscle, and was recently shown to induce mitochondrial rejuvenation in multiple tissues in the mitochondrial DNA mutator mouse, with aging [66]. Interestingly, these interventions have a similar rejuvenating effect on T cell-mediated immunity. Calorie restriction maintained a naïve T cell pool and improved T cell function in nonhuman primates [67] and mice [68]. Studies testing the effect of exercise on immune senescence in laboratory animals have shown an overall beneficial effect on immune potency, including enhanced responsiveness to vaccination and increased T cell proliferative capacity [69]. The ability of both exercise and calorie restriction to improve T cell immunity with aging could be partially mediated by their positive effect on mitochondrial function with age.

Although we are learning about the tight interaction between intracellular metabolic pathways and immune cell function, there is still a lot to discover regarding the role of mitochondria in aging immune cells. A better understanding of the role of mitochondrial metabolism in immune cell function may provide insights into mechanisms of immune senescence with the potential for developing novel therapeutic approaches to improve immune responses in aged individuals.

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